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Relationship of ABO Blood Groups and Rh Factor with Ischemic Heart Disease at Khartoum State

علاقة فصائل الدم والعامل الريصي بمرض القلب الاحتباسي في ولاية الخرطوم

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الايـــة

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قال تعالى:

(الرَّحْمَنُ {1} عَلَّمَ الْقُرْآنَ {2} خَلَقَ الْإِنسَانَ {3} عَلَّمَهُ الْبَيَانَ {4})

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Dedication

To my dear father and beloved mother to provided support.

To my husband for his understanding and encouragement.

To my wonderful supervisor Professor shadia Abdul Ati, for her great help and support.

To everyone who helped me to finish this work. Special thank to my sister Tassneim.

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Abstract

The aim of the study was to find out the relationship between ABO blood groups and Rhesus factors (Rh) with ischemic heart disease (IHD) and possible risk factors .The study was conducted in Ahmed Gasm and Alshaab Hospitals in Khartoum State during the period from April to September 2018.Prior to the study, ethical clearance was obtained from the authorized bodies and a written consent was taken from all the participants. The study included 40IHD patients, comprising 21(52.5%) males and 19(47.5%) females and 60 controls comprising 52(86.7%) males and eight (13.3) females.The participants age range was (40 -80) years and 67.5% of them were (40-60) years old. The patients were interviewed and relevant data were obtained through a questionnaire. Blood groups and Rh factor were determined by slide technique using specific antibodies. IHD was more common in males than female. The common blood group was O (57.5%) followed by A(25%),B (12.5%) and AB(5%) groups and the corresponding percentages for the control were 65% , 23.3%, 11.3% and zero, respectively. It was found that 85%of IHD patients and 90% of the control were Rh positive. No significant association was found between IHD and ABO blood group or Rhesus factor. It is concluded that ABO/Rh blood groups are not risk factors for IHD .Smoking , diabetes mellitus, hypertension and hyperlipidemia are considered risk factors for IHD. It is recommended that wide scale studies to be carried out in Sudan to investigate the prevalence and risk factors for IHD.

المستخلص

هدفت الدراسه الي التعرف علي العلاقه بين فصائل الدم والعامل الريصي بمرض القلب الاحتباسي وعوامل الخطر المحتملة.اجريت الدراسه في مستشفي احمد قاسم ومستشفي الشعب بولاية الخرطوم في الفترةمن شهرابريل الي شهر سبتمبر 2018 .قبل الدراسه تم الحصول علي تصريح اخلاقي من الجهات المعتمده وتم اخذ موافقة مستنيره من جميع المشاركين.اشتملت الدراسة اربعين مريض,تضم21 (52.5%) ذكرا و19(47.5%) انثى.وستين عينه ضابطه52(86.7%) ذكرا وثمانية(13.3%) اناث.تراوحت اعمار المشاركين بين 40–80 عاما وكان67.5% منهم(60–40) عاما. اجريت مقابلات مع المرضي وتم الحصول علي البيانات ذات الصله عن طريق استبيان.تم تحديد زمر الدم والعامل الريصي عن طريقة الشرائح بإستخدام أمصال مضادة . كان مرض القلب الاحتباسي اكثر شيوعا في الرجال من النساء. وكانت أكثر زمر الدم انتشارا في المرضى هي (57.5%)O,(25%)A, (12.5%)B,(5%)AB.

وكانت النسبه المئويه المقابله للعينات الضابطة,65%,23.3.%, 11.3 %,0% علي التوالي.وجد ان 85% من مرضي القلب الاحتباسي و 90% من المجموعة الضابطة موجبي العامل الريصي. لم يتم الحصول علي علاقه ذات دلاله احصائيه بين فصائل الدم ومرض القلب الاحتباسي .خلصت الدراسه الي فصائل الدم والعامل الريصي ليست عامل خطر لمرض القلب الاحتباسي. وجد ان التدخين, امراض السكري ,الضغط ,ارتفاع الدهون مثلت عوامل خطر للمرض. يوصى باجراء دراسات واسعه النطاق في السودان للتحقق في عوامل الانتشار وعوامل الخطر الخاصه بمرض القلب الاحتباسي.

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List of Abbreviations

IHD	Ischemic heart disease
VWF	vonWill brand Factor
HDFN	hemolytic disease of the fetus and newborn
RBC	Red blood cell
GAL	D-galactose
GAL NAC	N-acetyle-galactosemine
IgG	Immunoglobulin G
IgM	Immunoglobulin M
K2EDTA	Potassium Ethylene Diamine Tetra Acetic Acid
MI	Myocardial Infarction
Rh	Rhesus
VHD	Valve Heart Disease von
CHD	Congenital heart defect
SPSS	Statistical Package of Social Science

Chapter One

1. Introduction and Literature review

1.1 Introduction:

The ABO blood group system is widely credited to have been discovered by the Austrian scientist Karl Landsteiner, who found three different blood types in 1900.Blood groups form a comparatively small field of study but they have an important place in genetics, immunology, anthropology and clinical medicine .This is the most significant blood group system in transfusion practice and the only one for which reciprocal antibodies are consistently present in sera. These antibodies may cause severe intravascular hemolysis if ABO-incompatible blood is transfused into such patients. (Dean , 2005). Many reports (Lutfullah ., *et.al*.,2010, Banerjee and Datta 2011; Zhang *et. al*., 2012,Geggel and Writer 2015)have appeared in recent years suggesting an association between blood groups and various manifestations of heart disease. Most of these studies investigated patients with Ischemic heart disease (IHD) which is a worldwide health problem. Its incidence is on rise. It is one of the most common causes of death and morbidity in most regions of the world. In almost all the cases, atherosclerosis is the underlying cause of IHD and its different presentations(Bloomfield, *et al*., 2009).In addition, blood group was studied as a risk factor for many diseases like peptic ulcer, carcinoma of stomach periodontal diseases and diabetes mellitus .There is an evidence that individual's ABO group can predetermine the risk of thrombosis. However, significance of the association between the ABO blood group and IHD in the

clinical practice is not yet known (Clark, *et al.*2005,Demir, *et al.*2007,Faircloughand Silk, 2009*).*

In Sudan to the best of our knowledge there are no studies on the association between ABO blood group and ischemic heart disease, so the aim of this work was to investigate the association between ABO blood group and ischemic heart disease.

1.2 Literature Review:

1.2.1. 1Composition of blood:

Blood is a sticky opaque fluid with a characteristic metallic and salty taste. Depending on the amount of oxygen it is carrying, the color of blood varies from scarlet (oxygen-rich) to a dull red (oxygen-poor).Blood is heavier than water and about five times thicker, or more viscous ,largely because of its formed elements. Blood is slightly alkaline, with a pH between 7.35 to7.45, it's temperature (38^{o}c) is always slightly higher than the body temperature. Blood accounts for approximately 8 % of the body weight. The average adult body contains approximately 6 liters of blood.

Blood consists of 55% plasma and 45% cellular elements which are erythrocytes (red cells), leukocytes(white cells), and thrombocytes (platelets).Red cells (erythrocytes) in human are small, non-nucleated biconcave disc contain Hb (an O2-carrying pigments responsible for red color of fresh blood), transport oxygen from the respiratory epithelium to the sites of consumption, The Red cell membrane (or outer cover) is composed 3 bimolecular leaflet or phospholipids hydrophobic) covered 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 are situated largely on the external surface. (Bryant, 2002).

1.2.1.3 ABO Blood Group System:

ABO blood group was the first blood group system described, and remains the most important blood group system for transfusion purposes. ABO blood group has four major phenotypes (Table) derived from the two major antigens (A and B) of the system. These phenotypes group A, group B, group AB and group O (Hoff brand *et al*, 2006) .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. (Gutman, 2011). The different blood groups react differently to environmental stimuli and have different susceptibility to human disease, depending on type of antigens on red cell surface. If blood enters the system with antigens that are not found, the body will create antibodies against it. However, some people can still safe 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 will not attack (Krans , 2015).

The ABO Blood Group System. (Hoff brand *et al*,2006)

phenotype	genotype	Antigens	**Naturally occurring anti bodies**	**Frequency (UR) (%)**
O	OO	O	Anti-A, Anti-B	46
A	AA or AO	A	Anti-B	42
B	BB or BO	B	Anti-A	9
AB	AB	AB	None	3

1.2.1.4 History of blood group discovery:

The ABO blood group system was first discovered by Karl Landsteiner in 1901; he mixed the sera and red cells from his coworkers and discovered that they reacted in different ways .He named three groups A, B and O. The fourth less frequent blood group AB was discovered in 1902 by Decastello and Struli (Mourant,1983). The genes for A and B blood group are found on chromosome 9p and expressed in Mandalian co-dominant manner (Hauser,*et al*.,2005).

1.2.1.5 Antigens of ABO blood group system

They are mainly A, B and H antigens which are proteins in nature embedded in a mosaic pattern without any fixed position on fluid lipid layer of cell membrane .The formation of ABH antigens results from the interaction of

genes at three separate loci (ABO, Hh , and Se). These genes do not actually code for the production of antigens but rather produce specific glycosyl transferases that add sugars to a basic precursor substance. H, A and B antigens are formed from the same basic precursor material (called aparagoboside) to which sugars are attached in response to specific enzyme tranferases elicited by an inherited gene(Seltsam, *et al* 2003,). ABH antigens on the RBC are constructed on oligosaccharide chains of type2 precursor substance .The ABH antigens develop as early as the 37th day of human fetal life but do not increase much in strength during the gestational period .The expression of A and B antigens on the RBCs is fully developed by 2 to 4 years of age and remains constant for life (Dodd, *et al.,* 2002).

1.2.1.6 Antibodies of ABO blood group system:

ABO antibodies are produced in response to common chemical structures, identical to the A and B antigens, which are present widely in the environment. Naturally occurring antibodies occur in the plasma of subjects who lack the corresponding antigen and who have not been transfused or been pregnant. The most important are anti-A(from a group B individual) and anti B(from a group A individual); these are usually immunoglobulin M (IgM) that react optimally at cold temperatures (4ºC) but there may be small quantities of IgG present .Group O serum contains anti-A and anti-B. Only IgG antibodies are cable of trans placental passage from mother to fetus ,the antibodies develop after birth .Children of less than 3 to 4 months usually have little or no antibodies in their serum due to their underdeveloped immune system and lack of antigenic exposure .Antibody production peaks when an individual is between 5 and 10 years of age and declines later in life (Daniels,2002).

1.2.1.7 ABO Sub Groups:

Red blood cells of 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. The cells of approximately 80 percent of all group A (or AB) individuals are A1 (or A1B), and the remaining 20 percent are A2 (or A2B) or weaker subgroups (A_3, A_x, A_{end}, A_m, A_y, and A_{el}), The difference between A1 and A2 is both quantitative(fewer A antigens on A2 than A1) and qualitative (structure differences),because1 to 8 percent of A2 individuals produce anti-A1 in their serum, and 22 to 35 percent of A2B individuals produce anti-A1. This antibody can cause discrepancies in ABO testing and incompatibilities in cross matches with A1 or A1B cells. (Shaz *et. al*., 2013).

1.2.1.8 Secretor and Non-Secretor:

about 80% of the UK populations are ABH secretors as they have H antigen plus A or B according to their ABO genotype in body fluids such as saliva, sweat, tears, serum and the gastrointestinal mucous secretions .The remaining 20% are non – secretors or weak secretor who have no or little antigen present in their body fluids .A recent paper suggests that individual ABO blood groups and secretor status are part of human's innate immunity against infectious disease.(Hoff Brand *et al*., 2000).

1.2.1.9 The Rhesus System:

Rh is the most important blood group system after ABO in transfusion medicine with more than 50 different Rh antigens but Only 5 antigens D, C, c, E and e are inherited in various combinations and account for most of the Rh-related problems encountered in practice. The clinical importance of the

Rh blood group systems arise from the fact that the antigen D of the system is highly immunogenic If a unit of D -positive blood is transfused to a D-negative recipient, the recipient forms anti-D in some 90% of cases and there after cannot safely be transfused with D-positive red cells. The Rh antigens are thought to play a role in maintaining the integrity of the RBC membrane. They may also be involved in transport of ammonium across the RBC membrane (Avent and Reid ,2000) .

1.2.1.10The nomenclature of Rh blood group system

Three different systems of nomenclature 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 phenotyping information rather than genetic information. In this system, the antigens are numerically named in the order of their discovery or assignment to the Rh blood group system. (Greer *et al.*, 2009

1.2.1.11. Rh blood group system antigens:

Rh antigen is a protein surrounded by lipid, Rh activity is not lost when lipid is extracted from red cells membranes the lipid doesnot carry the antigenic

determination but may be essential for confirmation of the determinants (Nevillen*et al.*,1994). The Rh antigens are thought to play a role in maintaining the integrity of the RBC membrane. They may also be involved in transport of ammonium across the RBC membrane (Avent and Reid ,2000) .

1.2.1.12 Rhesus Antibodies:

Rh system antibodies are usually made by exposure to Rh antigensthrough transfusion or pregnancy. Antibodies to Rh system antigens show similar serologic characteristics. Most antibodies are IgG and bind at 37° C; agglutination is observed by the IAT. The majority of Rh antibodies are IgG and usually IgG1, IgG3 or combination of these subclasses, the majority of Rh antibodies are IgG1 and IgG3 activate complement, Exceptional examples of Rh antibodies anti-D and antic (Rudmann , 2005).

1.2.2.1 Heart:

The heart is one of the most important organs in the entire human body. It is composed of four chambers two thin-walled atria on top, which receive blood, and two thick-walled ventricles underneath, which pump blood. Veins carry blood into the atria and arteries carry blood away from the ventricles. Between the atria and the ventricles are atrioventricular valves, which prevent back-flow of blood from the ventricles to the atria. The left valve has two flaps and is called the bicuspid (or mitral) valve, while the right valve has 3 flaps and is called the tricuspid valve. The valves are held in place by valve tendons (“heart strings”) attached to papillary muscles, which contract at the same time as the ventricles, holding the valves closed. There are also two semi-lunar valves in the arteries (the only examples of

valves in arteries) called the pulmonary and aortic valves .The left and right halves of the heart are separated by the inter-ventricular septum. The walls of the right ventricle are three times thinner than the left and it produces less force and pressure in the blood. This is partly because the blood has less far to go (the lungs are right next to the heart), but also because a lower pressure in the pulmonary circulation means that less fluid passes from the capillaries to the alveoli. The heart works as a pump moving blood around in our bodies to nourish every cell. Used blood, that is blood that has already been to the cells and has given up its nutrients to them, is drawn from the body by the right half of the heart, and then sent to lungs to be re oxygenated. Blood that has been re oxygenated by the lungs is drawn into the left side of the heart and then pumped into the blood stream. It is the atria that draw the blood from the lungs and body, and the ventricles that pump it to the lungs and body (Bridget, 2010).

1.2.2.2 Type of Cardiovascular diseases:

Cardiovascular disease is the term for all types of diseases that affect the heart or blood vessels (vascular disease). The vascular diseases include , Coronary artery disease (known as coronary heart disease and ischemic heart disease).,Peripheral arterial disease Cerebrovascular disease (disease of blood vessels that supply blood to the brain-includes stroke),Renal artery stenosis ,and Aortic aneurysm .The cardiovascular diseases that involve the heart include: Congenital heart disease ,Cardiomyopathy ,Hypertensive heart disease, Heart failure ,Pulmonary heart disease, Cardiac dysrhymias and Valvular heart disease .In addition there are Inflammatory heart disease i.e Endocarditis, Inflammatory cardiomegaly and Myocarditis (Mendis *et al*., 2011 ; G.B.D., 2013).

1.2.2.3 Myocardial Infarction (MI)

There are two types of MI, with different morphology, pathogenesis and clinical significance: transmural infarct which involves the full thickness of the ventricular wall; it is usually caused by severe coronary atherosclerosis, with acute plaque rupture superimposed occlusive thrombosis, and sub endocardial infarct which is typically limited to the inner third of the ventricular wall; it is caused by increased cardiac demand in the setting of limiting supply due to fixed atherosclerotic disease. (Mitchell et al., 2005).

1.2.2.4 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 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, 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)

1.2.2.5 Valve Heart Disease (VHD)

Valve heart disease in adults is typically caused by degeneration, immunologic inflammatory processes or infection. The failure of compensatory hypertrophy mechanisms is heralded by angina, syncope with onset of such symptoms, and if left untreated, there is a 50.0%risk of death within 2 to 5 years; urgent surgical valve replacement is clearly indicated (Mitchell *et al*., 2005).

1.2.2.6 Cardiomyopathy:

When the abnormality is primary in and localized to the myocardium, the condition is called cardiomyopathy. Cardiomyopathy is not synonymous with CHF; the latter represents a consequence of many forms of cardiac disease. Cardiomyopathy is divided into three main categories: dilated, hypertrophic, and restrictive (Mitchel *et al*., 2005).

1.2.2.7Heart Failure:

Heart failure means that the heart is not 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 cough. (American Heart Association, 2015)

1.2.2.8Ischemic Heart Disease:

(IHD) is a condition of an imbalance between myocardial oxygen supply and demand. It Occurs when there's an inadequate supply of blood and oxygen and inadequate perfusion by one or more coronary arteries to a portion of the myocardium supplied by it (Long .,*et al*2005).Ischemic heart disease (IHD) which is the most common presentation of heart disease is the most important and largest single cause of premature death in many developed countries, but seen to be rare in most developing countries (Trustwell .,2003,Colledge .,*et al* 2010).The clinical manifestations of IHD can be divided into four syndromes**:**

- the most important form of IHD is MI, in which the duration and severity of ischemia is sufficient to cause death of heart muscle.

-Angina pectoris, in which the ischemia is less severe and does not cause death of cardiac muscle, has three variants; stable angina, Prinzmetal angina, and unstable angina ,The latter is the most threatening as a frequent harbinger of MI, Chronic stable angina is generally caused by fixed, obstructive at heromatous plaque in one or more coronary arteries. Unstable angina and acute MI are also known as acute coronary syndromes and result from distinct pathophysiologic mechanisms, most commonly rupture of an unstable atherosclerotic plaque with subsequent platelet aggregation and Thrombosis .In Prinzmetal angina atherosclerotic plaques are absent and the with from ischemia intense vasospasm that reduces myocardial oxygen supply Then cause Chronic IHD with heart failure and the fourth is Sudden cardiac death defined as unexpected cardiac death within 1hour of symptom onset (Mitchell.*et al*., 2005).

1.2.2.9 Symptoms of Ischemic Heart Disease:

-Chest pain is described as tightness in the chest which may radiate to the base of the neck, the jaw, arms (normally the left arm) or back. It is sometimes accompanied by shortness of breath, dizziness, cold sweats, nausea and vomiting, palpitations or even loss of consciousness.

-Dyspnea may result as the heart becomes weaker and can no longer pump blood towards the rest of the body. This result in congestion, edema and difficulty in breathing.

-There are many types of palpitation of varying severity, may occur which tend to produce a rapid pulse and a fluttering or thumping feeling in the chest. Palpitations could be secondary to an arrhythmia.

-Sweating, nausea and vomiting. These symptoms may all appear together or individually. They are caused by the body, response , specifically that of the nervous system, to the heart muscle ischemia.

-Loss of consciousness, in the context of an infarction is due to problems with the heart's electrical conduction or the presence of severe arrhythmias (Mitchell.*et al*., 2005).

1.2.2.10 Risk Factors:

There are several risk factors of IHD, some are modifiable risk factors which are smoking, hypertension, diabetes mellitus obesity medical history, alcoholism, lack of exercise, stress and hyperlipidemia. Other non modifiable risk factors, which are age, sex and family history of IHD (Long *et al*., 2005, Abdollahi *et al*., 2009).

1.3: Correlation between ABO blood group system with disease:

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).

1.3.1Correlation between ABO blood group system and malaria:

The association of genetic markers with malaria has been the subject of numerous investigations, since the protection afforded by sickle-cell hemoglobin against infection by falciparum malaria parasite. A broad range of available evidence suggests that the origin, distribution and relative proportion of ABO blood groups in humans may have been directly influenced by selective genetic pressure from Plasmodium falciparum infection(Christine *et al*.2007). Clinical reports of ABO blood groups and P. falciparum infection, revealed a correlation between disease severity and ABO groups. However, several studies undertaken have been unable to link ABO blood groups to the incidence of malaria or to the repeat attacks of malaria (singh *et al,* 1995).In study done by (Montoya *et al,* 1994) they showed that there was no correlation between ABO blood groups to the incidence of malaria parasitemia.

1.3.2 Correlation between ABO blood group system and cardiovascular disease:

Over time, the development of sciences introduced a relationship between blood grouping system and cardiovascular diseases.. In Iran, Geggel and writer (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 followed 50,000 middle age and elderly people in northeastern Iran for an average of seven years and found that 9% of people with non-O blood types were more likely to die for any health-related reason, and 15 % more likely die for cardiovascular disease compared to people with O blood type .They also 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 a substantial historical association between non-O blood group status and an increase in some cardiovascular disorders. have confirmed ABO as a locus for venous thrmbo embolism (VTE), (MI) and multiple cardiovascular disease.

1.3:3 Correlation between ABO blood group system and ischemic heart disease:

In Italy, Carpeggiani *et al*,(2010), found in their study of ABO blood group alleles found that A blood group is a risk factor for coronary artery disease A significant association was found between non - O blood group and ischemic heart disease and presence of coronary atherosclerosis .Higher prevalence of A and B alleles were found in patients with myocardial infarction.

In sudan , (Reela, 2014), performed a descriptive cross-sectional study of the frequency of ABO blood groups and Rh factor among 70 cardiovascular disease patients ,she found that the Most common cardiovascular diseases was ischemic heart disease (30.0%) followed by heart failure (17.1%) and least frequent was valvular heart disease .no association found between ABO blood group and Rh factor and any of the cardiovascular diseases. However O blood group was more common in IHD patients. In western countries(stakishaitis, *et al* 1991, Akhund., *et al* 2001,Stakishaitis , *et al* 2002)all reported lower incidence of coronary heart disease with blood group O as compared with group A and men belonging to groups O or B had a lower mean cholesterol level than group A, a finding that emphasizes the genetic pathogenesis of ischemic heart disease .

Studies done by Amirzadegan, *et al* 2006 and Sari., *et al*(2008), no correlation between various blood group and development of coronary artery

disease .They observed that the prevalence of major risk factors was equal in patients with different blood groups and had no impact on development of premature coronary artery disease in individual subject. In Pakistan Khan *et al* (2005) and Wazirali , *et al* (2005) found a strong association of Ischemic heart disease with blood group A as compared with other blood group. Other study done in British men by Whincup , *et al* (1990) showed that the incidence of ischemic heart disease is higher in patients with blood group A. In UK Meade, *et al* (1994) found that the incidence of ischemic heart disease is significantly highest in patients with blood group phenotype AB than in those with groups O, A or B.

1.8 Justification:

In Sudan there is an increase in the incidence of ischemic heart disease (Reela , 2014).To the best of our knowledge there is a scarcity of data concerning any relationship between ischemic heart disease and ABO/Rh blood group in Sudan.

This work will be undertaken to contribute to the knowledge in this aspect.

There is an evidence that incidence of ischemic heart disease is highest in blood type A in India .It was found that having a non O blood group is associated with increase risk of coronary events and risk of myocardial events.

1.9 Objectives:

1.9.1General objective:

To assess the association between ABO blood group and ischemic heart

1.9.2. Specific Objectives :

1. To determine the frequency of ABO blood group in ischemic heart disease
2. To investigate whether ABO/Rh blood group is a risk factor to IHD.
3. To investigate whether DM, hypertension, hyperlipidemia and smoking are risk factors for ischemic heart disease.

Chapter Two

Materials and Method

2. Study type and duration:

This is a prospective analytical study conducted in Khartoum State during the period of April to septmber2018 in Sudanese patients with ischemic heart diseases.

2.2Study area:

The study was conducted at Ahmad Gasm Hospital and Alshaab Hospital - Khartoum State.

2.3 Study population:

Forty patients with confirmed IHD of both sexes attended Ahmad Gasim Hospital and Alshab Hospital during the study course and sixty matching healthy individuals as the control group.

2.4 Inclusion criteria:

Adult male and female who were diagnosed with ischemic heart disease (IHD)who attended Ahmad Gasim Hospital and Alshab Hospital during the study period were enrolled in this study .Control group were healthy individuals free from IHD .

2.6 Participants Data

Demographic data and some characteristics of the participants were collected by using a questionnaire which was filled by verbal direct interviewing of the participants.

2.7Method

2.7.1Blood collection:

Two and half m1 of venous blood was collected in K_2 EDTA (Potassium Ethylene Di amine Tetra Acetate) and mixed gently. (Kathen *et al*., 1998).

2.7.2 ABO slide agglutination test

2.7.2.1 Principle:

When red cells are 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 occurredin the red cells not containing the corresponding antigen (Walker *et al*., 1999).

2.7.2.2 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 was slowly tilted for one minute, then agglutination was observed.
6. Result was read and recorded.

2.7.2.3. 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.

2.7.2.4. Quality control:

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

2.7.3 Rh (D) red blood cell typing:

2.7.3.1. 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 should be performed.

2.7.4. DU Method (The indirect anti globulin):

2.7.4.1. 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.

The technique of D" method:

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

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

3-The tube was incubated at 37°C for 15 minutes in LISS.

4-After incubation, the mixture was centrifuged.

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

6-Two drops of antiglobulin reagent were 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).

2.7.4.2. Interpretation:

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).

2.8 Ethical consideration:

Study was approved by Ethical and Scientific Committee, Medical Laboratory Science College-Sudan University of Science and Technology .Participant were verbally informed about the research, its benefits and method of sample collection .The participants were assured that the results will be confidential and will not be used for any other purpose than of this study.

2.9 Data analysis

Data were statistically checked and analyzed using Statistical Package of Social Science (SPSS) program version 20.Chi- square test was performed for determining the association between ischemic heart disease and ABO/Rh blood group and some other possible risk factors.

3.1 RESULTS:

3-1 Participants particulars:

The study included 40IHD patients, comprising 21(52.5%) males and 19(47.5%) females and 60 controls comprising 52(86.7%) males and eight (13.3) females. Most of the patients (67.5%) were aged (40-60) years and the mean age was57.73, while the control mean age was 53.85.Table (1)

Table1Distribution of study group according to gender and age group:

Age group year	**IHD**		**Control**	
	M NO (%)	F NO (%)	M NO (%)	F NO (%)
40-60 61-80	14(35%) 7(17.5%)	11(27.5%) 8(20%)	44(73.3%) 8(13.3%)	6(10%) 2(3.3%)
Total	21(52.5%)	19(47.5%)	52(83.3%)	8(11.7%)

3-2.Occupation of Participants:

The frequency Occupation status in IHD patient is equal in the cases who had have occupation officer and house wife 35.5%; it was only 30.0% among cases manual occupation. While 56.7 of the control had have occupation officer worker, and 43.3%have manual worker, and the relation between IHD prevalence and occupation was significant p=0.000.showed table2.

Table 2 Occupation status for the participants:

Occupation	Case		control	
	NO	%	NO	%
Official workers	14	35.0	34	56.7%
Manual workers	12	30.0	26	43.3%
House wife	14	35.0	0	00.0%
Total	40	100.0	60	100.0
$X^{2=}$24.470,p=0.000				

3-3Nutritional aspect diet and body condition:

According to the type of the diet the distribution of IHD patients and the control was eating high fat diet (52.5% vs.15.0%) and eating low fat diet was(47.5 vs.85%). Obesity in IHD was 22% while in the control was 5%. Table (3).

Table 3 Distribution of study groups according to diet and body condition:

Parameters		Case%	Control%
Type of diet	High fat	52.5%	15.0%
	Low fat	47.5%	85.0%
Body condition	Obese	22.5%	5.0%

3-4. ABO blood groups and Rh factors of participants:

The most frequent ABO blood type among all the participants was O .Distribution of IHD patients and the control according to their ABO blood type was O (57.5%vs.65%) A (25% vs23.3%), B (12.5%vs 11.3%) and AB (5%vs zero. 34(85.0%) of IHD patients were Rh positive (+ve) ,six (15.0%) were Rh negative(-ve) ,and in the control group 54(90%) were Rh positive(+ve) and six (10%) were Rh negative (-ve).

Table (2) shows that there is no significant association between ABO and ischemic heart disease (P= 0.353) as well as between Rhesus factor and ischemic heart disease, the (P =0.451) show in table(4).

Table 4. ABO blood groups and Rh factors of participants:

Blood group	**Case**		**control**		**Total**		**P value**	**X^2**
	NO	**%**	**NO**	**%**	**NO**	**%**		
A	10	25.0	14	23.3	24	24.0		
	5		7		12			
B	2	12.0	0	11.7	2	12.0		
	23	5.0	39	0.00			0.353	3.259
AB		57.0		65.0	62	2.0		
						62.0		
O								
Total	40	100.0	60	100.0	100	100.0		
Rh +ve	34	85.0	54	90.0	88	88.0	0.451	0.568
	6	15.0	6	10.0	12	12.0		
Rh – ve								
Total	40	100.0	60	100.0	100	100.0		

3-5.Duration of IHD:

The most common duration of the disease was less than one year(37.5%), while 12-17(7.5%) was the least disease duration and the mean duration was 2.05 (Table 5).

Table 5 Distribution of study groups according to duration of disease:

Duration of disease	frequency	percent
Less than 1	15	37.5%
1-5	14	35%
6-11	8	20.0%
12-17	3	7.5%

3-6Association between IHD and some risk factors:

A significant association was found between and diabetes mellitus (P=0.004), hypertension (P=0.002), total serum cholesterol level (P=0.000) and smoking (P=0.041) as risk factors for IHD. No significant association was observed between family History of IHD (p=0.270) show table (6)

Table (6): Association between ABO blood group and some risk factors of IHD:

Risk factors	IHD patients	Control	Total	P value	X^2
Diabetes mellitus Yes No	18(45%) 22(55%)	11(18.3%) 49(81.7%)	29(29.0%)	0.004	8.289
Hypertension Yes No	20(50.0%) 20(50.0%)	12(20.0%) 48(80.0%)	32.0 68.0	0.002	9.962
hyperlipidemia Yes No	14(35.0%) 26(65.0%)	2(3.3%) 58(96.7%)	16(16.0%) 84(84.0%)	0.000	17.907
Family history with IHD Yes No	7(17.5%) 33(82.5%)	9(15.0) 51(85.0%)	16(16.0%) 84(84.0%0	0.270	2.259
Smoking Yes No	12(30.0%) 28(70.0%)	8(13.3%) 52(86.7%)	20(20.0%) 80(80.0%)	0.041	4.167

Chapter Four

4. Discussion

This is a prospective study conducted in Khartoum State during the period from April to September 2018 to determine frequency of ABO and Rh blood groups in Sudanese patients and to investigate their association with ischemic heart diseases .The mean age of the study population was 57.73,Most of the patients(67.5%) were aged (40-60) years ,this accords with a study carried out in Iran; in which the mean age of the patients was 58.98 years(Anvari., *et al* 2009).Distribution of IHD patients and the control according to their ABO blood type was O (57.5%vs.65%) A (25% vs23.3%), B (12.5%vs 11.3%) and AB (5%vs zero). No significant association was found between ABO blood group type and IHD in this study. Fathelrahman (2010) investigated the distribution of ABO blood group in Sudanese people and concluded that blood group O is the predominant group in Sudan(52.7%) followed by A (23.3%), B (13.2%), while AB was the least frequent blood group(10.8%) .Similar findings were reported by Shahata *et al*, (2012), who undertook a cross sectional study investigating the distribution of ABO blood group and Rhesus antigens among blood donors attending the Central Blood Bank-Khartoum. No significant association was found between ABO blood group type and IHD in this work which accords with the findings of Sari *et al.*, (2008) who did not find any correlation between ABO blood group and coronary artery disease in Turkey. Swapnali *et al*, (2014) in India investigated the relationship between ABO blood group, secretor status and the incidence of IHD in a population in and around Davangere, and they did not find any significant association between ABO blood group, secretor status and IHD. Contradicting finding to this

study were found by many other researchers. Whincup *et al.*, (1990) found higher incidence of IHD in British men with group A than those with non group-A.; also Wazirali *et al*.,(2005) in Pakistan found a significant association between blood group Awith cardiac disease independent of the conventional risk factors for cardiovascular diseases. Bronte- Stewart. *et al.,(* 2003) found that blood group A (37.8%) and B (32.6%) are associated with higher risk of ischemic heart disease compared with group O (20.7%).In India Biwas *et al*.,(2013) found a significant association between O blood group and IHD, Banerjee and Daltta (2014) reported that IHD was highest in blood group A in Sikkimese as well as Bengaless population .Also Garg.*et al*(2012) studied the possible association between ABO blood group and myocardial infarction(MI), and observed a significant association between blood group B and MI . Chen *et al*.,(2016) have written a systematic review and meta-analysis of ABO blood group and the coronary artery disease; they concluded that both blood group A and non-O were the risk factors for coronary artery disease. The variation between the current work and the previous studies may be attributed to the sample size and the studied population was heterogeneous

The results of Rh factor shows that Rh positive (+v) frequency was higher than Rh negative (-ve) frequency in the study group, this aords with Tesfaye *et al.*, (2015), who studied the frequency distribution of ABO and Rhesus (D) blood group alleles in Silte Zone, Ethiopia, and they found that O blood type is predominant and also Rh positive was (92.06%) and Rh negative was (7.94%). The result presented in this study shows significant association between diabetes mellitus, hypertension, total serum cholesterol level, and Amirzadegan., *et al* (2006) showed that smoking is a risk factor for IHD,

which agrees with this study .other study done Lutfullah **,** *et al*,(2010) Association of ABO Blood Groups and major IHD Risk Factors show no significant association between D.M, hypertension, hyperlipidemia, Which dis agrees with this study.

4.2 Conclusion:

It is concluded from this study that:

1 blood group O is common IHD patients and controls

1- The ABO and Rhesus factor positive blood groups were Rhesus positive are common in IHD patients and controls.

2-There is no association between ABO blood group and Rhesus factor and ischemic heart diseases.

3-Thereis a significant association between IHD and diabetes mellitus, hypertension, total serum cholesterol level, and smoking.

4.3 Recommendations:

It is recommended that:

1. Minor blood grouping tests should be done for ischemic heart disease patients.

2. Cross sectional studies should be undertaken all over the country to investigate the possible risk factors of IHD like:

ABO blood group and Rhesus factor

Family history of coronary heart disease.

Occupation and pattern of life.

Education level.

Some chronic diseases like diabetes mellitus, hypertension , lipidemia etc.

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Appendix 1

Sudan University of Science and Technology

College of Graduate Studies

Medical Laboratory Science

Hematology Department

Questionnaire about relation of ABO blood group and Rhesus factor in Sudanese patients with ischemic heart diseases

Sample No. :……..

Pt .Name:……

Gender:…… Age:……

Other diseases: (if found)…….

Occupation:…… Smoking: Yes …….No.

Type of diet:…….

Body condition:……..

Pt. history of IHD:Yes ..No. Duration of illness:……

Laboratory Results:

ABO group:…….

Rhesus factor:……..

Smoking:……

Date:

Figure (1) Distribution of study group according to age group:

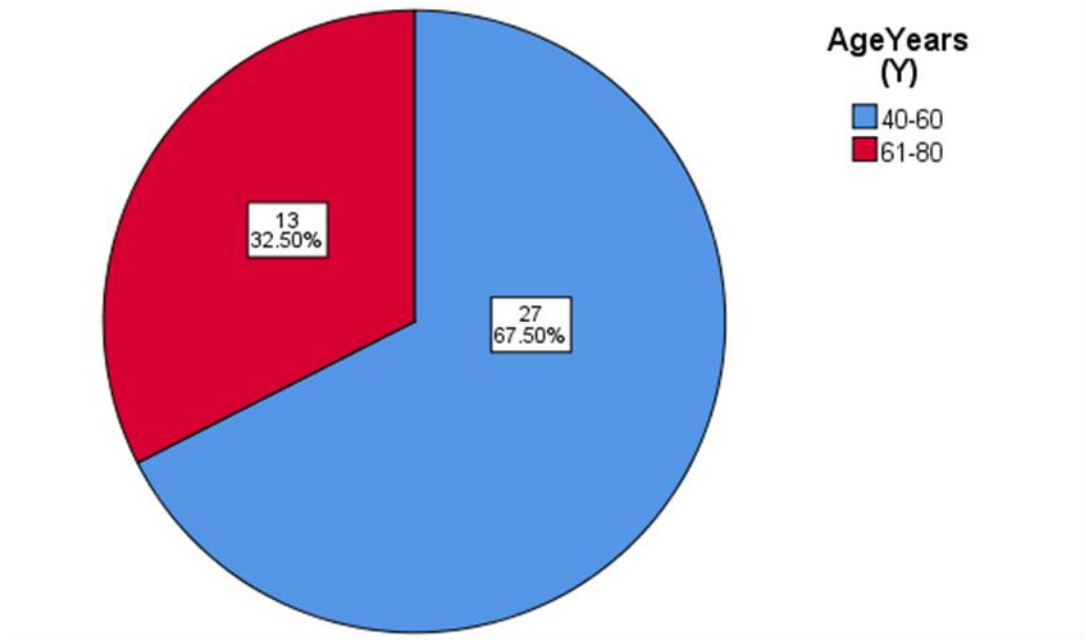

Figure (2) Distribution of study groups according to duration of disease

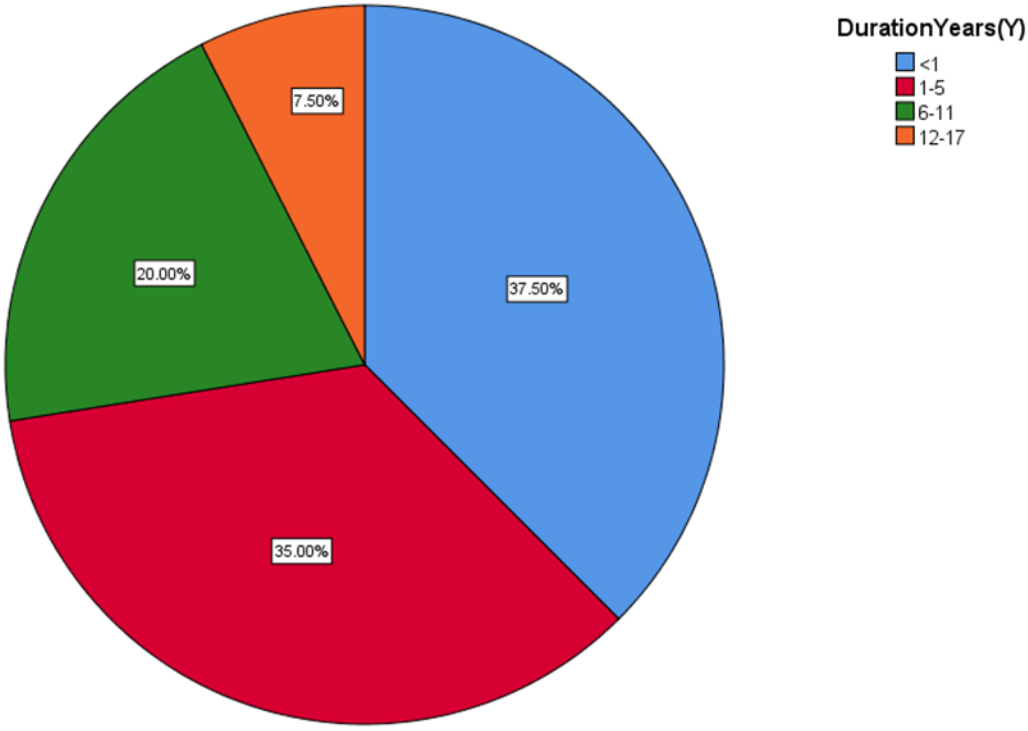

Figure (3) Distribution of study groups according to Occupation status: