بسم الله الرحمن الرحيم

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Frequency of Red Blood Cells Alloimmunization among Multigravida Women

A thesis submitted for partial fulfillment of the degree of M.Sc. in medical Laboratory Sciences

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بسم الله الرحمن الرحيم

قال تعالى:

﴿ الَّذِينَ يَحْمِلُونَ الْعَرْشَ وَمَنْ حَوْلَهُ يُسَبِّحُونَ بِحَمْدِ رَبِّهِمْ وَيُؤْمِنُونَ بِهِ وَيَسْتَغْفِرُونَ لِلَّذِينَ آمَنُوا رَبَّنَا وَسِعْتَ كُلَّ شَيْءٍ رَّحْمَةً وَعِلْمًا فَاغْفِرْ لِلَّذِينَ تَابُوا وَاتَّبَعُوا سَبِيلَكَ وَقِهِمْ عَذَابَ الْجَحِيمِ ﴾

صدق الله العظيم

الآية (7) من سوره غافر

Dedication

I would like to dedicate this work to…..

My parents whose affection, love, encouragement and prays of day and night make me able to get such success and honor.

My husband, my lovely son, and my sisters.

First and finally complete thanks to Allah for all countless gifts , who asked us to thank him to give us more and more.

A lot of appreciation to my research supervisor

Dr. Mohammad Osman ALi,

For his support guidance and suggestions that benefited in the completion and success of this study.

I would like also to thank all the participants of this study who agreed to provide me with samples

List of Abbreviations

Abbreviation	Term
Abs	Antibodies
CDRs	Complementarity-determining regions
Fab	fragment, antigen-binding
Fc	Fragment, crystallizable
HDFN	Hemolytic Disease of Fetus and Newborn
IAT	Indirect Antiglobulin Test
Ig	Immunoglobulin
IgA	Immunoglobulin A
IgD	Immunoglobulin D
IgE	Immunoglobulin E
IgG	Immunoglobulin G
IgM	Immunoglobulin M
ISBT	International Society of Blood Transfusion
LMP	last menstrual period
RBCs	Red blood cells
Rh	Rhesus
SPSS	Statistical Package for Social Science

ملخــص البحث

أجريت هذه الدراسة الوصفية التحليلية في مدينة شندي في الفترة من يونيو وحتى أغسطس عام 2018م لرصد ظهور أجسام مضادة مكتسبة ضد المستضادات الموجودة على كريات الدم الحمراء في النساء السودانيات متعددات الولادة.هدفت هذه الدراسة إلى إيجاد تكرار تكوين الأجسام المضادة المكتسبة في 60 سيدة حامل .

تم جمع عينة مصل من كل سيدة حامل وأجري لها اختبار مسح الاجسام المضادة باستخدام الطريقة اليدوية .

تم جمع البيانات بواسطة استبيان يحتوى على بيانات العمر ،تكرار مرات الحمل، مراحل الحمل ووجود حالات إجهاض في السابق، من نساء حوامل متعددات الولادة تم تسجيلها.

النتائج: وجدت الأجسام المضادة المكتسبة في واحدة من النساء الحوامل متعددات الولادة(1.7%).أظهرت الدراسة عدم وجود ارتباط ذو دلالة إحصائية مابين العمر ،وعدد مرات الولادة ووجود الأجسام المضادة(P.value =0.601,0.818)على التوالي، كما أظهرت وجود ارتباط ذو دلالة إحصائية مابين وجود إجهاض ووجود الأجسام المضادة(P.value =0.037).

خلصت الدراسة إلى أهمية بإجراء مسح الأجسام المضادة لكل السيدات الحوامل.

وبذالك أوصى بعمل هذه الفحوصات لكل سيدة حامل في المراحل الأولى من الحمل.

Abstract

This study was descriptive, cross sectional study, carried out during the period of June to August 2018 in Shendi city, to determine the frequency of allo –antibodies against red cell antigen among multigravida women. The frequencies of alloantibodies in 60 multigravida women were studied. The samples were tested for antibody screening using manual methods. Data was collected using structured questionnaire for age, presence of abortion, number of pregnancies and stage of pregnancy from multigravida.

Results: Antibodies were detected in one multigravida women (1.7%).In this study there was no correlation between ages, number of pregnancies and alloimmunization with (P.value=0.601 and 0.818)respectively. Current study revealed that significant correlation between history of abortion and alloimmunization (P. value=0.037).

Conclusion: The study conclude that the frequency of alloantibodies were found in 1.7% of all pregnant women participated this study, it founds that there was relationship between alloimmunization with history of abortion, also it showed that there was no association between alloimmunization and age, number of pregnancy and stage of pregnancy.

This present study recommended that routine antibody screening should be performed for each pregnant woman during the early stage of pregnancy.

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Chapter One:

1-1: Introduction:

An antibodies (Abs), also known as an immunoglobulins (Ig), is a large, Y-shaped protein produced mainly by plasma cells that is used by the immune system to neutralize pathogens such as pathogenic bacteria and viruses.(1)

Serum containing antigen-specific antibodies is called antiserum. There are five classes of immunoglobulins including IgM, IgG, IgA, IgD, and IgE. The basic structures of all antibodies are same. There are four polypeptide chains held together by disulfide bonds. These four polypeptide chains form a symmetrical molecular structure.(2)

There are two identical halves with the antigen binding sites between the ends of the heavy and light chains on both sides. There is a hinge in the center between heavy chains to allow flexibility to the protein. The two light chains are identical to each other. There are two types of light chain among all classes of immunoglobulin, a lambda chain and a kappa chain. Both are similar in function. Each type of immunoglobulin has a different type of heavy chain. (2)

Alloimmunization is defined as an immune response to foreign antigens after exposure to genetically different cells or tissues. Although alloimmunization is a natural event during pregnancy, frequently it is the undesirable outcome of a blood transfusion and/or transplant.(3)

Red blood cell (RBC) alloantibodies can develop after exposure to foreign RBC antigens in the context of transfusion therapy or pregnancy/delivery.(3)

Pregnancy is the time from fertilization of an egg to birth. Getting pregnant and growing a human from scratch is very complicated biological process that takes a lot of resources. As a result pregnancy can have wide range of effects of the mother both physically and emotionally. Fertilized egg develops into atiny human embryo on the way .On reaching the uterus .The embryo implants itself in the uterine wall. Develops into a fetus and steadily grows

until about nine months later it is ready to emerge in to the outside world as anew born baby. [4]

Alloimmunization in pregnant women has been extensively studied in different areas of the world, with the frequency being found to range from 0.4% to 2.7% worldwide. [5]

1.2 Rationale:

Red cell immunization during pregnancy is a challenge that continues to task obstetricians and blood transfusionists even 50 years after the introduction of Rhesus (Rh) D prophylaxis. Anti-D prophylaxis had brightened the hopes that hemolytic disease of fetus and newborn (HDFN) due to D antigen incompatibility was in the last throes of life. However, we have reached the 21st century and the burden of alloimmunization in pregnancy is still on our backs. Apart from the D antigen, other blood group antigens of the Rh system (C, c, E, e, C^{w}) and other blood group systems have come into limelight. Universal screening of all antenatal women, including D antigen-positive pregnant ones, is highly debated and controversial, however screening and detection of clinically significant antibodies among antenatal women plays an important role in transfusion safety and preventing hemolytic disease of fetus and newborn.

Routine screening of antenatal women for antibodies is do not usually done in many transfusion centers in Sudan, so for this reason the immunization rates are un known in pregnant women.

1.3 Objectives:

1.3.1 General objective:

To detect erythrocyte alloimmunization among multigravida women in Shendi city.

1.3.2 Specific objective:

- To evaluate red cell alloimmunization among multigravida women attending Shendi town.
- To determine the frequency of red cell alloantibodies in multigravida women.
- To correlate the frequency of alloimmunization with the stages of pregnancy.
- To correlate history of abortion and alloimmunization.

Chapter two

Literature review

2.1.1 Structure and function of immunoglobulins:

Immunoglobulins are heterodimeric proteins composed of 2 heavy and 2 light chains. They can be separated functionally into variable domains that bind antigens and constant domains that specify effector functions, such as activation of complement or binding to Fc receptors. The variable domains are created by means of a complex series of gene rearrangement events and can then be subjected to somatic hyper mutation after exposure to antigen to allow affinity maturation. Each variable domain can be split into 3 regions of sequence variability termed the complementarity-determining regions (CDRs) and 4 regions of relatively constant sequence termed the framework regions. The 3 CDRs of the heavy chain are paired with the 3 CDRs of the light chain to form the antigen-binding site, as classically defined. The constant domains of the heavy chain can be switched to allow altered effector function while maintaining antigen specificity. There are 5 main classes of heavy chain constant domains. Each class defines the IgM, IgG, IgA, IgD, and IgE isotypes. IgG can be split into 4 subclasses, IgG1, IgG2, IgG3, and IgG4, each with its own biologic properties, and IgA can similarly be split into IgA1 and IgA2.[(6)]

Antibodies are heavy (~150 kDa) globular plasma proteins. The size of an antibody molecule is about 10 nm. They have sugar chains (glycans) added to conserved amino acid residues .In other words; antibodies are glycoproteins .The attached glycans are critically important to the structure and function of the antibody. Among other things the expressed glycans can modulate an antibody's affinity for its corresponding FcR(s). The basic functional unit of each antibody is an immunoglobulin (Ig) monomer (containing only one Ig unit); secreted antibodies can also be dimeric with two Ig units as with IgA, tetrameric with four Ig units like teleost fish IgM, or pentameric with five Ig units, like mammalian IgM. Several immunoglobulin domains make up the

two heavy chains (red and blue) and the two light chains (green and yellow) of an antibody. The immunoglobulin domains are composed of between 7 (for constant domains) and 9 (for variable domains) β-strands.The variable parts of an antibody are its V regions, and the constant part is its C region.[7]

2.1. 2 Immunoglobulin domains:

The Ig monomer is a "Y"-shaped molecule that consists of four polypeptide chains; two identical heavy chains and two identical light chains connected by disulfide bonds .Each chain is composed of structural domains called immunoglobulin domains. These domains contain about 70–110 amino acids and are classified into different categories (for example, variable or IgV, and constant or IgC) according to their size and function.[23] They have a characteristic immunoglobulin fold in which two beta sheets create a "sandwich" shape, held together by interactions between conserved cysteines and other charged amino acids.[7]

2.1.3 Heavy chain:

There are five types of mammalian Ig heavy chain denoted by the Greek letters: α, δ, ε, γ, and μ.[The type of heavy chain present defines the class of antibody; these chains are found in IgA, IgD, IgE, IgG, and IgM antibodies, Distinct heavy chains differ in size and composition; α and γ contain approximately 450 amino acids, whereas μ and ε have approximately 550 amino acids. Each heavy chain has two regions, the constant region and the variable region. The constant region is identical in all antibodies of the same isotype, but differs in antibodies of different isotypes. Heavy chains γ, α and δ have a constant region composed of three tandem (in a line) Ig domains, and a hinge region for added flexibility; heavy chains μ and ε have a constant region composed of four immunoglobulin domains .The variable region of the heavy chain differs in antibodies produced by different B cells, but is the same for all antibodies produced by a single B cell or B cell clone. The variable region of each heavy chain is approximately 110 amino acids long and is composed of a single Ig domain.[7]

2.1.4 Light chain:

In mammals there are two types of immunoglobulin light chain, which are called lambda (λ) and kappa (κ). A light chain has two successive domains: one constant domain and one variable domain. The approximate length of a light chain is 211 to 217 amino acids. Each antibody contains two light chains that are always identical; only one type of light chain, κ or λ, is present per antibody in mammals.(7)

2.1.5 Complementarity-determining regions (CDRs), fragment, antigen-binding(Fab) and Fragment, crystallizable(Fc) regions:

Some parts of an antibody have the same functions. The arms of the Y, for example, contain the sites that can bind to antigens (in general, identical) and, therefore, recognize specific foreign objects. This region of the antibody is called the Fab (fragment, antigen-binding) region. It is composed of one constant and one variable domain from each heavy and light chain of the antibody. The paratope is shaped at the amino terminal end of the antibody monomer by the variable domains from the heavy and light chains. The variable domain is also referred to as the FV region and is the most important region for binding to antigens. To be specific, variable loops of β-strands, there each on the light (VL) and heavy (VH) chains are responsible for binding to the antigen. These loops are referred to as the complementarity determining regions (CDRs).In the framework of the immune network theory, CDRs are also called idiotypes. According to immune network theory, the adaptive immune system is regulated by interactions between idiotypes.(7)

The base of the Y plays a role in modulating immune cell activity. This region is called the Fc (Fragment, crystallizable) region, and is composed of two heavy chains that contribute two or three constant domains depending on the class of the antibody. Thus, the Fc region ensures that each antibody generates an appropriate immune response for a given antigen, by binding to a specific class of Fc receptors, and other immune molecules, such as complement proteins. By doing this, it mediates different physiological

effects including recognition of opsonized particles (binding to FcγR), lysis of cells (binding to complement), and degranulation of mast cells, basophils, and eosinophil's (binding to FcεR).

In summary, the Fab region of the antibody determines antigen specificity while the Fc region of the antibody determines the antibody's class effect. Since only the constant domains of the heavy chains make up the Fc region of an antibody, the classes of heavy chain in antibodies determine their class effects. Possible classes of heavy chains in antibodies include alpha, gamma, delta, epsilon, and mu, and they define the antibody's isotypes IgA, G, D, E, and M, respectively. This infers different isotypes of antibodies have different class effects due to their different Fc regions binding and activating different types of receptors. Possible class effects of antibodies include: Opsonization, agglutination, hemolysis, complement activation, mast cell degranulation, and neutralization (though this class effect may be mediated by the Fab region rather than the Fc region). It also implies that Fab-mediated effects are directed at microbes or toxins, whilst Fc mediated effects are directed at effector cells or effector molecules. [7]

The main categories of antibody action include the following :

- Neutralization, in which neutralizing antibodies block parts of the surface of a bacterial cell or virion to render its attack ineffective

-Agglutination, in which antibodies "glue together" foreign cells into clumps that are attractive targets for phagocytosis.

-Precipitation, in which antibodies "glue together" serum-soluble antigens, forcing them to precipitate out of solution in clumps that are attractive targets for phagocytosis.

Complement activation (fixation), in which antibodies that are latched onto a foreign cell encourage complement to attack it with a membrane attack complex, which leads to the following: Lysis of the foreign cell or encouragement of inflammation by chemotactically attracting inflammatory cells.[7]

2.2.1 Red blood cells:

Red blood cells-- also known as RBCs, red cells, red blood corpuscles, haematids, erythroid cells or erythrocytes (from Greek erythros for "red" and kytos for "hollow vessel", with -cyte translated as "cell" in modern usage), are the most common type of blood cell and the vertebrate's principal means of delivering oxygen (O_2) to the body tissues—via blood flow through the circulatory system. RBCs take up oxygen in the lungs, or gills of fish, and release it into tissues while squeezing through the body's capillaries.[8]

The cytoplasm of erythrocytes is rich in hemoglobin, an iron-containing biomolecule that can bind oxygen and is responsible for the red color of the cells. The cell membrane is composed of proteins and lipids, and this structure provides properties essential for physiological cell function such as deformability and stability while traversing the circulatory system and specifically the capillary network.[8]

In humans, mature red blood cells are flexible and oval biconcave disks. They lack a cell nucleus and most organelles, in order to accommodate maximum space for hemoglobin; they can be viewed as sacks of hemoglobin, with a plasma membrane as the sack. Approximately 2.4 million new erythrocytes are produced per second in human adults. The cells develop in the bone marrow and circulate for about 100–120 days in the body before their components are recycled by macrophages. Each circulation takes about 60 seconds (one minute). Approximately a quarter of the cells in the human body are red blood cells. Nearly half of the blood's volume (40% to 45%) is red blood cells.**[8]**

2.2.2 Red blood cells antigens:

Since Landsteiner’s discovery in 1901, that human blood groups existed, a vast body of serological, genetic and biochemical data on red cell (blood group) antigens has been accumulated. More recently, the biological functions of some of these antigens have been appreciated. Each system is a series of red cell antigens determined either by a single genetic locus or very

closely linked loci. In addition to the blood group systems, there are six ‘collections’ of antigens (e.g. Cost), which bring together other genetically, biochemically or serologically related sets of antigens and a separate series of low frequency (e.g. Rd) and high-frequency (e.g. Vel) antigens which do not fit into any system or collection. A numeric catalogue of red cell antigens is being maintained by an International Society of Blood Transfusion (ISBT) working party. (

Apart from those of the ABO system, most of these antigens were detected by antibodies stimulated by transfusion or pregnancy. Alternative forms of a gene coding for red cell antigens at a particular locus are called alleles and individuals may inherit identical or non-identical alleles. Most blood group genes have been assigned to specific chromosomes (e.g. ABO system on chromosome 9, Rh system on chromosome 1). The term genotype is used for the sum of the inherited alleles of a particular gene (e.g. AA, AO) and most red cell genes are expressed as codominant antigens (i.e. both genes are expressed in the heterozygote). The phenotype refers to the recognizable product of the alleles.[9]

Red cell antigens are determined either by carbohydrate structures or protein structures. Carbohydrate-defined anti genes are indirect gene products (e.g. ABO, Lewis, and P). The genes code for an intermediate product, usually an enzyme that creates the antigenic specificity by transferring sugar molecules onto the protein or lipid. Protein-defined anti genes are direct gene products and the specificity is determined by the inherited amino acid sequence and/or the conformation of the protein. Proteins carrying red cell anti genes are inserted into the membrane in one of three ways: single pass, multi pass or linked to phosphatidylinositol (GPI-linked). Only a few red cell antigens are erythroid specific (Rh, LW, Kell and MNSs), the remainder being expressed in many other tissues. However, the main clinical importance of a blood group system depends on the capacity of alloantibodies (directed against the antigens not possessed by the individual) to cause destruction of transfused

red cells or to cross the placenta and give rise to hemolytic disease in the fetus or newborn. This in turn depends on the frequency of the antigens and the alloantibodies and the characteristics of the latter: thermal range, immunoglobulin class and ability to fix complement .[9]

On these criteria, the ABO and Rh systems are of major clinical importance. Anti-A and anti-B are naturally occurring and are capable of causing severe intra- vascular hemolysis after an incompatible transfusion. The RhD antigen is the most immunogenic red cell antigen after A and B, being capable of stimulating anti-D production after transfusion or pregnancy in the majority of RhD negative individuals.[9]

2.2.3 Blood Group Antibodies:

The discovery of almost universally present naturally occurring antibodies in blood plasma led to the discovery of the ABO blood group system which remains, more than 100 years later, the most important and clinically significant of all blood groups. Blood group antibodies play an important role in transfusion medicine; both in relation to the practice of blood transfusion and in pregnancy, but not all are clinically significant. Clinically significant antibodies are capable of causing adverse events following transfusion, ranging from mild to severe, and of causing hemolytic disease of the fetus and newborn following placental transfer from mother to fetus. Assessing the clinical significance of antibodies relies heavily on mode of reactivity and historical data relating to specificity; functional assays are sometimes employed. The principals of methodology for blood typing and antibody identification have changed little over the years, relying mainly on serological methods involving red cell agglutination.[10]

2.3.1 Pregnancy:

The state of carrying a developing embryo or fetus within the female body. This condition can be indicated by positive results on an over-the-counter urine test, and confirmed through a blood test, ultrasound, detection of fetal

heartbeat, or an X-ray. Pregnancy lasts for about nine months, measured from the date of the woman's last menstrual period (LMP). [11]

Pregnancy is typically divided into three trimesters. The first trimester is from week one through 12 and includes conception, which is when the sperm fertilizes the egg. The fertilized egg then travels down the fallopian tube and attaches to the inside of the uterus, where it begins to form the embryo and placenta. During the first trimester, the possibility of miscarriage (natural death of embryo or fetus) is at its highest. The second trimester is from week 13 through 28. Around the middle of the second trimester, movement of the fetus may be felt. At 28 weeks, more than 90% of babies can survive outside of the uterus if provided with high-quality medical care. The third trimester is from 29 weeks through 40 weeks.[11]

2.3.2 Physiological changes in pregnancy:

Every maternal organ adapts to pregnancy, each at a different time and in a different way. Maternal systems adapt as pregnancy progresses to accommodate the increasing demands of fetal growth and development. Management of both healthy and diseased pregnancy necessitates knowledge of the physiology of normal pregnancy. Understanding these adaptations enable clinicians to identify abnormal changes that lead to complications, as well as recognize changes that mimic disease, and understand altered responses to stress.[12]

In early pregnancy, the developing fetus, corpus luteum and placenta produce and release increasing quantities of hormones, growth factors and other substances into the maternal circulation. This triggers a cascade of events that transform the mother’s cardiovascular, respiratory and renal systems. The first trimester of pregnancy is therefore a transition period between the pregnant and non-pregnant state, during which changes in all these systems take place to prepare the mother to support fetal growth. Most pregnant women report symptoms of pregnancy by the end of the sixth week after the last menstrual period. It is assumed that most physiological adaptations are

completed during the first trimester, although studies examining early pregnancy physiological changes are limited, with few longitudinal measurements prior to conception and throughout the first trimester. (12)

Following implantation, the maternal adaptation to pregnancy can be categorized based on the following functions:

1. increased availability of precursors for hormone production and fetal–placental metabolism؛
2. improved transport capacity؛
3. maternal–fetal exchange
4 . removal of additional waste products.

Increased availability of metabolic substrates and hormones is achieved by increases in dietary intake, as well as endocrine changes that increase the availability of substrates like glucose. Transport capacity is enhanced by increases in cardiac output, facilitating both the transport of substrates to the placenta, and fetal waste products to maternal organs for disposal.

The placenta regulates maternal–fetal exchange by10-12weeks gestation, but transfer occurs through other mechanisms before this. Disposal of waste products (heat, carbon dioxide and metabolic by products) occurs through peripheral vasodilatation and by increases in ventilation and renal filtration.(12)

Many physiological changes occur with normal pregnancy and these changes impact every organ system, affecting both structure and function. Most are advantageous and allow the mother to cope with the increased physical and metabolic demands of the pregnancy. Some have important clinical implications: adjusting normal measurements and values, mimicking disease or altering responses to trauma and stress. (12)

2.3.3 Multigravida:

Multigravida is medical terminology for a woman who has been pregnant more than once, regardless of pregnancy outcome. (13)

2.3.4 Red Blood Cells Alloantibodies in Pregnancy:

Red blood cell alloantibodies are the unexpected immune- antibodies found other than the naturally occurring antibodies in the body, produced in response to the introduction of red cells possessing antigens that the subject lacks, as in cases of pregnancy, transfusion, transplantation or injection of any immunogenic material. In pregnancy alloantibodies appear when fetal RBCs carrying a paternal antigen that is foreign to the mother, enters the maternal circulation and this incompatibility of blood groups between the mother and fetus can lead to Alloimmune Haemolytic Disease of the newborn .[14]

Antibody screening is done in pregnancy to identify these pregnancies which are at risk of fetal and neonatal hemolytic disease resulting from clinically significant maternal alloantibodies [14].

Maternal alloimmunization, also known as isoimmunization, occurs when a woman's immune system is sensitized to foreign erythrocyte surface antigens, stimulating the production of immunoglobulin G (IgG) antibodies. The most common routes of maternal sensitization are via blood transfusion or fetomaternal hemorrhage (ie, transplacental passage of fetal erythrocytes) associated with delivery, trauma, spontaneous or induced abortion, ectopic pregnancy, or invasive obstetric procedures. Two recent studies found that intravenous drug abuse is also associated with alloimmunization. These antibodies can cross the placenta during pregnancies in alloimmunized women and, if the fetus is positive for these specific erythrocyte surface antigens, result in hemolysis of fetal erythrocytes and anemia.[15]

Most developed countries have guidelines for screening all pregnant women for irregular erythrocyte antibodies. According to the guidelines of the British Committee for Standards in Haematology , all pregnant women should be ABO and D antigen typed and

screened for the presence of red cell antibodies early in pregnancy and at the 28th week of gestation.According to guidelines in The Netherlands, it has been mandatory since 1998 to screen all pregnant women for the presence of irregular antibodies in the first trimester of pregnancy. However, no such guidelines are followed in developing countries.[16]

2.3.5 The aetiology of Rhesus disease:

The Rhesus system is coded on two adjacent genes ،which sit within chromosome one. One gene codes for antigen polypeptides C/c and E/e while the other codes for the D polypeptide (Rhesus antigen). Note that the d (little d) antigen has not been identified so it may be that women who are D negative lack the antigen altogether, as opposed to those with c (little c)or e (little e), where c is the allelic antigen of C and e is the allelic antigen of E. Antigen expression is usually dominant, whereas those who have a negative phenotype are either homozygous for the recessive allele or have a deletion of that gene .[12]

In practice, only anti-D and anti-c regularly cause HDFN. Anti-D is much more common than anti-c and is therefore the focus of this discussion.Occurrence of HDFN as a result of Rhesus isoimmunization involves three key stages a Rhesus negative mother must conceive a baby who has inherited the Rhesus positive phenotype from the father. Second, fetal cells must gain access to the maternal circulation in a sufficient volume to provoke a maternal antibody response. Finally،maternal antibodies must gain transplacental access and cause immune destruction of red cells in the fetus. Rhesus disease does not affect a first pregnancy as the primary response is usually weak and consists primarily of IgM antibodies that do not cross the placenta. Thereafter IgG antibodies are produced and these can cross the placenta. Rhesus antigens are well expressed by the fetus from as early as 30 days gestation so in a subsequent pregnancy،when maternal resensitization occurs (Rhesus positive red cells pass from the baby to the maternal

circulation, IgG antibodies cross from the mother to the fetal circulation. If these antibodies are present in sufficient quantities, fetal hemolysis may occur, leading to such severe anemia that the fetus may die unless a transfusion is performed. It is for this reason that Rhesus-negative women have frequent antibody checks in pregnancy; an increasing titer of atypical antibodies may suggest an impending problem.[12]

Potential sensitizing events for Rhesus disease:

• Miscarriage

• Termination of pregnancy.

• Antepartum hemorrhage.

• Invasive prenatal testing (chorionic villus sampling، Amniocentesis and cordocentesis).

• Delivery

Preventing Rhesus isoimmunization, the process of isoimmunization can be 'nipped in the bud' by the intramuscular administration of anti-D immunoglobulins to a mother, preferably within 72 hours of exposure to fetal red cells.Anti-D immunoglobulins 'mop up' any circulating rhesus-positive cells before an immune response is excited in the mother. The practical implications of this are that anti-D immunoglobulin must be given intramuscularly as soon as possible after any potentially sensitizing event. It is normal practice to administer anti-D after any of these events; the exact dose is determined by the gestation at which sensitization has occurred and the size of the fetomaternal hemorrhage. In the first trimester of pregnancy, because the volume of fetal blood is so small, it is unlikely that sensitization would occur, and a 'standard'dose of anti-D (the exact dose varies from country to country) is given; this will more than cover even the largest feto-maternal transfusion. In the second and third trimesters, fetal blood volume is greater and because there is a possibility of a fetomaternal transfusion of several millilitres, a larger dose is given and a Kleihauer test performed. A Kleihauer is a test of maternal blood to determine the proportion of fetal cells

present (relying on their ability to resist denaturation by alcohol or acid); it will allow calculation of the amount of extra anti-D immunoglobulin required should a large transfusion have occurred.(12)

In many countries, Rhesus-negative women are given anti-D at 28 and/or 34 weeks routinely. This is based on the finding that a small number of Rhesus negative women become sensitized during pregnancy despite the administration of anti-D at delivery and without a clinically obvious sensitizing event. The likelihood is that a small feto-maternal hemorrhage occurs without any obvious clinical signs; therefore، prophylactic anti-D would reduce the risk of isoimmunization from this event.(12)

2.3.6 The management of Rhesus disease in a sensitized woman:

Once a woman who is D Rhesus negative has been sensitized to the D Rhesus antigen, no amount of anti-D will ever turn the clock back. In a subsequent pregnancy, close surveillance is required. Rhesus disease gets worse with successive pregnancies, so it is important to note the severity of the disease in previous pregnancies. The management depends on the clinical scenario:

•The father of the next baby is D Rhesus negative.In this situation, there is no risk that the baby will be D Rhesus positive and therefore there is no chance of Rhesus disease .The father of the next baby is D Rhesus positive.He may be heterozygous and in this situation determining the paternal phenotype is useful in anticipating the likely fetal phenotype and, thus،the potential for development of HDFN. However،it is important to bear in mind that there are issues regarding paternal testing, and assuming paternity runs the risk of false prediction. Not with standing this issue, paternal blood grouping is frequently used and often useful .(12)

•In a sensitized woman, if the father is D Rhesus positive or unknown, standard management involves monitoring antibody levels every 2–4 weeks from booking. Antibody levels or quantity can be described using the titre or by using IU (international units) as a standard quantification method. The titre

simply refers to the number of times a sample has been diluted before the amount of antibody becomes undetectable; titre of 2, 4, 8, 16, 32, 64, 128, etc.Each time a sample is tested, it should be checked in parallel with the previous sample to ensure the detection of significant changes in the antibody15 IU/mL High risk of hydrops fetalis.[12]

•If antibody levels rise, the baby should be examined for signs of anemia. In the past، the bilirubin concentration of amniotic fluid was determined optically to give an indirect measure of fetal hemolysis. It has been found that titrations of anti-D do not correlate well with the development of HDFN, and that the standard quantification method (IU/mL) gives more clinically relevant levels. Anti-D level outcome 4 IU/mL HDFN unlikely 4-15 IU/mL. Moderate risk of HDFN invasive procedure with the attendant risks of miscarriage/preterm labour and further boosting of the alloimmune response.[12]

HDFN is a condition caused by maternal antibodies to fetal red cell antigens which cross the placenta and cause hemolysis in fetus. The sensitizing event causing alloimmunization is frequently a previous pregnancy or a transfusion, where the mother was exposed to the relevant antigen. HDFN due to alloimmunization shows wide spectrum of severity; some may have only mild jaundice on first day of life, but rapid fall of hemoglobin than other newborn infants. In others jaundice develops more rapidly, unless treated by exchange transfusion may lead to kernicterus and permanent brain damage. With a still more severe hemolytic process, profound anemia develops and the infant may die in utero at any time from about seventh week of gestation onwards.[17]

ABO blood group isoimmunization may occur when the mother is blood group O and the baby is blood group A or B. Anti-A and anti-B antibodies are present in the maternal circulation naturally, usually secondary to sensitization against A or B substances in food or bacteria. This means that ABO incompatibility may occur in a first pregnancy. In this situation, anti-A or anti-B antibodies may pass to the fetal circulation ،causing fetal hemolysis

and anemia. However, most anti-A and anti-B are mainly IgM and do not cross the placenta. In addition, A and B antigens are not fully developed in the fetus. Therefore ABO incompatibility generally causes only mild hemolytic disease of the baby, but may sometimes explain unexpected jaundice in an otherwise healthy term infant.[12]

2.4 Previous study:

A prospective cross-sectional study done by Suresh B *et al* was carried out on 2060 multiparous pregnant women attending the Government Maternity Hospital, Tirupati to detect prevalence of unexpected antibodies. The women were grouped and typed for ABO and rhesus (Rh) D antigens by tube method and screened for alloantibodies by column agglutination technology. The medical and detailed obstetric histories of these women were reviewed. They found that the overall prevalence of alloantibodies were 1.1%. There was a statistically significant difference between alloimmunization rates in the Rh D-antigen negative and D-antigen positive women (12.8% versus 0.3%). The antibodies detected in this study were, anti-D (63.8%), anti-D+C (13.7%), anti-C, anti-E, anti-M, anti-Le^a, and anti-Le^b (4.5% each). Anti-D contributed to 77.3% of total alloimmunization in this study. They concluded that: in spite of the introduction of prophylactic Rh- immunoglobulin, anti-D (77.3%) is still a common antibody identified in the antenatal women of our region. In developing countries like India, universal antenatal antibody screening, though desirable may not be justified at present as the cost and infrastructure required would be immense. However, it is necessary to impose properly formulated protocols to screen at least the pregnant women with adverse obstetric history. [17]

A cross-sectional study done by Mohd, Nazri Hassan, *et al* was carried out on a total of 5163 Malay pregnant women who attended labor room, from January to December 2009 were included in this study. The blood samples were subjected to the standard immunohematological procedure for ABO and Rhesus grouping and RBC antibody screening and identification. The T-test,

Pearson's Chi-square and Fisher's exact test were used for statistical analysis. They found Fifty one (0.99%) pregnant women were found to have RBC alloantibodies and when the specificities were further characterized, majorities are towards Rhesus and Lewis system. Most (66.7%) of the subjects had single alloantibody whereas 25.5 % of them had multiple alloantibodies. Among the single alloantibody, anti-E is the commonest whereas among multiple alloantibodies anti-Le",-Le^b are the commonest. There was significant association between RBC alloimmunization and Rhesus D blood group, miscarriage, preterm labor, ante partum hemorrhage, intrauterine death and blood transfusion.They concluded that in: Considering the low prevalence of RBC alloantibodies in Malay pregnant women, thus routine antenatal RBC antibody screening practice might not be advised as a routine practice at present. It is advisable to do antibody screening only for those at high risk of developing such antibodies. (18)

In this prospective study done by Sangeeta Pahuja, *et al* ,was carried out to detect the prevalence of alloantibodies among multigravida women in India, 3,577 multigravida women attending antenatal clinics were typed for ABO and D antigens and screened for alloantibodies by column agglutination technology. The medical history and detailed obstetric history of these women were reviewed and information recorded on any prior hemolytic disease of the fetus and newborn among siblings and/or blood transfusions.They found the overall prevalence of alloantibodies in this study was 1.25%. There was a statistically significant difference between alloimmunization rates in the D antigen-negative and D antigen-positive groups (10.7% versus 0.12%, respectively). Anti-D antibody contributed to 78.4% of total alloimmunizations in our study.(19)

This study was carried out in Khartoum State by Khartoum by Afra H and Mohammed, during the period from April-2012 to May-2012 to test alloimmunization against red blood cells among Sudanese multi-parous women. The aim of this study was to detect the frequency and type of

alloantibodies in randomly selected 80 pregnant women; all of them were attending Al-Turki Teaching Hospital during this period. Blood Samples were collected from pregnant women. Each sample was ABO Rh (D) grouped and screened for alloantibodies, then samples that gave positive antibody screening were tested for antibody identification using gel agglutination method. Red cell alloantibodies were found in 8 cases (10%). The identified antibodies 2(25.0%), anti-C^w 1(12.5%). Also were anti-Kell 1(12.5%), the results anti-S was 3(37.5%), 1(12.5%) revealed that, and no anti- E anti-Lea significant relationship was found between age and presence of alloantibodies with P.value (0.097). Also insignificant relationship was found between history of red cell transfusion and alloimmunization with P.value (0.556), thus may be for the small number of tested women. A significant relationship was observed between history of abortion, number of pregnancies and presence of alloantibodies with a P.values (0.027, 0.002) respectively.This study recommended that the importance of performing and identification of antibody screening in Sudan for all pregnant women. [20].

Another retrospective study conducted in turkey in 2013 enrolled 4840 pregnant women there were 4097 D antigen-positive women (84.65%) and 743 women with D-antigen-negative phenotype (15.35%). The prevalence of alloimmunization was found to be 8.74% in D-antigen negative group. (Anti-D antibodies represent 68.57% and non-D antibody 31.42%).[21]

In this nationwide population study in Iceland, the overall prevalence of RBC alloimmunization in pregnancy was 1.04%. This population prevalence is similar to that reported in the Netherlands, where the prevalence of positive antibody screens at first-trimester screening was 1.23%, and in Australia, which has an immunization rate of 0.73% of pregnancies; however, it is considerably higher than the prevalence reported in a Swedish study, with 0.48% prevalence, and in a Canadian study, with 0.36% prevalence. This difference in immunization frequency between studies is not surprising, because antibody prevalence rates are known to vary between countries,

probably due to variations in transfusion practices, testing techniques, and gene frequencies. Furthermore, lower immunization rates are reported with protocols that include antenatal RhIg dosing. [22]

This study was carried out in Khartoum State in Sa'ad Abu-Ela University Hospital by Abdiwahab, total of 70 pregnant women were investigated for Rhesus phenotyping and Rh alloimmunization, 8(11.4%) cases were positive for alloimmunization , There was no significant correlation between history of abortion and alloimmunization with P. value (0.102). There was no significant correlation between number of pregnancy and alloimmunization, with P. value (0.173). [23]

Chapter three

Materials and Method

3.1 Study design:

This descriptive analytical prospective cross sectional study was conducted in Shendi hospital, and aimed to detect the presence of irregular antibody in multigravida women .

3.2 Data collection:

Data was collected by using structural interviewing questionnaire.

3.3 Inclusion criteria:

Pregnant woman with past history of blood transfusion.

3.4 Data analysis:

Data was analyzed by statistic package for social science program (SPSS) and presented in form of tables.

3.5. Ethical consideration:

The permission for collection of sample taken verbally from the patients.

3.6 Study population:

A total of 60 venous blood samples were collected from multigravida women with age ranged between (20- 40 years) old in Shendi hospital.

3.7 Study area:

Shendi is located in the south of the River Nile on the sandy plan is just around the Nile River of the north and North West and beyond ,located in the River Nile State.

Shendi is about 100 miles to the north of Khartoum city and 23 miles to the south from the old city of Almosawarat. In the west is surrounded by the River Nile and Elmatmma city. Now there is a new bridge connects the two cities together across the river .

Shendi is the center of one of the largest Arab tribes in Sudan and the majority is Aljalia tribe, also include a group of Arab and Nubian cultures, it has a very basic services and infrastructure and it's location make it a center for trade in agriculture goods from nearby farms.

A University exists within the city from 1990 and draws students from across Sudan to study there, the University consists of -:

(1) Faculty of medicine and health studies which include .

•Medicine and surgery.

•Medical laboratory.

•Higher nursing.

•Public health .

(2) Faculty of law .

(3) Faculty of art .

(4) Faculty of education .

(5) Faculty of community development .

(6) Faculty of economics.

(7) Faculty of sciences and technology .

There are also three hospitals within the city:

-Almek Nimer university hospital.

-Shendi teaching hospital .

-The military hospital

3.4. Methods:

3.4.1 .Sample collection:

Ten milliliters (10 ml) of blood were withdrawn from antecubital vein of each patient after cleaning the patient skin with 70% alcohol and applying of the tourniquet above the vein a puncture site, using sterile non biogenic disposable syringe in a red tope blood container (No anticoagulant), then the serum was separated immediately by centrifugation after clot formation.

3.4.2. Equipment, and supplies:

•12 x 72 mm test tubes

•Plastic test tube holder

•Screening Cells I & II, and III

•Indelible marking pen

•Large bore dispose pipettes

•Anti-Human Globulin (Coombs serum)

•37^0C water bath

•Centrifuge

•Wash bottle with physiologic saline

3.4.3. Principle :

The patient's serum was tested for the presence of clinically significant antibodies using an indirect antiglobulin method. The serum was tested against un pooled Group O cells selected to possess the relevant blood group antigens.

3.4.4 Procedure:

•The patient information on the sample were verified to matches information on the worksheet .

•The samples were centrifuged and separated the serum to a labeled tube.

•tubes were labeled 1,2,3

•Two drops of patient serum was added to all tubes by using large bore pipette.

•one drop of Screening Cell I was added to tube I; one drop Screening Cell II was added to the II tube; one drop Screening Cell III was added to the III tube.

•all the tubes were incubated in water bath at 37°C for 45 minutes.

•all tubes were shaked , then washed 3 times, decanted well after each wash, mixed the cell button well between washes, and blotted the last drop of saline after the final wash .

•Immediately one drop of AHG was added to each tube, shaked to mix, and centrifuged .

•Immediately resuspend gently and checked macroscopically for agglutination .

•The results were read and recorded.

Chapter Four

Results

This descriptive analytical hospital based study was conducted in Shendi city, aimed to determine the frequency RBCs alloimmunization in multigravida women.

According to the table (4-1) RBCs alloantibodies was positive in (1/60) (1.7%) pregnant women, while (59/60) (98.3%) of pregnant women showed negative result .

Pregnant women that with age of (20 – 29 years) represent (46.7%), while (50%) of pregnant women with age of (30 - 39 years) and (3.3%) of pregnant women above (40 years) as demonstrated in table (4-2).

Regarding number of pregnancy, table (4-3) showed that pregnant women that have (1-3 pregnancies) was (11.7%), while (71.7%) of pregnant women have (4-6 pregnancies) and (16.7%) of them have more than (6 pregnancies).

Also according to table (4 - 4), (21.7%) multigravida women with once time history of abortion, and (13.3%) twice time of abortion.

About (1.7%) of pregnant women was in first trimester, while (40%) was in second trimester, and (58.3%) of them was in third trimester as noted in table (4 - 5).

Table (4 - 6) reveals no relationship between age and alloimmunization screening in pregnant women (P.value ≥ 0.05).

While table (4-7) showed a significant relationship between number of abortion and alloimmunization screening in pregnant women (P.value ≤ 0.05).

Finally table (4 - 8) reveals no relationship between number of pregnancy and alloimmunization screening in pregnant women (P.value ≥ 0.05).

Table (4.1) Antibody screening in pregnant women:

Antibody screening	Frequencies	Percentage
Positive	1	1.7%
Negative	59	98.3%
Total	60	100%

Table (4.2): Shows distribution of age among study group:

Age groups	Frequencies	Percentage
20 – 29 years	28	46.7%
30 – 39 years	30	50.0%
40 – 49 years	2	3.3%
Total	60	100%

Table (4.3): Shows number of pregnancy among test group:

No of pregnancy	Frequencies	Percentage
1 – 3	7	11.7%
4 – 6	43	71.7%
> 6	10	16.7%
Total	60	100%

Table (4.4): Shows number of abortion among study group:

No of abortion	Frequencies	Percentage
Once	13	21.7%
Twice	8	13.3%
No	39	65.0%
Total	60	100%

Table (4.5): Show distribution of study group according to stage of pregnancy:

Age groups	Frequencies	Percentage
First trimester	1	1.7%
Second trimester	24	40.0%
Third trimester	35	58.3%
Total	60	100%

Table (4.6): Relationship between age and alloimmunization screening in pregnant women:

Result of Abs screening		Age groups			Total	P.value
		20-29	30-39	40-49		
Positive	Count	0	1	0	1	
	% within Age groups	.0%	3.3%	.0%	1.7%	0.601
Negative	Count	28	29	2	59	
	% within Age groups	100.0%	96.7%	100.0%	98.3%	
Total	Count	28	30	2	60	
	% within Age groups	100.0%	100.0%	100.0%	100.0%	

Table (4.7): relationship between frequency of abortion and alloimmunization screening in pregnant women:

Result of Abs screening		No of abortion			Total	P value
		Once	Twice	No		
Positive	Count	0	1	0	1	0.037
	% within frequency of abortion	.0%	12.5%	.0%	1.7%	
Negative	Count	13	7	39	59	
	% within frequency of abortion	100.0%	87.5%	100.0%	98.3%	
Total	Count	13	8	39	60	
	% within frequency of abortion	100.0%	100.0%	100.0%	100.0%	

Table (4.8): relationship between number of pregnancy and alloimmunization screening in test group:

Result of Abs screening		No of pregnancy			Total	P value
		1-3	4-6	More than 6		
Positive	Count	0	1	0	1	
	% within No of pregnancy	.0%	2.3%	.0%	1.7%	0.818
Negative	Count	7	42	10	59	
	% within No of pregnancy	100.0%	97.7%	100.0%	98.3%	
Total	Count	7	43	10	60	
	% within No of pregnancy	100.0%	100.0%	100.0%	100.0%	

Chapter Five

5.1 Discussion

The objective of the current was to detect of RBCs alloimmunization and frequencies in pregnant women which is an important issue to minimize the complications of alloimmunization in pregnancy. It will also aid the development of screening and preventive management programs based on the calculated prevalence. Alloimmunization has been a focus of concern for obstetricians and hematologists for centuries in their quest to try and eliminate HDFN as a common obstetric problem; in this study the focus was screen the presence of unexpected alloantibodies in pregnant women.

This study observed that the frequency of alloantibodies in the study population (1.7%), this finding was similar to the result of study which done in India by Sangeeta Pahuja, *et al* .(1.25%). [18], it is also considerably higher than the prevalence reported in a Swedish study, with (0.48%) prevalence, and in a Canadian study, with (0.36%)[22] prevalence. This difference in immunization frequency between these studies was not surprising, because antibody prevalence rates were known to vary between countries, probably due to variations in transfusion practices, testing techniques, and gene frequencies. Furthermore, lower immunization rates were reported with protocols that include antenatal RhIg dosing.[22]

There was no significant relationship between number of pregnancy and alloimmunization, with P.value (0.818),this result was agree with previous study conducted in Khartoum by Abdiwahab I with P.value (0.137). [23]

An insignificant relationship observed between alloimmunization and number of pregnancy with P.value of (0.818). This disagree with previous study conducted in Khartoum by Afra H and Mohammed [19] with P.value of (0.002).

Regarding relationship between abortion and alloimmunization, the result of this study reveals that there was significant association with P.value of (0.037) this result agree with that of study done by Afra H and Mohammed[19]

5.2 Conclusion:

This present study concluded that:

- The frequency of alloantibodies were found in 1.7% of all pregnant women participated this study.
- The present study found that there was a relationship between alloimmunization with history of abortion.
- The present study also showed that there was no association between alloimmunization and age, number of pregnancy and stage of pregnancy.

5.3 Recommendations:

This present study recommended that:

- Routine antibody screening should perform for each pregnant woman during the early stage of pregnancy.
- Further studies are important to be conducted in order to specify type of antibodies which is common cause.
- Large studies regarding pregnancy and alloimmunization should be done with large sample size.

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Appendix (I)

Questionnaire

Shendi University

Faculty of medical laboratory science

College of graduate studies

Hematology department

Frequency of RBCs Alloimmunization among Multigravida Women

Age ……

Pregnant trimester first ( ) second ( ) Third( )

Number of pregnancies 1 ( ) 2 ( ) 3 ( ) 4 ( ) 5( )

more than 6( )

History of abortion Yes ( ) NO ( )

If yes how many abortions ..... ( )

Appendix II

إقـرار بالمـــوافقة

الاسم.....

العمر..... العنوان.....

أوافق بمحض إرادتي بالمشاركة في البحث العلمي المتعلق بدراسة مسح الأجسام المضادة عند السيدات متعددات الحمل في مدينة شندي.

اسم الباحث:

معالي الحاج عبد الله حاج احمد

بعد أن شرحت لـه بأنـه لا يترتب عليـه أي أذى جسدي أو نفسـي واعلم أن المشاركة فـي هذا البحث لن تؤثر بأي حال من الأحوال في الرعاية الطبية التي أتلقاها كما انـه يحق لي بدون إبداء أسباب الانسحاب من هذا البحث في أي مرحلة من مراحله.

البحث بإشراف:

د. محمد عثمان على محمد

التوقيع..... التاريخ.....