بسم الله الرحمن الرحيم

Sudan University of Science and Technology

Collage of Graduate Studies

Determination of Rhesus Antigens Frequency among Blood Group A and O of Pregnant Sudanese Women in Khartoum State.

تحديد تكرارالزمر الوظيفية الظاهرية للعوامل الريصية لدى النساء السودانيات الحوامل ذوات فصائل الدم (A) و (O) في ولاية الخرطوم

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الآية الكريمة

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قال تعالى :

"وَأَنزَلَ اللَّهُ عَلَيْكَ الْكِتَابَ وَالْحِكْمَةَ وَعَلَّمَكَ مَا لَمْ تَكُنْ تَعْلَمُ وَكَانَ فَضْلُ اللَّهِ عَلَيْكَ عَظِيماً"

سورة النساء الآية 113

Dedication

Idedicate this research with much love and appreciation

To …

The loving memory of I dears and beloved parentsTo…

My big and my small family

To…

My friends and teachers and

To…

Every one that I love

To …

All Patients who sustain the pain and their hope to become well and good.

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I would like to express sincers appreciation andgratitude to my supervisor

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for the unfailing guidance throughout the study and work on this project

and without this assistance this project would not have beenpossible.

Abstract

This is a descriptive cross-section al study carried out at Reproductive Health Care Centre in Khartoum state. during the period from August 2015 to November 2015, to determine the frequency of Rh antigens among Sudanese pregnant ladies group A and group O, compare the frequencies of rhesus antigens between group A and group O Sudanese pregnant ladies and to detect if there are significant role for c antigen in the previous abortion.by using direct tubes technique , One hundred blood samples were collected from participate enrolled in this study, 50 samples from Sudanese Pregnant Ladies group A, 50 samples from Sudanese Pregnant Ladies group O.

10 samples from Sudanese Pregnant Ladies group A with no previous abortion history ,10 samples from Sudanese pregnant Ladies group O with no previous abortion history Also were include in this study as control.

2.5 ml of venous blood were drown from each pregnant ladies in K2EDTA container. Rhesus phenotype was determined by using direct group method.

The result show that the frequency of Rh Antigens among pregnant Ladies group (A) with (+ve) Rh as(52) anti-D ,(41) anti-c ,(38) anti-e ,(23) anti-C ,(10) anti-E.and the pregnant Ladies from the same group with (-ve) Rh as (50) anti-E ,(37) anti-C , (22) anti –e , (19) anti –c ,(8) anti-D.

And pregnant Ladies group (O) with (+ve) Rh as (54) anti-D ,(40) anti-c ,(39) anti-e, (24) anti-C ,(13) anti-E.and the pregnant Ladies from the same group with (-ve) Rh as (47) anti-E ,(36) anti-C ,(21) anti-e ,(20) anti-c ,(6) anti-D.

The antigen-c can be consider as protective factor of abortion ,pregnant with –ve antigen-c are more susceptible to abortion when compare pregnant with +ve antigen-c.

ملخص الدراسة

أجريت هذه الدراسة خلال الفترة من أغسطس-نوفمبر عام 2015 لتحديد مدى تكرار النمط الظاهري (D,E, e, C, c) لعامل الدم الريصي بين السيدات الحوامل السودانيات ذوات فصائل الدم (A)و (O) واثبات احتمالية وجود علاقة مابين الاجهاض وانتجين c في المركز الاستشاري للامومة والخصوبة في ولاية الخرطوم.اجريت هذه الدراسة لمئة سيدة حامل مع سابقة اجهاض, كما اجريت على عشرون سيدة حامل ليس لديهم سابقة اجهاض كمجموعة ضمان جودة الدراسة تم جمع عينات الدم باستخدام وعاء محتوي على الايديتا المضادة للتخثر 2.5 مل.وأظهرت النتيجة ان ترتيب مدى تكرار النمط الظاهري (D, E, e, C, c) لدى النساء الحوامل من فصيلة A كالآتي: النساء من نوع الفصيلة A موجب العامل الريصي (52) انتيجين D, (41) انتيجين c, (38) انتيجين e, (23) انتيجين C, (10) انتيجين E والنساء من نوع الفصيلة A سالب العامل الريصي (50) انتيجين E, (37) انتيجين C, (22) انتيجين e, (19) انتيجين c, (8) انتيجين D, اما النساء الحوامل من فصيلة O كالآتي: النساء من نوع الفصيلة O موجب العامل الريصي (54) انتيجين D, (40) انتيجين c, (39) انتيجين e, (24) انتيجين C, (13) انتيجين E, والنساء من نوع الفصيلة O سالب العامل الريصي (47) انتيجين E, (36) انتيجين C, (21) انتيجين e, (20) انتيجين c, (6) انتيجين D.

من خلال هذا البحث نجد ان انتجين Cيعتبر كعامل حماية للنساء الحوامل من الإجهاض وذلك لأن الحوامل اللاتي لا يحملن هذا الانتجين قد تعرضنا للإجهاض بنسبة أكبر من اللواتي التي يحملن الانتجين نفسه

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Abbreviations

Ag : Antigen.

AHG : Anti Human Globulin.

DNA : Deoxyribonucleic Acid.

HDN : Hemolytic Disease of Newborn.

HTR : Hemolytic Transfusion Reaction.

I g G : Immunoglobulin G.

I g M : Immunoglobulin M.

RBCs : Red Blood Cells.

Rh : Rhesus.

Rh C, E : Rhesus C and E gene.

Rh D : Rhesus D gene.

SPSS : Statistical Package for Social Sciences

Chapter One

Introduction and Literature review

Chapter One

Introduction and literature review

1.1. Introduction:

TheRh (Rhesus)blood group system is one of thirty-three current human bloodgroup systems. It is the most important blood group system after the ABO bloodgroup system. At present, the Rh blood group system consists of 50 defined blood groupantigens, among which the five antigens D, C, c, E, and e are the most important. The Rhesus (Rh) blood group system was first described 60 years ago. A woman had a severe transfusion reaction when she was transfused with blood from her husband following the delivery of a stillborn child with Erythroblastosisfoetalis. Her serum agglutinated red blood cells (RBCs) of her husband and those of 80% of Caucasian ABO compatibledonors. The following year Landsteiner and Wiener (1940) found that sera from rabbits (and later guinea pigs) immunized with RBCs fromMacacamulatta(Macacus Rhesus in the original paper) agglutinated 85% of the human RBC samples. Initially, itwas thought that the animal and human antibodies identified a common factor (Rh) on the surface of Rhesus and human RBCs. It was soon realized that this was not thecase . Therefore the original terms (Rh factor and anti- Rh) coined by Landsteiner and Wiener, although being misnomers, have continued in common usage. The Rh blood group system is the most polymorphic of the human blood groups, consisting of at least 45 independent antigens and, next to the ABO blood group system, in terms of clinical significance in transfusion medicine. Apart from the importance

ofthe Rh antigensin blood transfusion and HemolyticDisease of the Fetus and Newborn (HDFN), Rh proteins are important in transporting ammonia across the RBCmembrane. It followsthatRBCs which lack Rh antigens will have abnormal shape, increased osmotic fragility and shortened life span. These often result in hemolytic anemia that ismild in nature. During pregnancy and also delivery, fetal RBCs may enter into the maternal circulation through the placenta. Some fetal cells may contain some antigens (C, D, and E) which may be lacking in the mother. These antigens may probably have been inherited from the father, and they are capable of stimulating the mother to produce Alloantibody. The type of RBC Alloantibodythat can be produced by the mother depends totally on the combination of antigens on the fetal RBCs. pregnant women potentially at risk of producing Rh antibodies against Rh antigens (C, D and E) present in the donor unit but which the pregnant woman lacks. This alloantibody developed put the woman potentially at risk ofHDFNif she is pregnant and carrying a baby that is positive for the antigen for which the maternal alloantibody is specific.

HDNiscausedbymaternalIgGantibody crossing the placenta, binding to the fetal antigen-positive RBCs, and initiating their destruction, thereby causing Anemia(Erhabor*etal;*2014), (Levine andStetson1939).

The aim of this present study is to determine the frequency of Rhesus antigens(D,C,E,c,e)among group A and O ofpregnant women attending Reproductive Health Care Center in Sudan.

1.2. Literature review:

1.2.1. ABO blood group:

1.2.1.1. History of ABO blood group system:

The ABO blood group system was widely credited to have been discovered bytheAustrian Karl Landsteiner, who found three different blood types in 1900. He was awarded theNobel PrizePhysiologyor medicine in 1930 for his work. Due to in adequate communication at the time it was subsequently found that Czech serologist JanJanskyhad independently pioneered the classification of human blood into four groups. but Landsteiner's independent discovery had been accepted by the scientific worldwhileJansky remained in relative obscurity (Harmening,2005), (Fisk andFoord, 1942).

1.2.1.2The inheritance of ABO blood group system:

The theory for inheritance of ABO groups was first described by Bernstein in 1924. He demonstrated that an individual inherited on ABO gene from each parent and these two genes determined which ABO antigens were present on theRBCs membrane.The inheritance of ABO gene followed Mendeliangenetics the ABO locus is located on chromosome9, it contain 7 exons thatspan more than 18 kb of genomic DNA; Exon7 is the largest and contains most of the coding sequence. The ABO locus has three main allele A,B and O, (Daniels, 2005).That the Aantigen could divided into sub groups A1, A2, and in proposed a four allele theory of inheritance to encompass these

sub groups. Under the terms of the theory of Thompson, four allelic genes A1, A2 ,B and O. since each individual inherits one chromosome from each parent for each chromosome pair, two genes are inherited characteristic and these four allelic genes give rise to ten possible genotypes(Hoffbrand, *etal;*2001), (Dutta, 2006).

1.2.1.3 Formation of ABO antigen:

Theexpression of the A and B genes depend on the action of another gene , known as H gene. Most individual are homozygous for this gene(HH), while other are heterozygous (Hh).The phenotype h (genotypehh) are extremely rare (Bombay phenotype)(Hoffbrand.*etal;*2001) .

When individual inherited H gene from parents, gene was responsible of production of enzyme called L- fucosy l- tranferase which transferred L fucoseto the terminal galactose of precursor chain (composed of 4 molecule of 3 different sugar N-acetyl-galaceosamine,N-acetyl- glucosamine and D-galactose)lead to form of H substance, other glycosyltransferasecoded by A orB antigen, located on chromosome 9 convert this H substance in to A or B antigen , the O gene was an morph gene and so the H sub stance remain unchanged in individual with O blood type. When individual inherited h gene (hh) the precursor substance not convert in to H substance so the antigen was not found(bombay phenotype), these individuals hadanti- A anti –B and anti H in their blood. H gene caused L-fucose to be added to the terminal sugar of precursor chain,

produced H antigen (shown in this diagram of type 2 H antigen saccharide chain) (Neville,1994).

1.2.1.4Nomenclature of ABO blood group:

In parts of Europe, the "O" in ABO blood type is substituted with "0" (zero), signifying the lack of A or B antigen. In the former USSR blood types are referenced using numbers and Roman numerals instead of letters. This isJanský's original classification of blood types. It designates the blood types of humans as I, II, III, and IV, which are elsewhere designated, respectively, as O, A, BandSubgroups of A(Erb,1940).

1.2.1.5 ABO Antigens:

TheABO blood groupswere four blood groups A, B, AB and O was racial variation in the frequency of these groups, the presence of A,B or O antigens on red cells was determined by the in heritance of the allelic genes A,B and O which inherited in pairs as Mendel an law. It was likely that the O and B were mutation of the A gene. O gene was identical to the A gene except for the deletion of single DNA base pair which causes a frame shift during transcription and translation resulting in an inactive enzyme. B gene differs from A gene by four consistent nucleotide substitutions. The cellular expression of A and B anti gens were determined by further gene, the H gene which inherited in dependently. This gene coded for an enzyme that converted a carbohydrate precursor into H substance .the A and B gene coded for specific

enzyme(glycosyltransferases)which converted H substance into A and B antigens by the terminal addition of N-acetylgalactosamineandD-galactoserespectively .the O gene produced an inactive transfers, O the H substance persistunchanged in group O individual. In the extremely rare on Bombayphenotype, the H genotype was (hh)and no H transfers produced, consequently, no H substance was made and therefore A and B genes, if present cannot be expressed, these individual had anti-A., anti-B and anti H in their blood ,all active at 37 degree C. and could only be safely transfused with other oh Bombay blood (DacieandLewis,2001).

Serologists had defined two common subgroups of A antigen about 20% of group A and group AB subject belong to groupA2 and A2B respectively,and the remainders belong to group A1andA1B, the distinction was most convent made using the lectinfrom Dolichosbiflorusseeds which only react with the A1cells. The H antigen content of the red cells depend on the ABO group and when assessed by agglutination reaction with anti-H, the strength of reaction tended to be graded: O > A_2>A_2B>A_1>A_1B ‘antigens weaker than A were occasionally found (called A_3, A_x, A_{eb} ,A_{enl}…. etc) (Dacie andlewis,2001).

These serological variations in the A antigen were due to the mutant from of glycosyltransferases produced by A gene , which was less efficient at transferring N-acetyl- D-galactosamine in the hexose ring of H substance(Dacie andlewis,2001).

The A, B and H antigens were detectable early in fetal life, but were not fully developed on the red cell at birth. The numberof antigen sites reach adult level at about one year of age and remain the same throughout life until old age when a slight reduction may occur (Dacie andlewis,2001).

O cell had no antigen in the ABO system. It would be more correct to say that they did not possess A or B antigen. However the H antigen was not thought to be the production of the O gene. Ti was regarded the product of H gene and was the precursor on which products of ABO gene act. The H gene present to some extent on all red cells regardless of the ABO group (Hoffbrand,*etal;*2001)

1.2.1.5.1.Bombayphenotype: Individuals with the rare Bombay phenotype(hh) did not express antigen H on their red cells.As H antigen serves as precursor for producing Aor B antigen as well(similar to O blood group). However, unlike O group.The HAntigen was absent; hence the individuals produceisoantibodies to antigen H as well as to both A and B antigen .In case they receive blood from O blood group , the anti-H antibodies will bind to H antigen on RBC of donor blood and destroy the RBCs by complement mediatedlysis.Therefore Bombay phenotype could be receiving blood only from other hhdonors(although they can donate as though they were type O(Reid and Lomas-Francis,2004).

1.2.1.5.2 Subgroups of A:

Inaddition to thecommon phenotypes A1 and A2,numerousphenotypes with weak expression of A on the red cells have been found and multitude of names has been adopted. Most of these phenotypes can be fitted in to the followingcategories; A_3,A_X,A_mA_y,and A_{el}.Theserological characteristicsofthese phenol types. All have normal or enhanced expression ofH.In most cases the variant phenotype results from inheritance of rare allele at the ABO locus,which can be detected when paired with 0ur B,but notA_1 orA_2(Denils,2002).

1.2.1.5.3The secretary status:

The Se locus encodesaspecific fucosyltransferasethat is expressed in the epithelia of secretary tissues, such as salivary glands, the gastro intestinal tract, and therespiratory tract. The enzyme encodes catalyzes the productionofH antigen in bodily secretions (secretors) have at least one copy of these antigen which depending on theirABO genotype,is then processed in toA and / or B antigen.Non-‘’secretors are homozygous for null alleles at this locus ‘’SE/SE’ they are unable to produce a soluble from of H antigen and hence do not produce A and B antigen.About 80% of the UKpopulations are ABH secretors as they have H antigen,plus A or B according to their ABO genotype in water-soluble from in their body secretion. The remaining 20% are non-secretors and have no secreted ABH antigens, regardless of ABO genotype(Yamato*etal;*2002).

1.2.1.6 Antibodies of ABO blood Group system:

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 and anti-B. They are usually immunoglobulin M (IgM), and react optimally at cold temperatures 4^0C) so, although reactive at 37^0C, are called cold antibodies (Hoffbrand*et al;*2001).

1.2.1.7 Classification of ABO blood group system:

Classification of blood groups was based on the reaction that agglutination had occurred because the red cells possessed an antigen and the corresponding specific antibody was present in the serum, when no agglutination had occurred, either the antigen or the antibody was missing from the mixture from these observation Landsteiner recognized four separate groups, named according to the antigen present on the red cells. Individual who possessed the B antigen (their red cells shows agglutination with anti-B) were classified as belonging to group B. red cells from certain individuals showed no agglutination with either anti-A or anti-B and were classified as belonging to group O (the symbol O denoting zero or lack of A and B antigens on red cells).The red cells of individuals which showed agglutination with both anti-A and anti-B the blood group was called AB. Individuals who possessed the A antigen on their red cells also possessed the anti-B in their serum, individual who possessed neither A nor B antigens (group AB) had neither anti-A no anti B in their serum (Race and Sanger,1975)

1.2.1.8Clinical signification of ABO blood group systems:

The discovery of theABO blood group, over 100 years ago, caused great excitement .the world of blood transfusionbecame a great deal safer, and has importance in genetics, immunology, anthropology, patemity and clinical medicine they were also valuable tools in forensic medicine and organ transplantation (Kathleen,*etal;*1988).

The clinical importance of blood group system antigens depend on the frequency of occurrence of the corresponding antibody and its ability to hemolytic the red cells invivo. ABO antibodies are of major clinical significance for two reasons they are naturally occurring and are found universally,and, they are highly reactive. The routine practice of blood typing and cross matching blood products should prevent adverse transfusion reactions caused y ABO antibodies, However, clerical error can result in the ‘’the wrong blood being 1990).If a recipient who has blood group O is transfused with non-groupORBCs, the naturally occurring anti-A and anti- B in the recipient’s serum binds to their corresponding antigens on the transfused RBCs. The antibodies fix complement and cause rapid intravascular hemolytic, triggering an acute hemolytic transfusion reaction that can cause disseminated intravascular coagulation, shock, acute failure, and death. Anti-A1 is a less significant cause of transfusion reaction and doesnot appear to fix complement. Most cases of hemolytic disease of thenewborn (HDN) that arise from an ABO in compatibility require and fetal hydrops is rare. Hemolytic disease of newborn caused by ABO antibodies occurs almost

exclusively in infants of blood group A or orB who are born to group O mothers .This is because the anti-A and anti-B formed in group O individuals tendto be of theIgGtype(and therefore can cross the placenta,whereas the anti-A and anti-B found in the serum of group B and Aindividuals,respective and Aindividuals,respective tends to be of the IgM type. Although uncommon Cases of HDN have been reported in infants born to mothers with blood groupA$_2$ and blood group B. hemolytic disease of new born tends to be relatively mild in nature mainly because fetal RBCs blood group antigens can vary,and therefore the degree of the race of a neonate was a risk factor for developing ABO,HDN.However,later studies showed that the prevalence of disease that required treatment did not differ significantly among Asian, Black,Hispanic,and Caucasian infants (Ozolek*etal*;1994), (Bowman, 1997).

1.2.2 Rhesus blood group:

1.2.2.1 The history of Rhblood group:

The Rhblood group is one of the most complex blood groups known in humans. From its discovery 60 years ago where it was named (in error) after the Rhesus monkey, it has become second in importance only to the ABO blood group in the field of transfusion medicine. It has remained of primary importanceinobstructs, being the main cause of hemolytic disease of the newborn (HDN) (Chapman and Waters,1981).

In 1939, a mother who had just given birth to a still-born child needed a blood transfusion. Although the mother was transfused with

ABO compatible blood from her husband, she still experienced an adverse reaction to the transfusion. Her serum was found to contain antibodies that agglutinated her husband's RBCs, even though they were ABO compatible. The death of the mother's fetus and her adverse reaction to a blood transfusion from her husband was related. During the pregnancy, the mother had been exposed to an antigen on the fetal RBCs that was of paternal origin. Her immune system attacked this antigen, and the destruction of the fetal RBCs resulted in fetal death. The mother re-encountered the same paternal antigen when she received a blood transfusion from her husband. This time her immune system attacked the transfused RBCs, causing a hemolytic transfusion reaction. The antibodies responsible led to the discovery of the Rh blood group. It was wrongly thought that the agglutinating antibodies produced in the mother's serum in response to her husband’s RBCs were the same specificity as antibodies produced in various animals' serum in response to RBCs from the Rhesus monkey. In error, the paternal antigen was named the Rhesus factor. By the time it was discovered that the mother's antibodies were produced against a different antigen, the rhesus blood group terminology was being widely used. Therefore, instead of changing the name, it was abbreviated to the Rhbloodgroup(Reidand Lomas-Francis,2004).

Remarkably, only 20 years after the discovery of Rh incompatibility in pregnancy, effective treatment became available. Today, the Rh status of mothers-to-be is checked during pregnancy to identify those at risk of HDN. In addition, all blood transfusions are matched for

the Rh status. The significance of the Rh blood group is related to the fact that the Rh antigens are highly immunogenic. In the case of the D antigen, individuals who do not produce the D antigen will produce anti-D if they encounter the D antigen on transfused RBCs (causing a hemolytic transfusion reaction, HTR) or on fetal RBCs (causing HDN). For this reason, the Rh status is routinely determined in blood donors, transfusion recipients, and in mothers-to-be (Bowman*etal*;1992).

1.2.2.2. Inheritance of Rhesus antigen:

The complexity of the Rh blood group antigens begins with the highly polymorphic genes that encode them. There are two genes, RHD and RHCE, which are closely linked and it is located on the long arm of chromosome 1. RHD gene encode for Rh D antigen while RHCE gene encode for C, c, E and e.(Reid and Lomas-Francis,2004).

The Rh locus is located onthelongarm of chromosome 1.The RHD and RhCE genes arestructuralhomologsand result from a duplication of a common gene ancestor. RHD and RHCE each contain 10exonsand span a ~75-kb DNA sequence(Wagner andFlegel,2000).

1.2.2.3Formation of Rhesus antigen:

The RHD and RHCE genes each encode a transmembrane protein over 400 residues in length that traverses the RBC membrane 12 times. The RhD protein only differs from the common form of the RhCE protein by about 35 amino acids.TheRhD protein bears the D

antigen which has over 30 epitopes. The RhCE protein carries the epitope for the C or c antigen on the second extracellular loop, and the epitope for the E or e antigen on the fourth extracellular loop. A number of nucleotide substitutions in the RHCE gene in turn cause a number of amino acid changes in the RhCE protein, but two polymorphisms are thought to be key in producing the polymorphic antigens on this protein, i.e., the S103P polymorphism (produces the C or c antigen, respectively), and the P226A polymorphism (produces the E or e antigen, respectively) (Wagner, *etal*,2005).

1.2.2.4 Terminology of Rhesus system:

1.2.2.4.1 Fisher-Race Theory:

The Fisher-Race theory involved the presence of 3 separate genes D, C, and E and their allele'sc and e and the absence of D since an anti-d has never been found. These three genes are closely linked on the same chromosome and are inherited as a group of 3. The most common group of 3 genes inherited is CDeand ce (D negative) is the second most common (Cartron and Agre,1993).

1.2.2.4.2 Weiner Theory:

Weiner believed there was one gene complex with a number of alleles resulting in the presence of various Rh antigens. According to Weiner there were 8 alleles, R^o, R^1, R^2, R^z, r, $r^{'}$, $r^{''}$, r^y , which ended up with different antigens on the red cells that he called Rh_o, rh', rh'', hr', hr''. Weiner terminology is not use as often today, but you will often see Rh_o(D) when a person considered to be Rh-positive. At

times the gene terms are easier to use than Fisher-Race. If a person has the Fisher-Race genotype of DCe/DCe, it is easier to refer to that type as R^1R^1(Cartron and Agre,1993).

1.2.2.4.3 Tippett Theory:

In 1986, Tippett predicted that there are two closely-linked genes - RHD and RHCE. The RHD gene determines whether the D antigen that spans the membrane is present. Caucasians who are D negative have no gene at thatgeneloci. In the Japanese, Chinese, and Blacks of African descent have an inactive or partial gene at this site (Cartron andAgre.1993).

1.2.2.5 Rhesus antigen:

The Rh blood group contains the D antigen which differs from the C/c and E/e antigens by 35 amino acids. This large difference in amino acids is the reason why the Rh antigens are potent at stimulating an immune response;therefore the Rh antigens are highly immunogenic (Westhoff,2004).

1.2.2.5.1 Uncommon Rh antigen:

The D antigen contains over 30 epitopes. Variations of the D phenotype arise when these epitopes are only weakly expressed ("weak D phenotype") or when some are missing ("partial D phenotype")(Avent andReid ,2000).

1.2.2.5.2 Weak D antigen:

"Weak D" is a Rh phenotype found in less than 1% of Caucasians and is only slightly more common in African Americans It is typically caused by a single amino acid switch in the transmembrane region of the RhD protein. This disrupts how the RhD protein is inserted into the RBC membrane, reducing the level of expression of RhD. In most cases, adequate levels of D antigen are present and because there has been no change in D epitopes, the formation of anti-D is prevented. Therefore, individuals with the weak D phenotype can receive Rh D-positive blood (Avent andReid ,2000).

1.2.2.5.3 Partial D antigen:

In contrast, people who have been identified as having the "partial D" phenotype should not receive Rh D-positive blood but in practice, people with partial D are difficult to identify. This phenotype is usually caused by the creation of a hybrid RhD and RhCE protein. The hybrid protein is similar enough to RhD to be correctly inserted in the RBC membrane, but it lacks several epitopes found on the complete RhD protein. If a person with the partial D phenotype encounters the complete D antigen on transfused RBCs, they may form anti-D and suffer from a transfusion reaction(Aventand Reid,2000).

1.2.2.5.4 Other Rh System Variants:

There are presently 46 Rh antigens identified and named. The following are the most common of those variants:

- **C^w**is a low frequency antigen found in approximately 2% of Whites and 1% of Blacks. It is not an allele of C and c. Its allele is MAR, which is found in 99.9% of the population.

- **V and VS**are low frequency alleles found in 1% or less of the Whites, but are more common in Blacks. V is found in 30% of the Blacks and VS in 32%.

- **G** is present when D or C present due to the present of serine at the 103 position of the Rh polypeptide. Anti-G will react with both D+ and C+ cells.

- **f**is present when c and e together on same chromosome: Dce or ce. This is the most common of what are called cis product antigens.

- **Rh_{null}**has no Rh antigens on their red cells but these individual can transmit normal Rh antigens to their offspring. In the most common type the core Rh polypeptide is missing. A less common type has the regulator gene that turns off the expression of Rh. There have been at least 43 individuals in 14 families that are Rh_{null}. In these individuals the red blood cell membrane is abnormal and some of these have been identified when it was observed that they had haemolytic anaemia and abnormal red cell morphology. If these

individuals develop an Rh antibody following a transfusion or pregnancy, it is considered a anti-total Rh antibody (Huang ,1997).

1.2.2.6 Rhesus antibody:

The majority of antibodies formed against the Rh antigens are of the IgG type. They are capable of causing significant HTR and HDN. Rh antibodies rarely, if ever, bind complement, and therefore RBC destruction is mediated almost exclusively via macrophages in the spleen (extravascular hemolysis). There are a few examples of Rh alloantibodies that are naturally occurring and are of the IgM type, but they are in the minority(Westhoff,2004).

1.2.2.7 Clinical significance of Rh antibodies:

Most of the Rh antibodies should be considered as potential causes of hemolytic transfusion reactions and HDN.

1.2.2.7.1 Blood transfusion reactions:

Routine blood typing for Rh D status in both blood donors and transfusion recipients has reduced the incidence of transfusion reactions caused by anti-D. But sensitization to other Rh antigens can be a problem in transfusion medicine, particularly in patients with sickle cell anemia (SCA). SCA is more common in Blacks, and the treatment of SCA involves blood transfusions. Blacks are also more likely to express variants of the Rh e antigen, and therefore produce anti-e, along with other Rh alloantibodies, which increases the difficulty in finding Rh-compatible blood donors(Urbaniak andGreiss,2000).

1.2.2.7.2 Hemolytic disease of the newborn:

Anti-D causes the most severe form of HDN and it used to be a major cause of fetal death. Since the introduction of anti-D immunoglobulin along with careful monitoring of at-risk pregnancies, the prevalence of HDN because of Rh D incompatibility has decreased dramatically. However, all cases cannot be prevented, and RhDalloimmunization remains a major cause of disease(Urbaniak andGreiss,2000).

Other Rh alloantibodies that are capable of causing severe HDN include anti-c, which clinically is the most important Rh antigen after the D antigen (Appelman,*etal;*1990)(Katiyar,*et al;* 2007)

1.3 Frequencies of ABO blood group and Rh D in Sudan:

Several studies have been carried out to determine the frequency of ABO blood group and Rh D among the Sudanese ethnic groups. Such study done among Alshwiga Sudanese tribe and the frequencies were as follow: A (36%), B (20%), AB (4%), O (40%), Rh D +ve (92%) and Rh D –ve (8%) (Mohammed,2013).

Similar study was done among ElgomoiaSudanese tribe and the frequencies were as follow:A (43%), B (17%), AB (3%), O (37%), Rh D +ve (96%) and Rh D –ve (4%) (Abuhorira, 2011).

1.4. Rationale:

In pregnant ladies the migration routes were variable because Sudan inhabitedby different population that migrate from other part of Africa and Arabia so the interaction is likely, this resulted in population with different characteristics, and different gene complexes in Rhesus phenotype would be expected. This research will provide frequency of Rhesus phenotype an essential data about the pregnant ladies which have problem in their pregnancieslead to abortion or death of babies in uterus or in early hours after deliveryfor a scientific and proper protocol to be used in Rhesus phenotype practice, the collected information can be used as guide for other studies like the association between problem of abortion and death of the babies of pregnant ladies and antigen -c.

Thatbecause they are found that may antigen –c have the same significant and antigenicity of D antigen.

1.5. Objectives:

1.5.1. General objective:

Determination offrequency of Rhesus antigens in Blood group A and OSudanesepregnant ladies.

1.5.2.Specific objectives:

- To compare the frequencies of Rhesus antigens between group A and group OSudanese pregnant ladies with previous abortion history.
- To detect if there are significant role for c antigen in the previous abortions because it have the same effect of D antigen which lead to hemolytic disease of new born to the Blood group A and group O pregnant ladies with previous abortion history.

Chapter Two

Materials and Methods

Chapter two

Materials and Methods

2.1 Studydesign:

Adescriptivecross-sectional study type.

2.2 Study area:

Study was conducted in Reproductive Health Care Centre during the period from August 2015 to November 2015, to determine thefrequency of Rh antigens among group A and O pregnant ladies.

2.3 Study population:

The data and samples were collected from One hundred volunteers, 50 samples from group A pregnant ladies and 50 samples from group O pregnant ladies.

2.3.1 Inclusion criteria:

- Healthy Sudanese Pregnant ladies with previous abortion history with A and O blood group.

2.3.2 Exclusion criteria:

- Other blood groups than A and O

- All those origin other Sudanese national

2.4 Methods:

2.4.1 Equipments:

-small testtubes(75×10 ml).

- pasture pipette

- Cotton

- 70% alcohol

- syringes(5 ml)

- Centrifuge

2.4.2 Collection of blood samples:

Venus blood was drawn after sterilization by 70% alcohol, syringe was used to withdrawn the blood from the arm.

2.4.3 Determination of Rhesus phenotype:

The tube method for antigenic determination was taken as gold standard.

2.4.4 Reagents:

Monoclonal IgM antibodies ready to use, in 5ml and 10 ml vials with 0.1% NaN_3 preservative the following reagents were used:

- Monoclonal anti-E

- Monoclonal anti-e

- Monoclonal anti-C

- Monoclonal anti-c

-Monoclonal anti-D

2.4.5 Controls:

Positive and negative controls for each antiserum used must be included with every test.

2.4.6 Principle of Rhesus phenotype:

The test procedure is based on the principle of agglutination. Red cells possessing the specific antigen will agglutinate when tested with the corresponding reagent (positive test), when used by the recommended technique. Lack of agglutination of red cells demonstrates the absence of specific antigen (negative test).

2.4.7 Test procedure of tube method:

1- In a clean tube equal volume of blood sample and antiserum is added.

2- Then mixed by using wooden stick.

3- Incubated for 5 minutes.

4- The result is read.

2.4.8 Interpretation of the result:

- Positive: agglutination found.

- Negative: no agglutination found.

2.5 D^u method:

D^u method used for detection of weak D antigen.

2.5.1 Steps of the method:

1- The cells were washed three times with saline after incubate for 15 min with modification IgG anti D.

2- Two drops of AHG reagent were added to the washed cells.

3- Centrifugation at 100 rpm for 60 sec was done.

4- Agglutination was read macroscopically.

2.5.2 Interpretation of result:

- Positive: agglutination found.

- Negative: no agglutination found.

2.6 Quality control measures (QCM):

According to the quality control measurement rules were involved and constructed in lab structure manual of The National Ribat University Laboratories and calibrated all the machines and reagents used.

2.7 Data analysis:

Different variables were presented and analysis of significant variance, were applied for comparison between different results.

Statistical analysiswere doing by using statistical package for social science (SPSS) program (version.20) under windows(IBM)computer system.

2.8 Data presentation;

The obtained data were presented in form of tables.

Chapter Three

Results

Chapter three

Results

The frequencies of blood group A pregnant ladies were 60; 10 (16.7 %) control pregnant ladies without abortion history and 50 (83.3 %) test pregnant ladies with abortion history as shown in table (3-1).

The frequencies of blood group O pregnant ladies were 60; 10 (16.7 %) control pregnant ladies without abortion history and 50 (83.3 %) test pregnant ladies with abortion history as shown in table (3-1).

Frequencies and percentage of blood group A pregnant ladies according to age were as follow; 5 (8.3%) were less than 20 years, 25 (41.7%) were from 20 - 30 years and 30 (50%) were more than 30 years as shown in table (3-2).

Frequencies and percentage of blood group O pregnant ladies according to age were as follow; 2 (3.3%) were less than 20 years, 22 (36.7%) were from 20 - 30 years and 36 (60%) were more than 30 years as shown in table (3-2).

Frequencies and percentage of Rhesus(c) phenotyping in A and O pregnant ladies were as follow; pregnant ladies who positive with anti c were 81 (67.5%) which contain 41 (34.2%) were A blood group and 40 (33.3%) were O blood group while pregnant ladies who negative with anti c were 39 (32.5%) which contain 19 (13.8%) were A blood group and 20 (16.%) were O blood group as shown in table (3-3).

Frequencies and percentage of Rhesus (D) phenotyping in A and O pregnant ladies were as follow; pregnant ladies who positive with anti D were 106 (88.3%) which contain 52 (43.3%) were A blood group and 54

(45.0%) were O blood group while pregnant ladies who negative with anti D were 14 (11.7%) which contain 8 (6.7%) were A blood group and 6 (5%) were O blood group as shown in table (3-3).

Frequencies and percentage of Rhesus(e) phenotyping in A and O pregnant ladies were as follow; pregnant ladies who positive with anti e were 77 (64.2%) which contain 38 (31.7%) were A blood group and 39 (32.5%) were O blood group while pregnant ladies who negative with anti e were 43 (35.8%) which contain 22(18.3%) were A blood group and 21 (17.5%) were O blood group as shown in table (3-3).

Frequencies and percentage of Rhesus(E) phenotyping in A and O pregnant ladies were as follow; pregnant ladies who positive with anti E were 23 (19.2%) which contain 10 (8.3%) were A blood group and 13 (10.8%) were O blood group while pregnant ladies who negative with anti E were 97 (80.8%) which contain 50 (41.7%) were A blood group and 47 (39.2%) were O blood group as shown in table (3-3).

Frequencies and percentage of Rhesus(C) phenotyping in A and O pregnant ladies were as follow; pregnant ladies who positive with anti C were 47 (39.2%) which contain 23 (19.1%) were A blood group and 24 (20%) were O blood group while pregnant ladies who negative with anti C were 73 (60.8%) which contain 37(30.8%) were A blood group and 36 (30%) were O blood group as shown in table (3-3).

The crosstab describe the distribution of antigen-c among research groups as follow: the -ve antigen-c in group A (36) were with abortion history while(5) only with non-abortion history.the -ve antigen-c in group O (33) were with abortion history while(7) only with non-abortion history.

The +ve antigen in group A(14) were with abortion history while (5) only with non-abortion history.the +ve antigen-c in group O (17) were with with abortion history while (3) only non-abortion history.

The risk estimation of abortion among research groups were:

The Odd ratio for –ve antigen-c is (2.024) mean ≥ 1.

The Odd ratio for +ve antigen-c is (.655) mean ≤ 1.

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Table (3-1) : represent frequency and percent of pregnant female with previous abortion history (group A and O) , control (group A and O).

The Groups	A		O	
	frequency	**percent**	**frequency**	**Percet**
A control	10	16.7%	10	16.7%
A Test	50	83.3%	50	83.3%
Total	60	100.0%	60	100.0 %

Table (3-2): represent frequency and percent of group A and O pregnant female according to age .41.7% group A between (20-30) years, 60% group O female above 30 years.

Age			Frequency	Percent
A		Less than 20 years	5	8.3%
		20 – 30 years	25	41.7%
		More than30 years	30	50%
		Total	60	100%
O		Less than20 years	2	3.3%
		20 – 30 years	22	36.7%
		More than30 years	36	60%
		Total	60	100%

Table (3-3): represent frequency and percent of presence (+ve) and absence(–ve) of D, E,C,c,e antigen I n group A and O pregnant females

		The Groups		Total
		A	O	
Anti c	+ve	41	40	81
		34.2%	33.3%	67.5%
	-ve	19	20	39
		15.8%	16.7%	32.5%
Total		60	60	120
		50%	50%	100%
Anti D	+ve	52	54	106
		43.3%	45.0%	88.3%
	-ve	8	6	14
		6.7%	5.0%	11.7%
Total		60	60	120
		50%	50%	100%
Anti E	+ve	10	13	23
		8.3%	10.8%	19.2%
	-ve	50	47	97
		41.7%	39.2%	80.8%
Total		60	60	120
		50%	50%	100%
Anti e	+ve	38	39	77
		31.7%	32.5%	64.2%
	-ve	22	21	43
		18.3%	17.5%	35.8%
Total		60	60	120
		50%	50%	100%
Anti C	+ve	23	24	47
		19.1%	20%	39.2%
	-ve	37	36	73
		30.8%	30%	60.8%
Total		60	60	120
		50%	50%	100%

Table (3-4):represent and describe the risk estimation of abortionamong research group.

Antigen-c	value	95%Confidence interval	
		Lower	Upper
+ve Odds Ratio for group(A/O)	.655	.189	2.265
-ve Odds Ratio for group(A/O)	2.024	.410	9.990
Total Odds Ratio for group(A/O)	1.000	.383	2.612

Odd ratio for –ve antigen-c is ≥ 1.

Odd ratio for +ve antigen-c is ≤ 1.

Table(3-5): represent crosstab describe the distribution of antigen-c among research groups:

Antigen-cc		Abortion history		Total
		No Abortion	Abortion	
-ve	groupA	5	36	41
	groupO	7	33	40
	Total	12	69	81
+ve	groupA	5	14	19
	groupO	3	17	20
	Total	8	31	39
Total	groupA	10	50	60
	groupO	10	50	60
	Total	20	100	120

Chapter Four

Discussion, Conclusion and Recommendations

Chapter four

Discussion,Conclusion and Recommendation

4.1 Discussion:

This study carried out at Reproductive Health Care Centre in Khartoum state. during the period from August 2015 to November 2015,the study group include 50 group A and 50 group O pregnant ladies with history of abortion, and 10 group A and 10 group O pregnant ladies without history of abortion, as control group.50 % of group A test group age more than 30 years,60% of group O test group age more than 30 years. Rhesus phenotype in Group A pregnant women reveal (34.2% +ve for c antigen,43.3% +ve for D antigen,8.3%+ve for E antigen,31.7%+ve for e antigen and 19.1%+ve for C antigen).

Rhesus phenotype in group O pregnant women reveal(33.3%+ve for c antigen,45%+ve for D antigen,10.8%+ve for E antigen,32.5%+ve for e antigen and 20%+ve for C antigen).

All Rhesus antibody show insignificant result (P.value>0.05) when compared with control group.In this study, in study by (Erhabor.,*etal*,2014) in sokoto , that the prevalence of Rhesus (D) positive and negative prevalence of 92.9% and 7.1% respectively among our cohort of pregnant women tested .in other study showed that Out of 155 pregnant women phenotyped for rhesus C antigen 40 (25.8%) out of 155 were positive,while 115 (74.2%) of the samples were negative.

Out of the 155 women tested, 44(28.4%) ofthe samples phenotyped were positive for Rh (E) whereas 111(71.6%) were negative

4.2 Conclusion:

- In group A pregnant ladies the highest frequency of Rhesus phenotyping was D antigen 52 (43.3%) and the lowest frequency of Rhesus phenotyping was E antigen 10 (8.3%).
- In group O pregnant ladies the highest frequency of Rhesus phenotyping was D antigen 54 (45%) and the lowest frequency of Rhesus phenotyping was E antigen 13 (10.8%).
- The antigen-c can be consider as protective factor of abortion,pregnant with –ve antigen-c are more susceptible to abortion when compare pregnant with +ve antigen-c.

4.3 Recommendations:

- Determination of Rhesus genotype and the most probable one.
- Rhesus typing should be considered in special cases such as pregnant women with previous transfused and known irregular antibodies.
- It is necessary to determine blood groups of different ethnic groups and geographical areas which is useful in Rhesus phenotyoing using large sample size.
- Determination of other bloodgroup system rather than that included in this study.

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