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EC EMERGENCY MEDICINE AND CRITICAL CARE
Research Article

ABO and Rhesus Grouping among Sudanese Patients with Coronary Artery Disease at Sudan Heart Center- Khartoum State

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Received: November 26, 2019; **Published:** December 09, 2019

Abstract

Background: Many reports have appeared in recent years suggesting an association between blood groups and various manifestations of heart diseases. Many risk factors have been suggested as risk factors for Coronary Heart Disease. Among them is the ABO blood grouping.

Materials and Methods: This a descriptive analytical study aimed to determine the frequency of blood group and Rhesus factor of patients with cardiovascular diseases at Khartoum state, during the period from October 2018 to March 2019. Five hundred patients with coronary artery diseases attended Sudan center of Heart and 500 apparently healthy controls were enrolled in this study. An informed consent was obtained from each participant before blood sample collection. ABO and Rh factor were performed by slide techniques using specific anti-sera. Thereafter, data was analyzed and organized by Microsoft Excel and SPSS computer program.

Results: The results showed that most common blood group in patients with coronary artery diseases was O followed by A, and then B and least frequent was AB. Majority of patients with coronary artery diseases were Rh positive. Most common blood group was O (44.2%) followed by A (30.4%) and then B (21.4%) and least frequent was AB (4%). Finally, the results of the present study showed that there is no association of ABO blood group and Rh factor with cardiovascular diseases. Although the frequent of coronary artery diseases was higher in O group, the difference was statistically insignificant. We noticed that there's an association between Coronary heart disease and ABO blood group due to the result of the P value (0.0003).

Conclusion: We concluded that the risk of Coronary heart disease for individuals with non O blood grouping is higher than that of people with O blood grouping. And no association of Rhesus factors with Coronary Heart disease.

Keywords: *ABO; Coronary Arteries Disease; Sudan*

Background

The ABO and Rhesus blood group system are clinically the most important blood donors and patients must be correctly ABO grouped because transfusing ABO incompatible blood may result in the death of a patient. Rhesus grouping is also performed routinely except where the population is known to be with few exceptions, Rhesus {Rh D antigen} positive blood to a Rhesus negative person [1].

A person ABO blood group depends on the A, B, or O gene {located on chromosome 9} inherited from each parent [1]. The A blood group has merely the A antigen and the B has both A and B antigens, and the O blood type has neither A nor B antigen [1].

From the time when the discovery of the ABO blood group system by Karl Landsteiner in 1901, several reports have suggested an important involvement of the ABO blood group system in the susceptibility to infection, neoplastic disease, bleeding, eclampsia and even life expectancy [2-4]. The distribution of each of the four ABO blood types varies between racial groups, however, group O is the most common and AB is the least common [1].

Blood has two main components cells and plasma. Cells consist of 40 to 45% of the total amount of blood and plasma consists of 55 to 60% of total amount of blood. Cells are the formed elements and are of three types' red cells (erythrocyte), white cells (leucocytes) and platelet (thrombocytes) and each has its own characteristic [5].

Blood is a fluid which is continuously on movement. This process of movement of blood is known as circulation and the system that sustains the process is known as circulatory system and Dr. William Harvey was first person to describe circulation in 1616. It consists of mainly the heart and blood vessels viz. arteries and vein _ cardio vascular system. The whole system works under supreme control of nervous system and cardio respiratory centre is believed to be located in medulla oblongata or brain stem. The circulatory activity is also indirectly influenced by some hormones produced in our body. So it is a co-operative function of nervous and humeral mechanism that co-ordinate various activities of heart and blood vessels ensuring proper supply of blood in every corner of our body there by feeding the vital organs and tissues with oxygen, nutrient and other essential substances.

Blood vessels are of three types – arteries, capillaries and veins. Arteries are vessel that carries blood away from the heart and are having thicker walls consisting tunica adventitia, media and intima from outside in ward respectively composed smaller arterioles tissue and endothelium. Artery gradual becomes smaller arterioles and then capillary as they move away from heart. Capillaries are very small, capillary wall consists single layer of endothelium through which inter change between blood and tissue fluid takes place.

As blood flow through capillary wall into the tissue the arterial blood changes into venous blood after exchange of substance between blood and tissues. This changed blood drains into vein which is brought back to heart and thence to the lung for purification. So veins are also part of circulatory system but unlike arteries all the three coats of venous walls contains less elastic and less muscle tissue and so the veins are less resilient and easily collapses, it also consists of valves to allow un directional flow of blood towards heart, valves prevents back flow of blood [5].

The heart is one of the most important organs in the entire human body. It is really nothing more than a pump, composed of muscle which pumps blood throughout the body, beating approximately 72 times per minute of our lives. The heart pumps the blood, which carries all the vital materials which help our bodies function and removes the waste products that we do not need. For example, the brain requires oxygen and glucose, which, if not received continuously, will cause it to lose consciousness. Muscles need oxygen glucose and amino acids, as well as the proper ratio of sodium, calcium and potassium salts in order to contract normally. The glands need sufficient supplies of raw materials from which to manufacture the specific secretions. If the heart ever ceases to pump blood the body begins to shut down and after very short period of time will die. The walls of the heart are made up of three layers, while the cavity is divided into four parts, three are two upper chambers, called the right and the left atria, and two lower chambers, called the right and the left ventricles. The right atrium, as it is called, receives blood from the upper and lower body through the superior vena cava and the inferior vena cava, respectively, and from the heart muscle itself through the coronary sinus. The right atrium is the larger of the two atria, having very thin walls. The right atrium opens into the right ventricle through the right atrioventicular valve (tricuspid), which only allows the blood to flow from the atria into the ventricle, but not in the reserve direction. The right ventricle pumps the blood to the lungs to be reoxygenated. The left atrium receives blood from the lungs via the four pulmonary veins. It is smaller than the right atrium, but has thicker walls. The valve between the left atrium and the left ventricle, the left atrioventicular valve (bicuspid), is smaller than the tricuspid. It opens into

the left ventricle and again is a one way valve. The left ventricle pumps the blood throughout the body. It is the Aorta the largest artery in the body, which originates from the left ventricle. 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 reoxygenated. Blood that has been reoxygenated 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. The output of each ventricle per peat is about 70 ml, or about 2 table spoons. In a trained athlete this amount is about double, with the average heart rate of 72 beats per minute the heart will pump about 5 liters per ventricle, or about 10 liters total per minute. This is called the cardiac output. In a trained athlete the total cardiac output is about 20 liters [6].

Cardiovascular diseases are a group of disorders of heart attacks and strokes are usually acute events and are mainly caused by a blockage that prevents blood from flowing to the heart or brain. The most common reason is a build-up of fatty deposits on the inner walls of the blood vessel in the brain or by blood clots [6-9].

The cardio vascular system includes the heart and the blood vessels. A functional cardio vascular system is vital for survival, because without blood circulation, the tissue lack oxygen and nutrients, and wastes accumulate. Under such condition, the cell soon begins irreversible, which quickly leads to death. Cardio vascular disease {also called heart disease} is a class of disease that involves the heart, the blood vessels {arteries, capillaries, and veins} or both [6-9].

Chronic coronary artery disease is most commonly due to obstruction of the coronary arteries by athermanous plaque. No uniform syndrome of signs and symptoms is initially seen in patient with coronary artery disease. Chest discomfort is usually the predominant symptom in chronic (stable) angina, unstable angina, prinzmetal (variant) angina, micro vascular angina and acute myocardial infarction [10].

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Non modifiable risk factor like age, gender, family history but there is other risk factor like Altered levels of blood cholesterol, raised triglycerides with low HDL-cholesterol, High blood pressure, Diabetes, Smoking, being overweight, being inactive, excessive alcohol, Excessive stress. Moreover, of this the risk factor of coronary artery disease is high levels of C-reactive protein (CRP), Lipoprotein, Homocysteine, Small dense LDL-C particles and fibrinogen for all cardiovascular disease but one of the unknown reasons is ABO grouping for all cardiovascular disease [11].

Studies have identified the ABO blood group as a risk factor for the development of coronary heart disease [12]. Individuals with blood group A, B, or AB were 5% to 23% more likely to develop coronary heart disease compared with subject with O blood type, and the association was not altered by multivariate adjustment of other risk or dietary factors [12].

Risk factors include

CVD occurs more often in men than women until women reach menopause, at which time the relative risks are the same for both genders. Researcher have not concluded whether the increased risk for women is solely due to menopause because separating risks associated with age from menopause is difficult [15].

Aging is an important factor in the development of hardening of the arteries. The longer one lives and the more one's heart and blood vessels work, the greater is the chance of developing 'hardening of the arteries or atherosclerosis''(Alpert,1978).General risk for CVD increase with the aging process (men older than 45 years, women older than 55 years) [13].

A positive family history is defined as a history of premature (before age 55 years in father or before 65 years in mother) CVD or high blood cholesterol above 240mg/dl in a parent or first degree relative. Early screening for children and adolescents with a high risk resulting from family history is important so that appropriate therapy may be started when the fatty streaks in coronary arteries are just beginning [13].

Stress and anxiety have been shown to increase risk of heart disease complication and management [14].

Diabetes is metabolic disorder characterized by chronic hyperglycaemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both [15].

Poor diabetes control might increase CHD risk. The definition of diabetes is arbitrary, linked to a blood glucose level above which microangiopath occurs; however, a consuming interest in normoglycaemia and micro vascular disease has led to insufficient attention being paid to other metabolic changes (such as lipid and lipoprotein abnormalities) relevant to macro vascular disease, that occur at lower glucose levels. Diabetic control should normalize triglyceride 11 and HDL cholesterol level, and ameliorate insulin resistance and hyperinsulinaemia. Clustering of conventional risk factor partly accounts for excess risk in diabetes [16].

Cholesterol is a waxy substance made by the body and found in some animal based foods. Blood cholesterol levels describe a group of fats also known as lipoproteins which includes HDL cholesterol and LDL cholesterol. Cholesterol is important to overall health, but when levels are too high, cholesterol can be harmful by contributing to narrowed or blocked arteries. People with diabetes are more prone to having unhealthy high cholesterol levels, which contributes to cardiovascular disease [17].

HDL cholesterol is inversely associated with risk factors of coronary heart disease. HDL protects against atherosclerosis by carrying out reverse cholesterol transport and, perhaps, by delivering endogenous antioxidant enzymes, such as paroxonase, to the vessel wall. Low HDL levels are associated with type 2 diabetes, obesity cigarette smoking, and lack of regular exercise. Weight loss may increase HDL levels, as there is a linear inverse relationship between body mass index and HDL. This should be achieved by a combination of reduced energy diet and increased physical exertion, as exercise increase HDL in its own right [18].

The incidence of coronary heart disease-induced events, such as myocardial infarction and cardiac death, was found to be higher in both men and women with triglyceride levels above the mean for population. The increase is most obvious as the baseline levels rise from approximately 150 to 350 mg/dl. This relationship of plasma triglycerides to the risk of coronary heart disease is often stronger in women than man [19].

The risk of developing coronary heart disease in males and cigarette smoking is approximately 2.14 times greater than in non-smoking. Cigarette smoking lowers HDL and increase the risk of myocardial infarction and coronary heart disease in women taking oral contraceptive.

Major effects of smoking on the cardiovascular system by nicotine and the displacement of oxygen from hemoglobin by carbon monoxide [20].

Cigarette smoking has been shown to damage the inner lining of the coronary arteries. This damage speeds the development of atherosclerosis. These two factors together, namely an increased propensity to develop atherosclerosis and a decrease ability of the blood to carry oxygen [21].

Has been well established as a major risk factor for development of coronary atherosclerosis. Risk increase if hypertension is present along with the other established risks of hypercholesterolemia and smoking [20].

The cells that form the lining of the coronary arteries are often damaged when the pressure within the vessel is abnormally high. They begin to degenerate, and masses of fat, or atheroma, are likely to form, tending to block the flow of blood through the vessel [22].

Dietary fibre refers to intact plant components that are not digestible by gastrointestinal (GI) enzymes, whereas functional fibre refers to non-digestible carbohydrates that have been extracted or manufactured from plants. Both of these types of fibre have been shown to have beneficial physiologic function in the GI tract and in reducing risk of certain disease states. Types of fibres are less soluble fibre (lignin, cellulose) in fruits and whole wheat mature vegetables, more soluble fibres (pectin) in apples, strawberries, carrot, oat and legumes and functional fibres (chitin). Serum lipid concentration can be modified by insoluble fibres such as cellulose, lignin, chitin and more soluble fibres because bind faecal bile acids and increase excretion of bile acid. Derived cholesterol, fibres prevent dietary fat and cholesterol absorption by binding bile acids or fat or lipids. Cholesterol lowering effects have been reported, but the effect varies with the type and amount of fibres [23].

The role of physical activity in preventing coronary disease and in decreasing mortality after myocardial infarction remains controversial. Long term physical activity is known to be important in maintaining body weight and muscle tissue, in lowering blood pressure and triglycerides, and in raising the level of HDL cholesterol [20].

The contribution of physical inactivity to CHD deaths is difficult to quantify, however people who are physically active appear to have a lower risk of CHD. Regular, aerobic exercise of moderate intensity should be undertaken >= 3 times per week for at least 30 minutes, but greater frequency and duration of exercise is associated with increasing benefits [24].

Many studies and research around the world have been conducted on the subject of the ABO blood group relationship with health in general and its relation to heart disease.

In 2017, an Egyptian researcher studied the association between blood group A and heart disease in some African countries and they found that there is a relation [25]. Also Zhonghua Xin in 2015 has studied the association between the ABO blood group and the risk factor myocardial infarction in Chinese people and the result was (non –O) blood group is associated with her increased risk of CHD [26] iin India 2013 (Kolkata) his study was in (250) person he suggest that AB blood group decreases the risk of Coronary Heart Disease (CHD), while the O blood group increases the risk of CHD. In Italy in 2012, Franchini has found that CHD increased risk in (– O) patient [27]. In 2005 in Iran there finding suggest that there is no correlation between various ABO blood groups and development of CHD [28].

Studies have identified the ABO blood group as a risk factor for the development of coronary heart disease. Individuals with blood group A, B, or AB were 5% to 23% more likely to develop coronary heart disease compared with subject with O blood type, and the association was not altered by multivariate adjustment of other risk or dietary factors [25].

In Sudan, we found only one work regarding ABO blood grouping in heart diseases. The work was published at the bibliography of University of Sudan for Science and Technology conducted by Reela A. abdelraheem; she has investigated seventy patients with different types of cardiovascular diseases for any association with blood grouping. Her results showed no association of ABO blood group and Rh factor with cardiovascular diseases, although the frequent of ischemic heart disease (IHD) was higher in O group, the difference were statically insignificant [29]. To our knowledge, this is the only study on this context which concluded with no association of CHD and ABO grouping. However, they used a small sample size and they did not include healthy controls for comparison. Therefore, we aimed to enroll a large sample size and healthy controls to compare the difference of frequency between CHD cases and controls.

Rationale

Cardiovascular diseases, in particular Coronary Heart Disease (CHD) are one of the ten most common diseases causing death in Sudan according to previous studies. Cardiac specialists and consultants revealed the increase in the percentage of people with Heart Disease in

Sudan and described it as frightening, attributing it to large numbers of people under the age of forty years, as well as the spread of diabetes and high blood pressure. And it is important to know the possible risk factors that could participate in the great increasing number of patients. ABO blood grouping is among the risk factors that have been explored in different countries.

There are few published studies have been done in Sudan to relate cardiovascular disease to risk factors. Risk factors have been previously suggested include diabetes mellitus and hypertension. Non modifiable risk factor like age, gender, family history was also suggested, however, ABO grouping was not sufficiently studied. This is why we aimed in this study to determine the frequency of ABO full blood grouping among CHD patients attended Sudan Heart Center at Khartoum and compared them with healthy controls to correlate it to association with coronary artery disease.

Objectives of the Study

General Objective

To study the relationship between ABO grouping and Coronary Artery Disease among Sudanese patients are attending Sudan Heart Center at Khartoum.

Specific Objective:

1. To determine the prevalence of different blood groups among Coronary Heart Disease compared to apparently healthy control.
2. To calculate the percentage of ABO blood grouping in patients with Coronary Heart Disease compared to apparently healthy control.
3. To correlate ABO and Rh blood group to Coronary Heart Disease.

Materials and Methods

Study design

Case control study.

Study area

Sudan Heart Center, Khartoum, Sudan.

Study period

Study was conducted from October 2018 until April 2019.

Study population

Sudanese Patients with cardiovascular disease.

Sample size

1000 participants will be included 500 patients of cardiovascular diseases and 500 of apparently healthy controls.

Inclusion criteria

Patients diagnosed (clinically or and Laboratory investigations) with coronary artery disease. Both gender and all available age groups of Sudanese patients were included.

Exclusion criteria

Patients diagnosed with other cardiovascular diseases than coronary artery disease. Patients were known with human immunodeficiency virus, chronic inflammation, renal disease or liver diseases or any other diseases.

Ethical considerations

Approval was taken from National University Ethical committee as well as permission from the hospitals manager at Sudan Heart Center in Khartoum. Informed consent signed by patients with cardiovascular diseases and healthy controls.

Laboratory experiments

Data collection

We collected the specimen from all patients with Coronary Artery Disease from Sudan Heart Center and performed full blood grouping at Hematology laboratory, The National University. For the control samples were collected specimen from apparently healthy individuals in different areas in Khartoum.

Methods

Sample collection

2.5ml venous blood was drawn after make sterilization by 70% alcohol use 20 or 21 G needle with limited occlusion of the arm by a tourniquet. The blood was collected in K2 EDTA (Potassium Ethylene Di amine Tetra Acetic) and mix gently.

ABO slide agglutination test

Principle

When red cells were mixed with various reagents of antisera (soluble antibody), agglutination occurred on the slides containing cells positive (possessing the antigen) for the corresponding antigen.

No agglutination occurred in the red cells did not contain the corresponding antigen.

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, and then agglutination was observed
6. Result was recorded.

Results interpretation

Agglutination (clumping) of the red blood cells is positive. 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.

Rh (D) red blood cell typing

Principle

Rh (D) typing is based on the principle of agglutination. Normal human red blood cells possessing antigen will clump in the presence of antibody directed toward the antigens. Agglutination of patient or control red blood cells with anti- D serum and no agglutination with the control reagent is a positive test result, which indicates the presence of the D antigen on the red blood cells. Absence of agglutination is a negative test result, which indicates the D antigen is not demonstrable. If Rh typing is negative, Du typing is automatically performed.

Du Method (The indirect anti globulin)

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 Du method

1. Two drop of mixture (IgG and IgM) anti- D was placed in 10 × 75 mm Test tube.
2. One drop of washed 5% suspension of the test cell was added.
3. Mix well, and the tube was incubated at 37°C for 15 minutes
4. After incubation, the mixture was centrifuged and then he result was Red 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 center fudged at 3400 rpm for 15 seconds.
8. The final results were read and recorded.

Requirements

- Test tubes
- Water bath at 37°C
- Anti- D sera
- Coomb's reagent
- Microscope
- Bench centrifuge

Interpretation

Agglutination in test sample and negative reaction in control sample shows a positive test and the sample are labeled Rh (d) positive.

Data analysis

Data was analyzed by using Statistical package of Social Science (SPSS), version (22) is a software for editing and analyzing all sorts of data, these data may come from basically any Statistical packages for social sciences [SPSS] was used for data analysis. Descriptive analysis was used to calculate the percentage of blood groups among control and cases Relative risk (RR) test was performed to examine the relationship between blood groups as risk factor of CHD.

Results

This was a descriptive analytical study aimed to determine the frequency of ABO and Rhesus blood grouping and if they are associated with Sudanese patients with Coronary Heart Disease. This study enrolled 500 patients with CHD and 500 apparently healthy individuals.

Among the 500 CHD patients there were 293 (55.4%) males and 207 (43.9%) females these findings showed that Coronary Heart Disease was more frequent in male than Female.

Regarding the 500 apparently healthy controls there were 236 (44.6%) male, and 264 (56.1%) female (Figure 1).

We found that the most frequent blood group in CHD patients was O 220 (43.6%) followed by a 154 (54.4%) followed by B 106 (59.9%) and least frequent was AB 20 (57.1%). Therefore, Coronary heart disease was most frequent among patients with Non-O blood

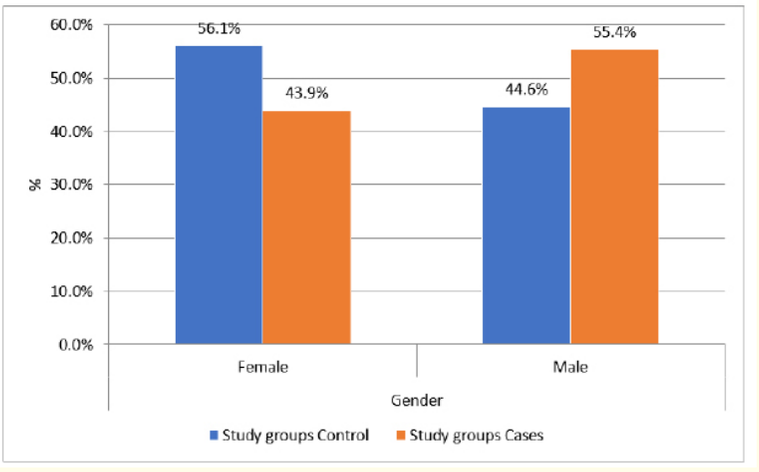

Figure 1: *Gender distribution among CHD cases compared to control.*

group 280 (56%) compared to O blood group 220(44%) (Figure 2) and the results were statically significant due to the result of the relevant risk (RR) which is 95% confidence interval. And for the control the non-O blood group was 173 (43.5%), and the O blood group was 225 (56.5%) (Figure 3).

A blood group was more frequent in the cases compared to the other non-O blood group.

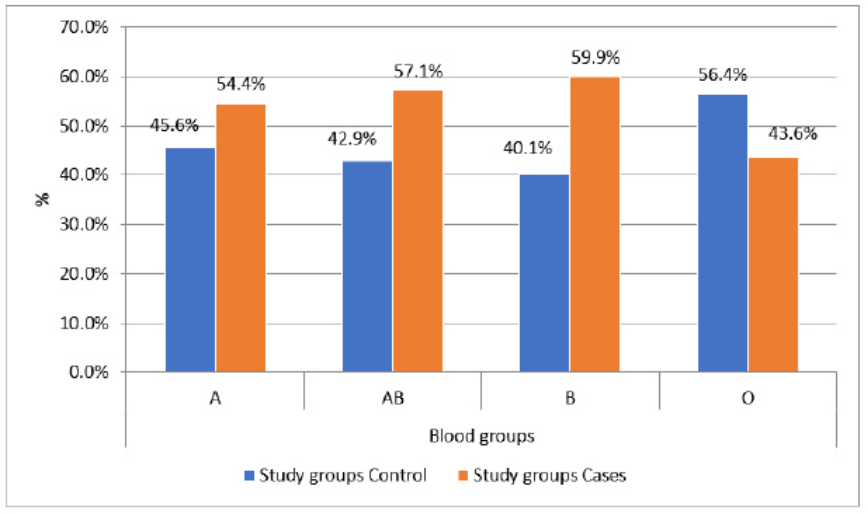

Figure 2: *The most frequent ABO blood group in both patient and control.*

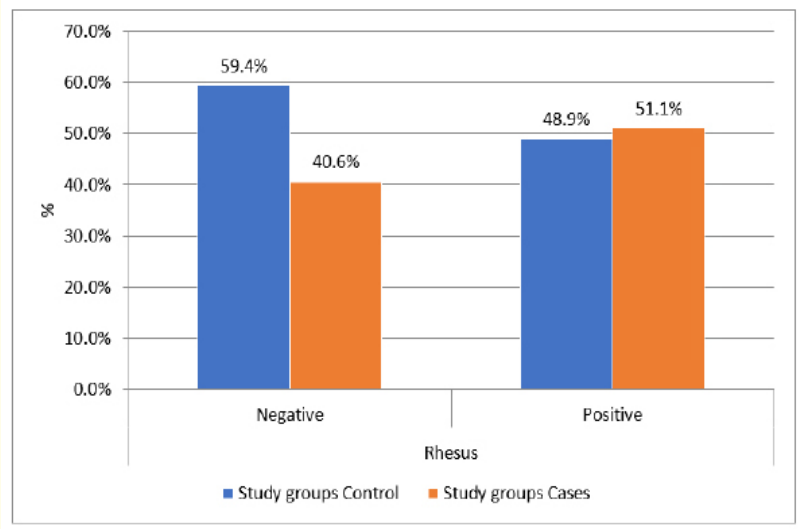

Figure 3: *The frequency of Rhesus factor among CHD patients and controls.*

The most frequent was positive Rhesus factor in patients 459 (50.1%) than negative which is 41 (40.6%), comparing to the control where we found 440 (48.9%) was positive Rhesus factor and 60 (59.4%) were negative (Figure 4).

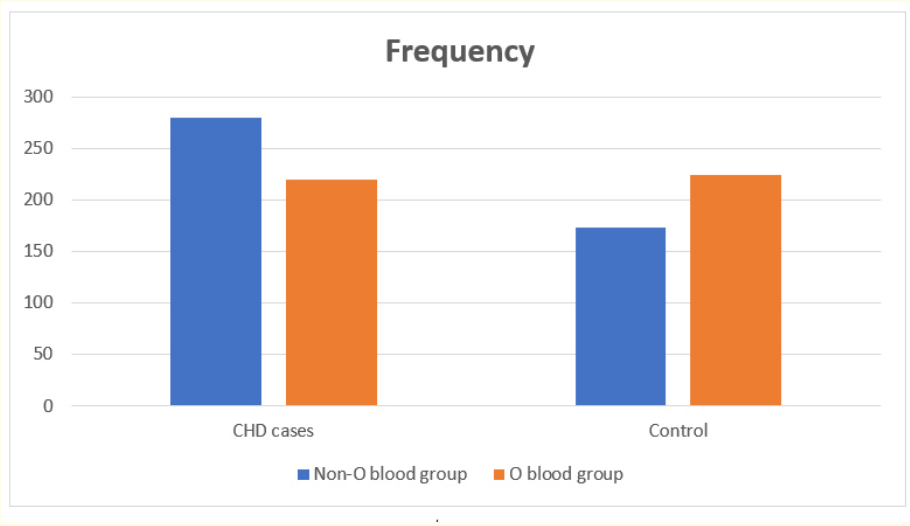

Figure 4: *Comparison between O and Non-O blood group in CHD cases and control.*

Discussion

This a descriptive analytical study aimed to determine frequency of blood group and Rhesus factor of patients with Coronary heart disease at Khartoum state during the period from October 2018 to March 2019.

According to our findings, CHD was more common in male 293(58.6%) than female 207(41.4%) statically significant . This finding agree with the study of Fath Elrahman , which concluded that blood group O was the predominant (52.7%) followed by A (23.3%), B (13.2%), while AB was the least frequent (10.8%). This finding in agreement with the only Study done by Abo Algasim; et al (2007). In Aldinga Sudanese Ethnic group, Where the workers reported highest frequency of blood group phenotype O (50%) followed by A (23%), B (18%) and AB (9%).

That ABO blood group considered as a risk factor for CHD. Most common blood group in males was O followed by A and least frequent was AB. Most common blood group in females was O followed by A and least frequent was AB. Most common Rhesus blood group in males was Rh (D) positive and most. Common Rh blood group in females also was Rh (D) positive.

Majority of patients with coronary artery diseases were Rh positive Rh result showed: control negative 60(59.4%) and positive 440(48.9%) and for the case: negative 41(40.6%) , positive 459(51.1%) . That means there is an association between CHD and blood grouping. t there's an association between Coronary heart disease and ABO blood groups due to the result of the P value (0.0003).

Recommendation

- ABO and Rh blood grouping should be done as routine investigation for patients with CHD.
- For further studies should include demographic and clinical information about both patients and control.

- Include bigger sample size.
- Raise awareness about CHD and its association with ABO and Rh grouping as a risk factor.

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Volume 4 Issue 1 January 2020