بسم الله الرحمن الرحيم

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College of Graduate studies

The Association of Blood Group ABO and Rhesus Factor with Glaucoma in Makka Hospital for Ophthalmology

العلاقة بين فصيلة الدم (أ ب و) و العامل الريصي

مع مرض الجلوكوما في مستشفي مكة للعيون

"A dissertation submitted in partial fulfillment of the requirements for the award of the degree of master in medical laboratory sciences - Haematology"

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(SUST)

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الاية

بسم الله الرحمن الرحيم

قال تعالي :

{ اللَّهُ نُورُ السَّمَاوَاتِ وَالأَرْضِ مَثَلُ نُورِهِ كَمِشْكَاةٍ فِيهَا مِصْبَاحٌ الْمِصْبَاحُ فِي زُجَاجَةٍ الزُّجَاجَةُ كَأَنَّهَا كَوْكَبٌ دُرِّيٌّ يُوقَدُ مِن شَجَرَةٍ مُّبَارَكَةٍ زَيْتُونَةٍ لاَّ شَرْقِيَّةٍ وَلا غَرْبِيَّةٍ يَكَادُ زَيْتُهَا يُضِيءُ وَلَوْ لَمْ تَمْسَسْهُ نَارٌ نُّورٌ عَلَى نُورٍ يَهْدِي اللَّهُ لِنُورِهِ مَن يَشَاء وَيَضْرِبُ اللَّهُ الأَمْثَالَ لِلنَّاسِ وَاللَّهُ بِكُلِّ شَيْءٍ عَلِيمٌ }

صدق الله العظيم

سورة النور, الاية 35

DEDICATION

TO

My Father, Mother, Brothers, and Sisters

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First of all great thank to AL-mighty ALLAH for offering the power to complete this work.

I would like to thank my supervisor **Prof. Shadia Abd Alatee Omer,** for her constant support and supervision during my study. I would also like to thank her for believing in my ability, for the freedom given to me to follow my instincts as well as for all the critical evaluation of my research

Abstract

This is a non – interventional case control study performed to assess the possible association between blood groups ABO and Rhesus factor with glaucoma in Makka Hospital for ophthalmology from March 2018 to May 2019. The study was performed on 100 glaucomatous patients who attended Makka Hospital during this period and 100 non - glaucomatous volunteer blood donors as control group. A Questionnaire was constructed to obtain some of the participant's characteristics. ABO and Rhesus blood group were determined by the slide method. The obtained data were analyzed by Chi square test using Statistical Package for Social Sciences (SPSS) version (16).

Males represent 63% of the glaucomatous patients and while females are 37%.

The age range of the glaucomatous patients was (32 – 82) years, the highest percentage (64%) was found in patients aged (50 – 70) years old and the least percentage was in patients aged less than 40 years.

The highest percentage of glaucoma occurrence (70%) was found in western Sudan and the least percentage (6%) was in central Sudan.

The order of ABO distribution was similar in both patients and control groups that is (O > A > B > AB). Blood group ABO occurrence in patients and control group were: (O 49% vs 44%), (A 29% vs 27%). (B 20% vs 23%), (AB 2% vs 6%) respectively.

No association was found between ABO blood group and glaucoma ($P > 0.05$).

Blood group Rh +ve in patients and control group are 94% vs 92%. Blood group Rh -ve in patients and control group were 6% vs 8% respectively.

No association was observed between Rhesus blood group and glaucoma ($P > 0.05$).

It is recommended that cross sectional studies from all the country should be done considering the form of the glaucoma and ethnicity.

ملخص البحث

هذه دراسة مراقبة حالة غير متداخلة أجريت لتقييم الارتباط المحتمل بين فصائل الدم (ABO) و العامل الريصي والجلوكوما في مستشفى مكه لجراحة العيون خلال الفترة من مارس 2018 إلى مايو 2019. أجريت الدراسة على 100 مريض جلوكوما الذين حضروا إلى مستشفي مكه خلال هذه الفترة و 100 من المتبرعين بالدم وهم لا يعانون من مرض الجلوكوما كمجموعة ضابطة. تم تصميم استبيان للحصول علي بعض خصائص المشتركين. خضع جميع المشاركين لاختبار فصيلة الدم عن طريق طريقة الشريحة. حللت البيانات بواسطة اختبار مربع كاي ببرنامج الحزمة الإحصائية للعلوم الاجتماعيه الإصدار (16) ، أظهرت النتائج أن:

مثل الذكور 63 ٪ من مرضى الجلوكوما بينما بلغت نسبة الإناث 37 ٪.

متوسط عمر مرضي الجلوكوما (32 - 82) عامًا ، أعلى نسبة (64٪) في مرضي الجلوكوما الذين تتراوح اعمارهم بين 50 و 70 عامًا ، وكانت النسبة المئوية الأقل للمرضى الذين تقل أعمارهم عن 40 عامًا.

وجدت أعلى نسبه مئويه للجلوكوما (70٪) في غرب السودان وأقل نسبه (6٪) في وسط السودان.

ترتيب فصائل الدم كان متشابها بين مرضي الجلوكوما و المجموعه الضابطه كالاتي: O تليها A تليها B تليها AB . فصيلة الدم O في المرضى و المجموعة الضابطة هي 49 ٪ مقابل 44 ٪ز فصيلة الدم A في المرضى والمجموعة الضابطة 29 ٪ مقابل 27 ٪. فصيلة الدم B في المرضى و المجموعة الضابطة هي 20 ٪ مقابل 23٪. فصيلة الدم AB في المرضى و المجموعة الضابطة 2 ٪ مقابل 6 ٪ علي التوالي.

لا يوجد ارتباط بين فصيلة الدم ABO والجلوكوما (القيمة ب > من 0.05)

فصيلة الدم ايجابيه العامل الريصي في المرضى و المجموعة الضابطة 94 مقابل 92 ٪. فصيلة الدم سالبه العامل الريصي في المرضى و المجموعة الضابطة 6 ٪ مقابل 8 ٪ على التوالي.

لا يوجد ارتباط بين فصيله العامل الريصي والجلوكوما (القيمة ب > 0.05)

وصي بإجراء دراسات مقطعية من جميع أنحاء البلد بحجم أكبر للعينه اخذا في الاعتبار نوع الجلوكوما و المجموعات العرقيه.

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Chapter One

1.1 Introduction

Glaucoma affects more than 70 million people world-wide and has been shown to be the second-leading cause of blindness after cataract. Glaucoma is classified into primary and secondary glaucoma; there are various types of primary glaucoma in which the eye does not have any preexisting disease, the most common being primary open-angle glaucoma (POAG) and primary closed-angle glaucoma (PCAG). As opposed to this, patients with glaucoma who had any preexisting eye disease are diagnosed with secondary glaucoma, which includes pseudo exfoliative glaucoma (PEXG). Significant associations have been observed previously between the blood groups and different diseases, which include, among others, coronary heart disease, ulcus ventriculi, gastritis, and leukemia. Eye diseases such as myopia, nuclear cataract, and convergent squint have also been shown to have an association with the blood group O. In contrast, some researchers have reported no significant association of myopia and cataracts with ABO blood groups. Several reports have appeared in recent years that have suggested a link between glaucoma and blood groups. In studies carried out in populations of India, Tunisia, and Iran, an association has been observed between glaucoma and blood groups. A possible explanation for this is that the glaucoma susceptibility loci have been mapped to chromosomes 1 and 9, which also contain the loci for the ABO blood group antigen and the Rh factor. (Khan *et. al.*, 2009)

1.2 Rationale

A survey was carried out in Blue Nile state which investigated prevalence and causes of blindness in Sudan; they have been estimated prevalence of blindness which was about 660 per 100,000. The main causes of blindness were found to be cataract (39.6%), anterior segment diseases (22.1%) and glaucoma (19.4%).

During the last few years, interest in the association between ABO blood groups and certain diseases has been increasing, In cases of some diseases this association has been proved.

1.3 Objectives

1.3.1 General objectives:

To study association of ABO and Rhesus blood group with glaucoma

1.3.2 Specific objectives:

- To study distribution of A, B, AB and O blood group in study population
- To study distribution of Rh positive and Rh negative in study population
- To study distribution of study population according to age group
- To study distribution of study population according to gender
- To study distribution of study population according to residence
- To study distribution of study population according to chronic disease

Chapter Two

Literature Review

2.1 Anatomy of the eye

Anatomy of the eye described by Khurana (2007) as follow:

Each eye ball is a cystic structure kept distended by the pressure inside it. Although, generally referred to as a globe, the eyeball is not a sphere but an ablate spheroid. The central point on the maximal convexities of the anterior and posterior curvatures of the eyeball is called the anterior and posterior pole, respectively. The equator of the eyeball lies at the mid plane between the two poles

The eye ball comprises three coats: outer (fibrous coat), middle (vascular coat) and inner (nervous coat).

The fibrous coat it is a dense strong wall which save the intraocular contents. Anterior one sixth of this fibrous coat is transparent and is called cornea. Posterior five sixth opaque part is called sclera. Cornea is set into sclera like a watch glass. Junction of the cornea and sclera is called limbus. Conjunctiva is firmly attached at the limbus. Vascular coat (uveal tissue) are supplies nutrition to all structures of the eyeball. It consists of three parts which from anterior to posterior are: iris, ciliary body and choroid. Nervous coat (retina) it is role visual functions.

The eyeball can be structured into two segments: anterior and posterior.

Anterior segment it includes crystalline lens (which is suspended from the ciliary body by zonules), and structures anterior to it, viz., iris, cornea and two aqueous humour-filled spaces: anterior and posterior chambers.

Anterior chamber is bounded anteriorly by the back of cornea, and posteriorly by the iris and part of ciliary body. The anterior chamber is about 2.5 mm deep in the centre in normal adults.

It is shall ower in hypermetropes and deeper in myopes, but is almost equal in the two eyes of the same individual. It contains about 0.25 ml of the aqueous humour.

Posterior chamber is a triangular space containing 0.06 ml of the aqueous humour. It is bounded anteriorly by the posterior surface of iris and part of the ciliary body, posteriorly by the crystalline lens and its zonules, and laterally by the ciliary body.

The posterior segment includes the structures posterior to lens, viz., vitreous humour (a gel like material which fills the space behind the lens), retina, choroid and optic disc.

Each eyeball acts as a camera; it perceives the images and transports the sensations to the brain (occipital cortex) through visual pathway which comprises optic nerves, optic chiasma, optic tracts, geniculate bodies and optic radiations. (Khurana, 2007)

2.2 Glaucoma

Glaucoma may be characterized as a bunch of disease that leads to dynamic loss of retinal ganglion cells. This loss is characterized by characteristic loss of useful visual areas related with optic neuropathy. Glaucoma can be classified as primary (70%) and secondary (30%). Primary glaucoma can be another classified as primary open-angle glaucoma (POAG) (55%), primary angle-closure glaucoma (12%), and congenital glaucoma (3%). Angle-closure glaucoma can be classified as angle-closure glaucoma with pupillary block and angle closure glaucoma

without pupillary block, each of which can be assist partitioned into primary and secondary forms. (Agarwal, 2006)

2.2.1 Pathogenesis

The pathogenesis of glaucomatous change is ascribed to a combination of variables influencing axonal wellbeing, with each figure being interlinked in its impacts to all the others, the two main influences are:

- Mechanical damage due to the elevated intraocular pressure

- Vascular perfusion of the optic nerve head.

- Other factors. (Sihota and Tandon, 2015)

2.2.1.1 Mechanical Changes

The coats of the eye can with stand fairly high intraocular pressures except at the lamina cribrosa, the fenestrated region through which optic nerve fibres enter the eye. Here, the nerve fibres are supported by glial tissue and have to bend over the edge of the disc. A raised intraocular pressure causes mechanical pressure on the lamina cribrosa altering capillary blood flow and decreasing axoplasmic flow in the initial stages. Later, significant backward displacement and compaction of the laminar plates narrows the openings through which the axons pass, directly damaging the nerve fibre bundles. (Sihota and Tandon, 2015)

2.2.1.2 Vascular Perfusion

The perfusion of the optic nerve head may be affected because of a lack of an adequate auto regulatory mechanism.

A substantial rise in intraocular pressure can also decrease the capillary blood flow due to mechanical compression of the vessels at the lamina cribrosa or a decreased

flow in the annulus of Zinn, which supplies nutrition to the laminar and post-laminar optic nerve head. A fall in perfusion pressure at the optic disc may additionally be caused by systemic factors such as hypotension, vasospasm and acute blood loss. (Sihota and Tandon, 2015)

2.2.1.3 Other Factors

2.2.1.3.1 Nitric oxide (NO)

This is a free radical which is formed from l-arginine by the enzyme NO synthetase (NOS). NOS have three isoforms: NOS-1 (this enzyme is associated with diminished bundles at the prelaminar region of the lamina cribrosa in glaucoma patients), NOS-2 (is associated with elevated IOP – the presence of this enzyme has a genetic predisposition), NOS-3 (vasodilator found in the prelaminar region of the optic nerve). It was shown that NOS-2 is absent in healthy patients, while NOS-1 and NOS-3 are up regulated in glaucoma patients. Studies showed that increased NO in the retina produces ischemia, inflammation and excitotoxicity. NO has the ability to pass from one neuron to another by passing synapses. The NO is a free radical with a moderate activity, the major problem being its growing effect on the other free radicals, which lead to retinal ganglion cells (RGC) apoptosis. (Ciotu, *et.al*., 2015).

2.2.1.3.2 Magnesium Deficiency and the Impact on Ocular Tissues

In experimental animal studies, it has been shown that Mg has a vital role in the development and normal functioning of the eye. Mg deficiency has been associated with multifocal necrosis in the retinal pigment epithelium of rats and hypomagnesemia was also found to be correlated with pigmentary retinal degenerations like Kearns-Sayre syndrome and retinitis pigmentosa. Additionally,

in experimental rats Mg deprivation during developmental phase may cause multifocal necrosis and myelination disorders in the optic nerve. Furthermore, Mg might be necessary for maintenance of healthy ocular surface in the prevention of infections and dryness and in inflammatory conditions of conjunctiva and cornea local application of Mg sulphate has shown some benefits. Additionally, in Mg deficient rats decreased microvilli in corneal epithelial cells and apoptosis-like nuclear changes in corneal epithelial and endothelial cells were observed . On the other hand, patients with keratoconus have also been shown to have low serum Mg levels. Furthermore, Mg taurate has been reported to reduce the progression of cataracts. Regarding the relationship of Mg with retinal disorders, patients with diabetic retinopathy were reported to have low serum Mg levels and those with the severest degree of retinopathy had more prominent hypomagnesemia. Mg deficiency may have a causative relation with several disorders of the eye and may point out a potential therapeutic value. (Ekici, *et.al*., 2014)

2.2.2 Forms of glaucoma

2.2.2.1 Primary open angle glaucoma

Chung, *et.al* found that glaucoma is the second leading cause of blindness worldwide. Although the mechanism of the development of primary open-angle glaucoma (POAG) is not fully understood, elevated intraocular pressure (IOP) is considered the most important risk factor. Several vascular factors have also been identified as risk factors and can lead to hypoperfusion of the optic nerve head and thus may play an important role in the pathogenesis and progression of POAG. The results suggest that both high and low blood pressure (BP) are associated with an increased risk of POAG based on a comprehensive literature review. Elevated BP is associated with elevated IOP, leading to increased risk of glaucoma, but

excessive BP lowering in glaucoma patients may cause a drop in ocular perfusion pressure (OPP) and subsequent ischemic injury. The relationship between IOP, OPP, and BP suggests that the relationship between BP and glaucoma progression is U-shaped. (Chung, *et.al*., 2015)

2.2.2.2 Primary closure angle glaucoma

Primary angle closure glaucoma (PACG) is more common in the Asian population and accounts for greater blindness than primary open angle glaucoma (POAG). with the advent of ultrasound biomicroscopy (UBM) and anterior segment ocular coherence tomography (AS-OCT). (Sinha, *et.al*., 2011)

2.2.2.2.1 Primary chronic angle closure

Is due to obstruction of the out flow of the anterior chamber, and may be accompanied by a rise in intraocular pressure (IOP). The obstruction of outflow is due to contact of the iris with the trabecular meshwork and this is usually identified by the examination technique of goniscopy. Iridotrabecular contact and obstruction of more than half of the trabecular meshwork drainage channel is defined as a closed angle. Advanced imaging techniques of the anterior segment can also identify the closure. usually presents painlessly. Treatment of pressure is with IOP-lowering medication. However, the underlying mechanism of primary angle closure is treated with pilocarpine to constrict the pupil and laser or surgical peripheral iridotomy to relieve pupil block. More recently, elective lens extraction is being evaluated in an ongoing RCT because this may increase the capacity of the anterior chamber and open the drainage angle. This has been proposed for both acute and chronic angle closure. (Foster, *et.al*. 2002)

2.2.2.2.2 Primary acute angle closure

Is a sudden rapid rise in IOP due to obstruction of the outflow of the anterior chamber. The presentation is acute, usually with pain, and may be associated with sudden vision loss. (Foster, *et.al*. 2002)

2.2.2.3 Pseudo exfoliative glaucoma

Pseudo exfoliative glaucoma (PEXG) occurs when pigment and abnormal basement membrane material from the anterior segment of the eye deposit in the trabecular meshwork (TM), which raises the intraocular pressure of the eye causing a degeneration of the optic nerve. In addition the presence of pseudo exfoliative material causes changes in the cornea, camerular angle, lens and zonules as well as a significant loss in the number of axons. The accumulation of exfoliative material in the juxtacanalicular tissue (JCT) results in the disorganization of the JCT and Schlemm's canal followed by dysfunction of endothelial cells, which appear to be the causative factor in the development of PEXG. (Micheal, *et.al*., 2012)

2.2.2.4 Congenital glaucoma

Primary congenital glaucoma refers to a specific form of developmental glaucoma characterized by an isolated maldevelopment of the trabecular meshwork (isolated trabeculodysgenesis) not associated with other developmental ocular anomalies or ocular disease that can raise the IOP. Also called primary infantile glaucoma, it is the most common form of developmental glaucoma. The condition is typically bilateral, but 25–30% of the cases may be unilateral.

Most western textbooks describe a classic triad of symptoms comprising epiphora, photophobia and blepharospasm (attributable to IOP-induced corneal epithelial

edema). However, one study conducted in a tertiary institution shows that large eyeball size and hazy eyes (from corneal edema) may be the more common presenting features in the Indian subcontinent. Occasionally, the child may also be present with a red eye, mimicking conjunctivitis. (Mandal and Chakrabarti, 2011)

2.3 ABO and Rhesus blood grouping

Blood is a body fluid in humans and other creature that supply fundamental substances such as nutrients and oxygen to the cells and transports metabolic waste products away from the same cells. In vertebrates, it is composed of blood cells suspended in the blood plasma. Plasma, which constitutes 55% of blood fluid, is mostly water (92% by volume), and contains dissaminated proteins, glucose, mineral ions, hormones, carbon dioxide , plasma being the main medium for excretory product transportation, and blood cells themselves. Albumin is the main protein in the plasma, and it functions to regulate the colloidal osmotic pressure of blood. (Igbenghu, *et.al.*, 2012)

ABO blood group system is hereditary controlled and pro protein of various ABO groups differs significantly in different population and ethnic groups. Thus, any national or universal study reporting association of ABO groups with a disease must use population frequency of ABO groups as the base for comparison. (Cserti and Dzik, 2007)

Almost always, an individual has the constant blood group for life, but very rarely an individual's blood type changes through addition or suppression of an antigen in infection, malignancy, or autoimmune disease. Another more common cause in blood type change is a bone marrow transplant. (Pathirana, *et.al.*, 2005).

2.3.1 ABO blood grouping

2.3.1.1 Historical Perspective

Karl Landsteiner truly opened the doors of blood banking with his discovery of the first human blood group system, ABO. This marked the beginning of the concept of individual uniqueness defined by the RBC antigens present on the RBC membrane. The ABO system is the most important of all blood groups in transfusion practice. It is the only blood group system in which individuals predictably have antibodies in their serum to antigens that are absent from their RBCs. This occurs without any exposure to RBCs by transfusion or pregnancy. Due to the presence of these antibodies, transfusion of an incompatible ABO type can result in the almost immediate lysis of donor RBCs. This produces a very severe, if not fatal, transfusion reaction in the patient. Even today, transfusion of the wrong ABO group remains the leading cause of death reported to the Food and Drug Administration (FDA). (Harmening, 2005)

In 1901 Landsteiner drew blood from himself and five associates, separated the cells and serum, and then mixed each cell sample with each serum. He was inadvertently the first individual to perform the forward and reverse grouping. (Harmening, 2005)

2.3.1.2 Molecular Biology in Transfusion Medicine

Proteins are macromolecules composed of amino acids, the sequences of which are determined by genes. Lipids and carbohydrates are not encoded directly by genes; genetic determination of their assembly and functional structures results from the action of different protein enzymes. Blood group antigens can be considered gene products, either directly, as polymorphisms of membrane associated proteins, or

indirectly, as carbohydrate configurations catalyzed by glycosyltransferases. (Brecher, *et.al*., 2005)

2.3.1.3 Blood Group Genetics

Landsteiner's discovery of the ABO blood group system demonstrated that human blood expressed inheritable polymorphic structure Shortly after the discovery of the ABO system, red cells proved to be an easy and accessible means to test for blood group polymorphisms in individuals of any age, as more blood group antigens were described, blood group phenotyping provided a wealth of information about the polymorphic structures expressed on proteins, glycoproteins and glycolipids on red cells, and the genetic basis for their inheritance. (Brecher, *et.al*., 2005)

2.3.1.4 Genetics and Heredity

Alternative forms of genes, any one of which may occupy a single locus on homologous chromosomes, are called alleles. The ISBT terminology distinguishes between the alleles for blood group antigens (for example the genetic polymorphisms) and the antigens that they encode. For example, the major antigens of the ABO system are A, B, and O, yet the alleles areA1, B1, and O1. In the Kell system, two alleles, K and k, determine the K and k antigens, respectively. Individuals who have identical alleles at a given locus on both chromosomes are homozygousfor the allele (for example A1/A1orK/Kork/k). In the heterozygous condition, the alleles present at the particular locus on each chromosome are non-identical (for example A1/O1orA1/B1orK/k). (Brecher, *et.al*., 2005)

Individuals who are homozygous for an allele in some blood group systems may have more antigen expressed on their red cells than persons who are heterozygous for that allele. (Brecher, *et.al*., 2005)

Table (2.1) Chromosomal Locations of Human Blood Group System Genes

System	ISBT No.	ISBT Symbol	Gene(s)Designation (ISGN)	Location
ABO	001	ABO	*ABO*	9q34.2
Rh	004	RH	*RHD, RHCE*	1p36.13-p34.3

(Brecher, *et.al*., 2005)

2.3.1.5 The ABO and Hh systems

The ABO blood group system, which was the first human blood group system to be discovered, remains the most important in transfusion practice. This is because of the regular occurrence of the antibodies anti-A, anti-B and anti A,B, reactive at 37°C, in persons whose red cells lack the corresponding antigens, so that if transfusions were to be given without regard to the ABO groups, about one-third (in white people) would be incompatible. The regular presence of anti-A and anti-B is made use of in the routine determination of ABO blood groups; in addition to testing red cells for A and B antigens, the group is checked, in serum or 'reverse' grouping, by testing the serum against red cells of known ABO groups. Although H is encoded by a gene on a different chromosome from ABO, the H blood group system is considered because H is a precursor of A and B. (Klein and Anstee, 2005)

2.3.1.6 ABO phenotypes in different populations

South American Indians all belong to group O; in Australian aborigines, only groups O and A are found; in some populations (for example Bengalese) the commonest group is B; and, finally, in some populations (for example Lapps) there is a relatively high frequency of A2. In Africans (black people), B is in general a much stronger antigen than in Europeans (white people) and black people have a higher level of B-specified glycosyl transferase in the serum . Based on

quantitative agglutination, about 50% of black people have stronger B than white people. (Klein and Anstee, 2005)

2.3.2 The Rh factor and antigens:

The Rh blood grouping system actually involves more than 50 antigens that are found on the surface of red blood cells. These antigens are proteins that, when introduced into a body that does not have the same type, can cause the person's immune system to respond by producing antibodies that attack the proteins. The Rh factor, Rh+ and Rh-, usually refers specifically to the presence or abs ence of one of these proteins, the D antigen. The D antigen tends to cause an especially strong immune response in people who do not have it. There are two alleles, or genetic variants, of this antigen: D and d. A person who is Rh negative have two recessive variants, dd. anyone who has at least one D= DD or Dd is Rh+. As with most genetic traits, one allele is inherited from each parent. (Anstee, 2010)

2.3.3 ABO and D grouping

ABO and D grouping described by (Dacie and lewis. 2011) as follow:

ABO and D grouping must be performed by a validated technique with appropriate controls. Before use, all new batches of grouping reagents should be checked for reliability by the techniques used in the laboratory. Grouping reagents should be stored according to manufacturer’s instructions. (Dacie and lewis, 2011)

2.3.3.1 ABO Grouping

ABO grouping is the single most important serological test performed in compatibility testing; consequently, it is imperative that the sensitivity and security of the test system are not compromised. The fact that anti-A and anti-B are naturally occurring antibodies allow the patient’s plasma to be tested against known A and B cells in a ‘reverse’ group. (Dacie and lewis, 2011)

This is an excellent built-in check for the ‘forward’ or cell group and has always been considered to be an integral part of ABO grouping, allowing the reading and recording of test results to be split into two discrete tasks. However, with secure, fully automated systems, linked to secure laboratory information management systems that in combination have the ability to prevent procedural ABO grouping errors, some laboratories now omit the reverse group when testing samples for which a historical group is available. Any discrepancy between the forward and reverse groups should be investigated further and any repeat tests should be undertaken using cells taken from the original sample rather than from a prepared cell suspension. (Dacie and lewis, 2011)

2.3.3.1.1 Reagents for ABO Grouping

Monoclonal anti-A and anti-B reagents have replaced polyclonal reagents in routine grouping tests. A1and B cells are used for reverse grouping; group O cells or an auto control may be included to ensure that reactions with A and B cells are not a result of the presence of cold auto antibodies. A diluents control should be included where recommended by the manufacturer. (Dacie and lewis, 2011)

2.3.3.2 D Grouping

D grouping is usually undertaken at the same time as ABO grouping for convenience and to minimize clerical errors that may arise through repeated handling of patients’ samples. (Dacie and lewis, 2011)

2.3.3.2.1 Reagents for D Grouping

Monoclonal reagents do not have the problem of possible contamination with antibodies of unwanted specificities, as was the case with polyclonal reagents.

Therefore, the duplicate testing may be undertaken using the same anti D reagent, although this should be dispensed as though it were two separate reagents.

DVI is the partial D with the fewest epitopes; therefore of all the D variants, DVI individuals are those most likely to form anti-D and a case of severe haemolytic disease of the fetus and newborn (HDFN) has been described. For this reason, anti-D monoclonal reagents that do not detect DVI should be selected for testing patients' samples. (Dacie and lewis, 2011)

2.3.3.3 Methods

There are several techniques available for routine ABO and D grouping including tube test, slide test, liquid-phase and solid-phase microplates and columns. Other techniques for blood grouping have been described but they are not in routine use. For example, molecular ABO typing is reserved for investigating anomalous ABO groups, in organ transplantation where red cells from the donor are not available, forensic practice and paternity testing. (Dacie and lewis, 2011)

Care should be taken to use the appropriate reagent because not all reagents have been validated by the manufacturer for all techniques. (Dacie and lewis, 2011)

2.3.3.3.1 Tube and slide tests

Spin-tube tests may be used for urgent testing, where small numbers of tests are performed at once. Slide or tile techniques are widely used in under-resourced countries for ABO and D grouping. Spin-tube tests should be performed in 10 or 12 mm plastic tubes. Immediate spin tests may be used in an emergency, whereas routine tests are usually left for 15 min at room temperature (about 20 C) before centrifugation for 1 min at 150g. Equal volumes (1 or 2 drops from either a commercial reagent dropper or a Pasteur pipette) of liquid reagents or plasma and

2%cell suspensions are used. The patient's red cells (diluted in phosphate buffered saline, PBS) should be tested against monoclonal anti-A and anti-B grouping reagents. The patient's plasma should be tested against A1and B reagent red cells (reverse grouping). In addition, the plasma should be tested against either the patient's own cells or group O cells (i.e. a negative control) to exclude reactions with A and B cells as a result of cold agglutinins other than anti-A or anti-B in the patient's sample. Mix the suspensions by tapping the tubes and leave them undisturbed for 15 min. Agglutination should be read. Any discrepancy between the results of the red cell grouping and the reverse grouping should be investigated further and any repeat tests should involve cells taken from the original sample rather than the prepared suspension. Reverse grouping is not carried out for infants younger than 4 months of age because the corresponding antibodies are normally absent or maternal in origin. (Dacie and lewis, 2011)

2.3.3.3.2 Slide method

In an emergency, rapid ABO grouping may be carried out on slides or tiles. The method is satisfactory if potent grouping reagents are used . An immediate spin-tube test is preferable. (Dacie and lewis, 2011)

2.3.3.3.3 Liquid-phase micro plate methods

Liquid-phase micro plate technology provides a cheap and secure method for batch testing when semi automation is utilized for dispensing and reading but it is no longer the grouping technique of choice in the UK. In 2009, a UK NEQAS survey showed that only 13% of responding laboratories were using micro plates for grouping, down from 41% in a similar survey. (Dacie and lewis, 2011)

2.4 Association of blood group with diseases

There are many studies which have demonstrated association between ABO and Rh blood group with different diseases

A study conducted in Bafeno area located in southern Ethiopia found that individuals of blood groups A, B and AB are more susceptible to *P. falciparum* infection as compared with individuals of blood group O. (Zerihun, *et.al*., 2011)

In China individuals with blood group "A" have been found to be highly susceptible to *falciparum* malaria whereas blood group "O" is said to confer protection against complicated cases. (Afoakwah *et.al.*, 2016)

The association between ABO blood group with hypercholesterolaemia, hypertension and diabetes mellitus was investigated in Iraq by Jassim (2012) and he showed that the levels of total cholesterol, glucose and systolic/diastolic blood pressure were all significantly higher in male and female patients in blood group O than the other groups, with a decreasing trend from group A to B then AB. (Jassim, 2012).

People with blood group "A" and "Rhesus +ve" have high risk of breast cancer, while blood type "AB" and "Rhesus –ve" are at low risk of breast cancer (Meo, *et. al*., 2017).

Tavasolian, *et.al*. (2014) studied the relationship between ABO blood group and acute lymphoblastic leukemia and they found a significant association between Acute Lymphoblastic Leukemia and blood group AB with a higher risk of acute lymphoblastic leukemia. (Tavasolian, et.al., 2014)

Chapter Three

Materials and Methodology

3.1 Study design:

This is an analytical non - interventional case control study.

3.2 Study area:

The study was conducted in Makka hospital for ophthalmology, located at Alrheyad Khartoum, during period between September 2018 to January 2019.

3.3 Study population:

On hundred Glaucoma patients who attended Makka hospital for ophthalmology. And on hundred normal blood donors as control group were enrolled in the study.

3.4 Exclusion criteria:

Glaucoma patient who attended to Makka hospital suffering from cataract, eye injury and who use steroid eye droplet.

3.5 Data collection tools:

A data were collected by using a designed questionnaire (appendix) was designed to obtain information needed for the study, it was filled by interviewingall the patient.

3.6 Blood collection:

Remnant of blood sample which were taken from patient of glaucoma for investigation**.**

3.7 Procedure of ABO and Rh blood groups:

With a grease pencil two circle were drawn on a clean dry slide, and labeled one (A) and another (B), and on another slide a circle was drawn and labeled (D), A drop of blood was placed on each circle, then to circle (A) drop of anti-serum A was added, drop of anti-serum B also was added to circle (B), and drop of anti-serum D was added to circle (D). Then each suspension was mixed with a different wood stick (Dietze et al. 1995).

3.8 Interpretation of ABO blood groups:

The interpretation of ABO blood group as follows as described by Dietze *et al*. (1995):

- Agglutination on (A) circle and no agglutination on (B) circle mean the ABO blood group is A.

- Agglutination on (B) circle and no agglutination on (A) circle mean the ABO blood group is B.

- Agglutination on Both (A) circle and (B) circle mean the ABO blood group is AB.

- No agglutination on both (A) circle and (B) circle mean the ABO blood group is O.

- Agglutination on (D) circle means the Rhuses factor (Rh-factor) is positive (+ve).

- No agglutination on (D) circle must be followed by Due technique then if no agglutination in test tube that means the Rhuses factor (Rh-factor) is negative (-ve).

3.9 Quality Control

Positive and negative controls included with every test or batch of manual tests.

The control samples loaded in the same way as the test samples

Reagent	**Positive control**	**Negative control**
Anti – A	A cell	B cell
Anti – B	B cell	A cell
Anti – D	D positive cell	D negative cell
A_1 cell	Anti – A	Anti – B
B cell	Anti – B	Anti – A

3.10 Data Analysis:

The Data were entered in Microsoft Excel, checked for its correctness, The data was analyzed by using chi- square test SPSS (Statistical Package for Social Sciences) version (16) to assess association of ABO and Rhesus blood group with glaucoma.

3.11 Ethical Consideration

The study was approved by the College of Medical Laboratory Science Sudan University of Science and Technology and verbal consent was taken from all the participant before being included in the study.

Each individual was informed on the nature of the study and was award for confentiality of the information and results.

Chapter Four

Results

Out of 100 glaucomatous participants the range of the age was between (32 – 82) years old. Less than 40 years old percentage were 6%, from 40 to 50 years old percentage were 16%, more than 50 to 60 years old percentage were 32%, more than 60 to 70 years old percentage were 32% and more than 70 years old percentage were 14%. Figure (4.1)

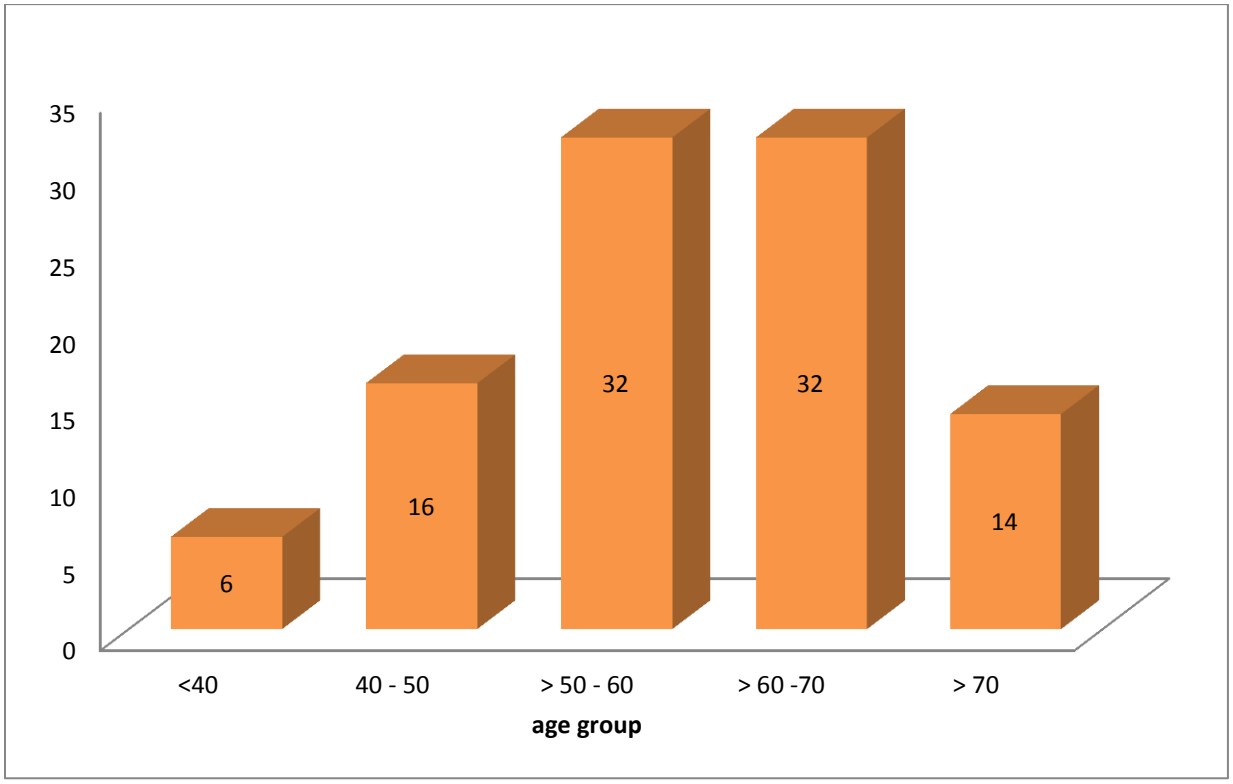

Figure (4.1) Distribution of study group according to the age.

63% of the study population are males and 37% are females. And controls were 65% males and 35% females. Table (4.1)

Table (4.1) Distribution of study group according to the gender.

Gender	**Percentage**	
	cases	Controls
Males	63%	65%
Females	37%	35%

This study involve all region of Sudan, there are participant from Center, north, east, south and west of Sudan, Table (4.2)

Table (4.2) Distribution of study group according to residence.

Region	Percentage
West of Sudan	70 %
North of Sudan	16 %
East of Sudan	8 %
Center of Sudan	6 %

Some participants suffer from chronic disease: diabetes and hypertension. Figure (4.2)

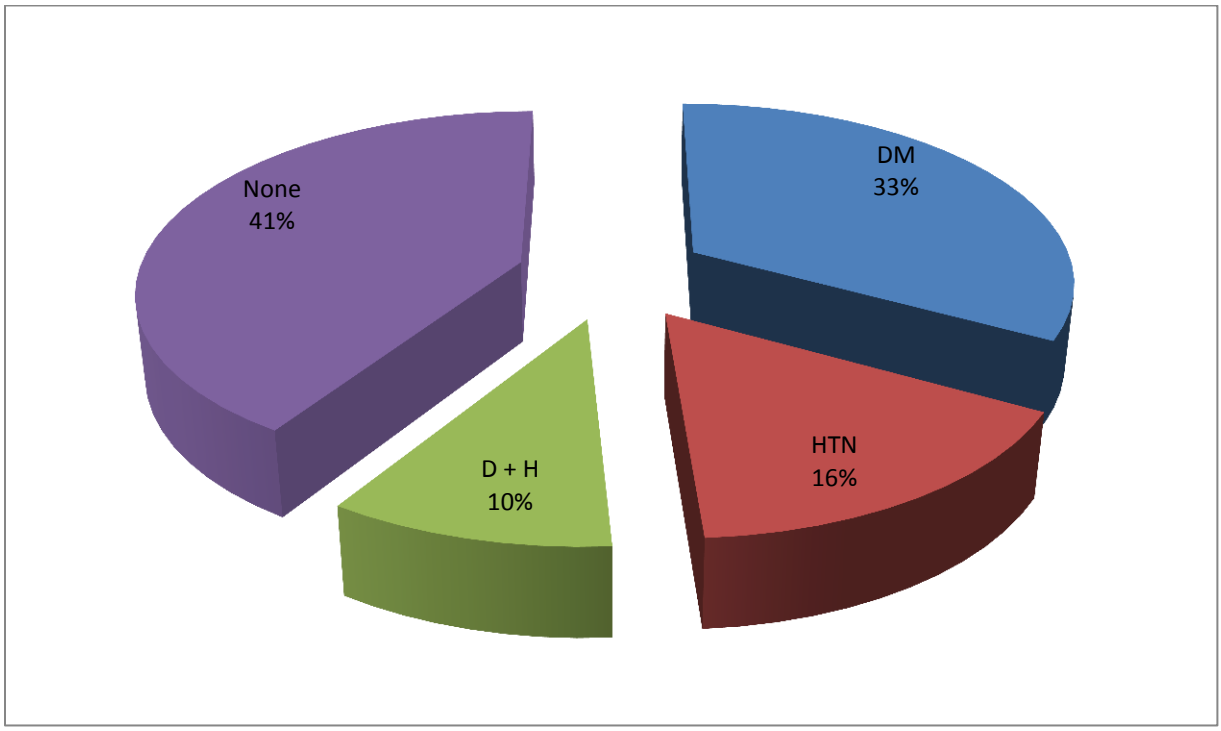

Figure (4.2) Distribution of study group according to chronic diseases

DM: Diabetes Mellitus D + H: Diabetes Mellitus + Hypertension

HTN: Hypertension None: have not chronic disease

Most of the parent's study participants are relatives, (89%) are relatives and (11%) are non- relatives. (Figure 4.3)

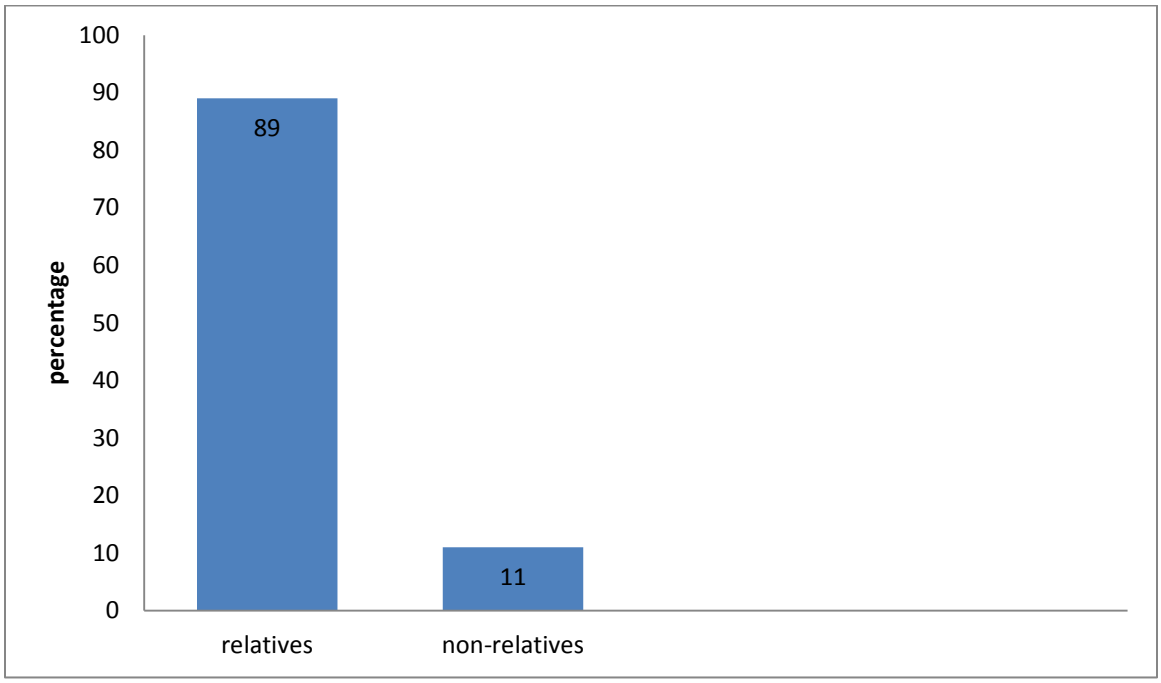

Figure (4.3) Distribution of study group according to relativeness

All patients underwent ABO and Rh blood group testing. The distribution of blood group A in patients and control group were 29%, 27% respectively. The distribution of blood group B in patients and control group were 20%, 23% respectively. The distribution of blood group AB in patients and control group were 2%, 6% respectively. The distribution of blood group O in patients and control group were 49%, 44% respectively (p value 0.466). The distribution of Rh +ve in patients and control group were 94%, 92% respectively. The distribution of Rh –ve in patients and control group were 6%, 8% respectively (p value 0.579).

Table (4.3) Distribution of ABO blood group among cases and controls

Blood group	Cases		Controls		Total	
	Frequency	Percentage	Frequency	Percentage	Frequency	Percentage
A	29	29	27	27	56	28
B	20	20	23	23	43	21.5
AB	2	2	6	6	8	4
O	49	49	44	44	93	46.5
Total	100	100	100	100	200	100

Table (4.4) Association of ABO blood group with glaucoma

Blood group	No. of cases	No. of Controls	Total	P value
A	29	27	56	0.466
B	20	23	43	
AB	2	6	8	
O	49	44	93	
Total	100	100	200	

Table (4.5) Distribution of Rh positive and Rh negative among cases and controls

Blood group	Cases		Controls		Total	
	frequency	percentage	frequency	percentage	frequency	percentage
Rh positive	94	94	92	92	186	93
Rh negative	6	6	8	8	14	7
Total	100	100	100	100	200	100

Table (4.6) Association of Rhesus blood group with glaucoma

Blood group	No. of Cases	No. of Controls	Total	P value
Rh positive	94	92	186	0.579
Rh negative	6	8	14	
Total	100	100	200	

Chapter Five

5.1 Discussion

In this study high percentage of O blood group (49%) phenotype was observed among the study participants followed by A (29%), B (20%) and AB (2%). Also high percentage of Rh (D) positive (94%) and only (6%) were Rh (D) negative. This agrees with some previous studies (Hassan, (2010) and Ismail, *et. al.*, (2017)). carried out in Sudanese population that also reported high frequency of group O and low frequency of group AB and predominance of Rh (D) positive. Hassan(2010) studied the distribution of ABO blood groups ,in both Rh (D) positive and negative subjects, and found that blood group O was the commonest (52.7 %), followed by A (23%), B (13%) and AB (10.8%).Ismail, *et. al*.(2017) described the distribution of ABO and Rh grouping as follow: the highest blood group was O (41%) followed by A blood group (32.1%), B blood group (21%) and the lowest percentage was AB blood group 23(5.9%). However, the allelic frequencies were in the order of O > A>B. The Rh positive and negative distribution trend was also similar. There is a minor difference between the two studies (Hassan, 2010) and (Ismail, *et. al.*, 2017) with regard to the frequency of the ABO blood groups but the order of the frequency O > A > B> AB was the same.

Males over numbered (63) females(37) with regard to the frequency of the disease without a significant variation .This may be attributed to men being more subjected to health risk factors ,like occupational hazards, smoking, alcohol consumption, than the females.

Most of the glaucomatous patients were from west of the Sudan with insignificant variation from the other regions and this may be due to the continuous movement of the people from their original areas or may be due to the sample size.

In this non – interventional case control study design there is insignificant variation between ABO and Rhesus blood group in the glaucomatous patients who participated in the study and the control group. Accordingly there is no significant association between blood group ABO Rh factor and glaucoma in the study population this is on line with the study of Reed and Platts. (1964) who did not find any association of blood group ABO with certain eye diseases in which glaucoma is included. Many researchers abroad found contradicting results to those of the current study. Khan *et.al.,* (2009) in Pakistan found a significant association between B blood group with different forms of glaucoma. (Khan *et.al*., 2009)

In Iran an association between B blood group and primary congenital glaucoma was observed by Zaree *et. al.*, (2006) which supports the findings of Khan *et. al.*, (2009).This is not surprising because Pakistan is known to share a genetic history with Iran.

AB Blood group was found to be a risk factor for primary open angle

glaucoma in Tunisia (Blouza, 2007). This discrepancy between the results of this work and that of the previous studies may be attributed to the variation in the study design, sample size. Another possible cause for these contradicting results is that in the previous studies the population was homogenous and may have the same genetic makeup in contrast to the population of the current work.

This study was limited by inability to investigate the form of the glaucoma and to get more demographic data of the participants.

5.2 Conclusion

- No association was found between glaucoma and ABO and Rhesus blood group in studied population

- Glaucoma was found to be most common in west of Sudan.

- Males are more susceptible to glaucoma more than females

- Chronic diseases have no effect on progression of glaucoma

5.3 Recommendations

Cross sectional studies from specific area of country should be done considering:

- The form of glaucoma .

- Family history of participants.

- Ethnicity of the participants.

- Occupation of the participants.

Confirmatory tests should be used

Advance technique like gel technology should be used

Chapter Six

6.1 References

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Appendixes

Appendix 1

6.2 Questionnaire

Sudan University for Sciences and Technology

Questionnaire about association between ABO and Rh blood group and glaucoma

1-Name: ……..

2-Age: ……

3- Residence: (a) North (b) center

(c) East (d) West

4- Sex (a) male (b) female

5- There are relation between mother and father?

(a) Yes (b) No

6- There is incidence of glaucoma in family?

(a) Yes (b) No

If answer yes what is relationship? .....

8- Presence of chronic disease?

(a) hypertension (b) diabetes

(c) None (d) both

Appendix 2

6.3 Informed Consent

Sudan University of Science and Technology

College of graduate studies

Study toward the degree of master

Name: ……

Remnant blood sample that taken for investigation for operation will be used and Questionnaire to assess the Association of Blood Group ABO and Rhesus Factor with Glaucoma

I am the above mentioned person; I agree to give remnant blood sample and information for this study

Signature: …….

Date: …../…../…….