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DISTRIBUTION OF RHESUS ANTIGENS (D,C, C, E, E) AMONG SUDANESE BLOOD DONORS ATTENDING CENTRAL BLOOD BANK IN WAD MADANI, GEZIRA STATE, SUDAN (2014-2015)

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ABSTRACT

Objective: To determine Rhesus antigens (D, C, c, E, e) distribution among Sudanese blood donors attending Central Blood Bank in Wad Madani, Gezira State, Sudan and to minimize Rhesus allo-immunization among blood recipients. **Material and Methods:** 2.5 ml of blood was collected from 132 blood donors under a septic condition in Ethylenediamine tetra-acetic acid (EDTA) containers, mixed well and labeled, ABO and Rhesus typing were performed using the classical slide method. **Results:** The study revealed that frequency percentage of ABO blood phenotypes in the total samples were as follows: O (55%), A (23%), B (20%) and AB (2%); whereas the frequency percentage of Rh antigens were e (100%), c (93%), D (92%), C(43%), E (16%). **Conclusion:** The study revealed that Rh e antigen had highest prevalence in all the study population while Rh E antigen is the lowest one.

KEYWORDS: ABO blood group, Rhesus factor (Rh), Rh D, Rh E, Rh e, Rh C, Rh c.

INTRODUCTION

ABO blood group system is a classification system for human blood that identified four major blood group base on the presence or absence of A and B antigens on the red blood cells (A, B, AB and O), It was first discovered in 1900 by Landsteiner and is remains most important in transfusion and transplantation because almost everybody over the age of about 6 months has clinically significant anti-A and or anti-B in their serum if they lack corresponding antigens on their red cells (Mollisonet al, 1997). Rh blood group system is one of the most polymorphic and immunogenic system known in human and most important blood group system after ABO at present, it consists of 50 defined blood-group antigens, among which the five antigens D, C, c, E, and e are the most important. Besides its role in blood transfusion, the Rh blood group system specifically, the D antigens used to determine the risk of hemolytic disease of new born, Blood donation in all circumstance shall be voluntary (Levine et al, 1939)

In general blood donors should be healthy adults between the ages of 17-70 years. These age limit vary slightly worldwide, but lower limit is set to take account of the high iron requirement of adolescence. Pregnant and lactating women are not accepted as the donors of allogeneic blood because of high iron requirements. The donors should be fit and healthy individual, Donation should not be accepted from individuals suffered from cancers, diabetes, heart or renal disease and those with severe allergic reaction (Barbara, 2002)

ABO was first blood group system to be discovered by Landsteiner in 1900 who mixed sera and RBCs from his colleagues and observed agglutination. On the basis of the agglutination pattern he named the first two blood group antigen A and B, using the first two letter of alphabet, while RBC's that not agglutinated by either sera were first called type C but become known as ohne A and ohne B (ohne is German for "without" finally named O (Landsteiner et al, 1900)

Antigens

In general ABO antigens were biochemically characterized in 1950s and 1960s as carbohydrate structure on glycoproteins and glycolipids The antigens are synthesized in stepwise fashion by glycosyl transferase enzymes that sequentially add specific monosaccharide sugars in specific linkage to growing oligosaccharide precursor chain.

The terminal sugar determines antigen specificity: N – acetylglactosamine residue results in expression of A antigen while the terminal glactose residue is responsible for B antigen. These structures are similar differing only in that A antigen has a substituted amino group on carbon 2. The procure substrate for A and B antigen is H antigen.

The terminal fuse in alpha (1, 2)lineage to galactose is responsible for H antigen specificity. Large amount of H antigen found in blood group O because it not converted to A or B. Some H antigen precursor also remain on A and B RBCs listed as follow in descending order of frequency A2,B, A2B,A1,A1B.RBCs membrane proteins carry well over 2\106A, B and H antigens. These antigens also found on many tissues, large amount are expresses on endothelial and epithelial cells of the lug and gut and on the epithelial cells of urinary and reproductive tract (Landsteiner et al, 1900). ABH antigens also found in secretion (particularly saliva) and fluid (milk and urine)of 80% of the population who have the secretor (Se) phenotype and ABH antigens also found on platelet with vary amount between individuals (Clausen et al, 1989).

ABO antibodies

Anti-A and anti- B are found on sera of individuals who lack the corresponding antigens. They are natural occurring antibodies because they produce in response to environmental stimulants such as bacteria. Their production begins after birth reaching at age of 5 to 10. These types of antibodies are mostly immunoglobulin M, which able to activate complement, which in conjugation with the high density of ABO antigens sites on RBCs, is responsible for severe, life threatening transfusion reaction that may be caused by ABO incompatible transfusion(Brecher, 2005). Hemolytic disease of the new born cause by ABO antibodies are usually mild for the following reasons: placental transfer is limited to fraction of IgG anti-A and anti-B found in the maternal serum, fetal ABO antigens are not fully developed and ABO tissue antigen provide additional targets for the antibodies(Brecher, 2005). ABO –HDN most often seen in non-group O infants of group O mothers because anti-A and anti-B of group O often have significant IgG component (Brecher, 2005).

Rh blood group system

The Rh blood group system is one of thirty-five current human blood systems. It is the most important blood group system after ABO but it remains primary importance in obstetrics, being the main cause of hemolytic disease of the newborn (HDN).

Rh blood group is one of the most complex blood groups known in humans; the complexity of the Rh blood group antigens begins with the highly polymorphic genes that encode them. There are two genes, RHD and RHCE that are closely linked. Numerous genetic rearrangements between them have produced hybrid Rh genes that encode a myriad of distinct Rh antigens. At present, the Rh blood group system consists of 50 defined blood-group antigens, among which the five antigens D, C, c, E, and e are the most important (Levine et al, 1939).

History

In 1939 Levine and Stetson described women who delivered stillborn fetus, and suffered severe reaction when transfused blood from her husband, her serum agglutinated the RBCs of him and 80 of 104 ABO-compatible donors (Levine et al, 1939). In 1940, Karl Landsteiner and Alexander Wiener in an effort to discovered additional blood group immunizing rabbits and guinea pigs with red blood cells from Rhesus monkey, the anti-serum agglutinated not only Rhesus celled but also the RBCs of 85% of a group of Caucasian subject from New York. The antigen that induced this immunization was designated by them as Rh factor to indicate that rhesus blood had been used for the production of the serum". Later it was realized that the rabbits anti-serum does not recognizing the same antigen but it was detecting an antigen found in greater amount on Rh positive than Rh negative. This antigen was named LW for Landsteiner and Wiener and the original human specificity become known as anti-D (Daniels, 2002). As early as 1941, it obvious that Rh was not simple single antigen system. Fisher named the C and c on the base on reactivity of two antibodies that recognized antithetical antigens, and used the next letters of alphabet D and E to define antigens recognized by two additional 2antibodies(Raceet al, 1975). Anti-e which recognized the e antigen was identified in 1945(Mourant, 1954)

Rh nomenclature

The Rh system has been acknowledged as the one of the complex blood group systems because it's large number of antigens and heterogeneity of antibodies. The Rh blood group system has two sets of nomenclatures: one developed by Ronald Fisher and R.R Race, the other by Wiener. Both systems reflected alternative theories of inheritance (Tippett 1986). The Fisher-Race system, which is more commonly in use today, uses the CDE nomenclature.

This system was based on the theory that three closely linked genes C/c,E/e and D were responsible for production of Rh antigens, each separate gene controls the product of corresponding antigen (e.g., a "D gene" produces D antigen, and so on). However, the d gene was hypothetical, not actual (Tippett 1986). The Wiener system used the Rh–Hr nomenclature. This system was based on the theory that there was one gene at a single locus on each chromosome, each contributing to production of multiple antigens (Tippett 1986). These different nomenclatures reflected the different in opinion concerning the number of gene encoded these antigens. A capital R indicate that D is present, and lowercase r indicate that is not. The C or c and E or e Rh antigens carried with D are represented by subscript 1for Ce (R1), 2 for cE(R2) 0 for ce (R0), and Z for CE (Rz) the Cc Ee antigens present without D (r) are represented by subscript symbols: prime for Ce(r,), double prime for cE(r,), and y for CE(ry) (Tippett 1986).

In 1962 Rosen field and associated introduced numerical designation to more accurately represent the serologic data to be free from genetic interpretation and to more

compatible for computer use. However this nomenclature with few exceptions (Rh 17, Rh32, Rh33) is not widely used in clinical laboratory (Rosen field et al, 1962).

Terminology

Current Rh terminology distinguished the genes and proteins from the antigens, which are referred to by the litter designation D,C,c, E, e. To indicate RH genes, capital letter with or without italics are used (i.e., RHD, RHCE and RHAG). The different alleles of RHCE gene are designated RHce, RHCe, RHcE, according to which antigen encode. Rh haplotypes are designated Dce, DCe, DcE, etc. or ce, Ce, CE when referring to specific CE haplotypes (Tippett 1986).

Rh proteins

The Rh proteins RhD and RhCE, are 417- amino acid, non-glycosylated, one carries the D antigen and the other carries various combination of CE antigen. RhD differ from RhCE by 32 to 35 amino acid depending on which form of RhCE is present and both are predicted to span RBCs membrane 12 times. They migrate in SDS-page (sodium dodecyl sulfate polyacrylamide gel electrophoresis) with an approximate molecular weight ratio (Mr) 30,000 to 32,000 (hence sometimes referred as Rh30 proteins). Also they are covalently linked to fatty acid (plamitate) in the lipid bilayer (Hartel-Schenk, 1992).

A 409 –amino acid glycosylated protein that co precipitates with Rh30 proteins and migrate with an approximate Mr of 40,000 to 100,000 is called RhAG protein(Rh associated glycoprotein) or Rh50. It shares 37% amino acid identify with RhD and RhCE proteins and has same predict membrane topology. RhAG is not polymorphic and does not carry Rh antigens. It is important for targeting the RhD and RhCE to the membrane (Ridgwell et al, 1992).

Rh genes

Two genes, designated RHD and RHCE encode Rhantigens, these genes are 97% identical. Each gene has 10 exons and the result of a gene duplication on chromosome 1p34-p36(Che,rifet al, 1991). The single gene, RHAG, located at chromosome 6p11p21-1 encodes RhAG. It is 47% identical to RH genes and also has 10 exons (Ridgwellet al, 1992).

Rh antigens

As previously described the major Rh antigens are D, C, c, E, e. The many other Rh antigens define compound antigens I cis (e.g., f (ce), Ce, CE), low incidence antigens arising from partial D hybrid proteins (e.g., Dw, GOa, BARG), high incidence antigens, and other variant antigens. The molecular bases of most Rh antigens have determined. Studies to estimate the number of D,C/c and E/e antigen sites on RBCs found differences between Rh phenotypes. The number of D range from 10,000 on Dce/ce RBCs to 33,000 on DcE/DcE. The number of C, c, e antigen per RBC from 8500 to 85000. Because C or c and E or e are carried on same protein their number should be equivalent (Daniels, 2002)

D antigen

Rh positive and Rh negative refer to the presence and absence respectively, of D antigen which is most immunogenic Rh antigen. The D-negative phenotype occur in 15-17% of white person. The absence of D in European is primarily the result of deletion of the entire RHD gene. However rare D- negative white person carry RHD genes but not expressed due to premature stop codon(Avent et al, 1997). A 4- base pair (bp) insertion at intron 3/exon 4 junction. Most of this are associated with un common Ce(r,) or cE(r,) haplotypes (Andrewes et al, 1996). D-negative phenotypes in Asian or African people mostly caused by inactive or silent RHD gene rather than complete gene deletion. Asian D-negative individual occur with a frequency less than 1% and most carry mutant RHD gene associated with Ce haplotypes (Okuda et al, 1997).

Only 3% to 7% of South African persons are D-negative but 66% of these group have RHD gene that contain a 37- bp internal duplication, which result in premature stop codon. In addition 15% of D-negative phenotype in African result from hybrid RHD-CE-Ds gene characterized by expression of VS, weak C and e and no D antigen (Singleton et al, 2000). Only 18% of D-negative African completely lack RHD gene.

Weak D

About 0.2% to1% of white persons (and greater number of African) have reduce expression of D antigen, which is characterized serologically as failure of RBCs to agglutinated directly with anti-D typing reagent and required the used the indirect antiglobulin test (IAT)for detection. Collectively weak D phenotypes have amino acid changes predicted to be intracellular or in the trans membrane region of RhD protein and not on the outer surface of RBC,s suggesting that the mutation affect the efficiency of insertion and therefore the quantity of RhD protein in the membrane but not affect D epitopes (Wanger et al, 1999).

The majority of individual with weak D phenotype can be safely receive D-positive blood and do not make anti D. However two weak D types (type 4.2.2 and type 15) have been reported to makeanti D. A very weak form of D designated Del, detected by absorption and elution of anti D, has high incidence in Chinese and Japanese persons. It is most often result from splice site mutation resulting in absence of amino acids encoded by exon 9 of RHD gene. These RBCs type as D negative (even when tested using the IAT), they are usually recognized if stimulate production of anti -D in D negative recipient (Yasuda et al, 2005).

Partial D antigens (D categories or D mosaics)

The D antigen describe as mosaic on the base of observation that some Rh- positive make alloanti-D

when exposed to D antigen. It was hypothesized that RBCs of these individual lack some part of D antigen means they can produced antibodies for missing portion. Molecular analysis show that this hypothesis is correct, but what was not predicted is that missing portions of RHD gene are replaced by corresponding portions of RHCE. Some replacement involved entire exons and novel sequence of amino acid generate new antigen (e.g. Rh32,Dw). Other partial D result from some amino acid conversion between RHCE and RHD, and some are result of single point mutation in RHD (DVI,DMH, DFW).

These point mutation are predicted are located on an external loop or portion of RhD protein. Individuals with partial D antigen can make anti-D and therefore should receive D negative donor blood (Huang, 1997).

Elevated D

Several phenotypes including D--, Dc-, and DCw-, have an elevated expression of D antigen and no, weak, and variant of CE antigens respectively. They are cause by replacement of RHCE by RHD. The additional RHD sequences in RHCE along with a normal RHD may explain enhanced D and account for reduced or missing of CE antigens(Daniels, 2002).

C/c and E/e antigens

There are four allelic forms of RHCE: Ce, ce, cE and CE. C and c differ by four amino acids: Cys16Trp (cysteine at residue 16 replaced by tryptophan) encoded by exon 1, and Ile60Leu, Ser68Asn and Ser103 Proencoded by exon 2. Only residue of 103 is predicted to be extracellular,. it is located on the second loop of RhCE. E and e differ by one amino acid, Pro226Ala. This polymorphism predicted to be reside on the fourth of the protein is encoded by exon 5. A single point mutation RHce resulted in RHcE(Mouro et al, 1993)

The antigens V and VS are expressed on RBCSs of more than 30% of black persons. They are result of Leu245Valsubstitution located in the predicted eighth transmembrane segment of Rhce. The close location of the e antigen Ala226 on the fourth extracellular loop, suggest that Leu245Val cause local conformational changes responsible for weakened expression of e antigen in many black persons who are V and VS positive. The V- VS+ phenotype result from aGly336Cyson the 245Val background while the V+ VS- is associated with additional amino acids changes and is the characteristic of the ceAR haplotype(Fasset al, 1997). Other modifications of RHCE which are uncommon are the hybrid rG,RNand several E/e variants. RN phenotype found in people with African origin and type as weak or negative e with polyclonal reagents but indistinguishable from normal e positive RBCs with some monoclonal ani-e. E variants –EI,EII and EIII are result either from a point mutation(EI) or from gene conversion events that lead to replacement of several extracellular RhcE amino acids with RhD residues (EII and EIII) and loss of some E epitope expression (Noizat-Pirenne et al, 1998).

Rh antibodies

Naturally occurring antibodies

Generally Rh antibodies are produced following immunization by red cells. However anti-E is often naturally occurring, about one-half may occur without a history of pregnancy or transfusion (Daniels et al, 2002).

Immune antibodies

The clinical importance of the Rh system lies in the readiness with in which anti-D arise after stimulation with D-positive cells by pregnancy or transfusion. Prophylaxis of Rh immunization with anti-D immunoglobulin led to significant decrease in the incidence of anti-D, but it still remains the most common immune antibody of clinical relevance detected in routine blood transfusion laboratory antigen considerably most immunogenic than other Rh antigens, which have the following order immunogenicity : c>E >e>C(Daniels et al, 2002).

Blood Donation

Blood donation is critical to all of transfusion therapy, as it provides the starting product. Blood donation can be divided in to five processes that are directly related to the donors.

1. Recruitment

It is specialized task, often performed by recruiters, and the massage delivered must be convincing and compelling to result in scheduled appointment to donate blood.

2. Screening

This process is carried out to make sure the donation process will be safe for the donor and that the collected blood also will be safe for the recipient. The screening process questionnaire that seeks to find medical condition and behavior that may make donation un safe for the donor and recipient. Critical information is confirmed by direct verbal question to ensure that answer is accurate (Sullivan et al, 1999).

3. Physical examination

A brief Physical examination should be following the screening process which includes examination of antecubital veins, measurement of body temperature, donor hematocrit or haemoglobin, and heart rate (Sullivan et al, 1999).

4. Collection

After the vein puncture is performed, blood is collected, labeled, and temporary stored until it can be transfer to manufacturing center for further processing and distribution. Specimen tube are draw at the time of collection for infectious disease testing(Hepatitis Viruses, Human Immunodeficiency Virus, Human T-

Lymph tropic Virus, Syphilis, Cytomegalovirus) (Sullivan et al, 1999).

5. Post donation care

After donation donors receive oral fluids and remain under observation under a period of time so that any post donation reactions may be treated appropriately(Sullivan et al, 1999)

Anticoagulant and preservative solutions

The common anticoagulant-preservative solutions used in transfusion practice are acid citrate dextrose(ACD-A), citrate phosphate dextrose (CPD and CP2D), and citrate phosphate dextrose adenine(CPDA-1). Anticoagulant –preservative solutions vary in the content and the approved length of RBC storage, with the RBC storage for CPD and CP2D at 21 days and CPDA-1 at 35 days. Additive solutions (AS-1,AS-3AS-5) approved RBC storage up to 42 days.

Each component of the preservative solution has specific metabolic support function. Dextrose and adenine serve as substrate for ATP production, and phosphateacts as PH buffer and substrate for 2,3-DPG formation. For a whole blood collection kits, the volume of Anticoagulant –preservative solution is specific for collection volume. For 450ml primary collection bag the Anticoagulant –preservative solution is 63ml. To extend the expiration time to 42 days, RBCs are suspended in an additive solution (100ml) that is transfer to primary bag after the plasma is expressed from the whole blood collection. Additive solutions decrease in vitro haemolysis by stabilizing the RBC membrane, either through the action of mannitol or citrate also enhance transfusion flow by decreasing the viscosity of packed RBCs.

Additive solutions include the saline-adenine-glucose-mannitol(SAGM) formulation, AS-1(Adsol) and AS-5(Optisol), and non-mannitol base solution AS-3 (Nutricel) (Hess et al, 2002).

Complication of blood transfusion

Transfusion of blood and its product is generally a safe and effective way of correcting haematological defects but adverse effects do occur during or after transfusion and they are commonly called blood transfusion reactions. These effects vary from being relatively mild to lethal and some of them can be prevented while others cannot. Medical personnel who order and administer transfusions should be able to recognize transfusion reaction so that appropriate actions can be taken promptly (Popovsky 1994).

Immunological Complication

Sensitization

As only the ABO and RhD antigens are routinely matched in blood transfusion; there constant possibility of sensitization to other red cell antigens. This is more likely in multiple transfusion patients (Popovsky 1994)

Haemolytic transfusion reactions

It is premature destruction of transfused red cells reacting with antibodies in the recipient. Red cell alloantibodies form in response to exposure, through previous transfusion or pregnancies (not naturally occurring). The reaction may occur immediately after transfusion or may be delayed up to 2-3 weeks. As only the ABO and RhD antigens are routinely matched in blood transfusion; there constant possibility of sensitization to other red cell antigens. This is more likely in multiple transfusion patients.

There are four broad categories of transfusion reactions:

1. Acute immunologic (<24hrs).
2. Acute non immunologic (<24hrs).
3. Delayed immunologic(>24hrs).
4. Delayed non immunologic(>24hrs).

Haemolytic transfusion reactions (HTR) can occur in the first three categories mentioned above (Popovsky 1994).

Acute immunologic haemolytic transfusion reaction

Very severe hemolytic reactions occur when transfused cells interact with preformed antibodies in the recipient. However the interaction of transfused antibodies with the recipient's red cells. Antigen antibody interaction on the red cell membrane can initiate a sequence of complement activation, cytokine and coagulation effects, and other elements of a systemic inflammatory response that result in clinical manifestations of a severe acute hemolytic transfusion reaction (HTR). Severe symptoms can occur after the infusion of even 10-15ml of ABO incompatible red cells. In anaesthetized patients the initial manifestations of an acute HTR may be hemoglobinuria, hypotension or diffuse bleeding at the surgical site. Severe acute HTRs are usually caused by ABO incompatibility, but occasionally anti-Rh or anti-Jka, capable of fixing complement. Rarely may be caused by anti-Pk, anti-Vel, anti-Lea (Lewis), anti-Jk b (Kidd) or anti-K1 (Kell) antibodies (Popovsky 1994).

Acute non immunologic haemolytic transfusion reaction

Causes

Thermal exposure of transfused red cells Red cells may undergo in-vitro haemolysis if the unit is exposed to improper temperatures during shipping or storage or is mishandled at the time of administration. Malfunctioning blood warmers, use of microwave ovens or hot water baths, or inadvertent freezing can cause temperature-related damage. Mechanically haemolysed red cells: may be caused by the use of roller pumps (such as those used in cardiac bypass surgery), pressure infusion pumps, pressure cuffs, or small-bore needles. Chemically effected red cells: Osmotic haemolysis in the blood bag or infusion set may result from the addition of drugs or hypotonic solutions. Inadequate deglycerolization of frozen red cells may cause the cells to haemolyze after infusion.

Blood infected with bacteria: Haemolysis may also be a sign of bacterial growth in blood units. Transfusion reactions due to bacterial contamination are commonly caused by endotoxins produced by bacteria capable of growing in cold temperature such as pseudomonos species, Escherichia coli and Y. enterocolitica (Beauregard et al, 1994).

Delayed hemolytic transfusion reactions

Delayed hemolytic transfusion reactions (DHTRs) may occur when there is an antigen mismatch between transfused RBCs and recipient RBC antibodies where sensitized RBCs are cleared by macrophages or complement activation leading to immunoglobulin G (IgG) mediated hemolysis. Some DHTR etiologies remain unknown since there are cases of DHTR when an RBC autoantibody or alloantibody is absent. Mechanisms have been proposed to explain these types of cases of DHTR, including bystander or reactive haemolysis by hyperactive macrophages. Studies in patients with sickle cell disease (SCD) have shown abnormalities in the structure and function of the RBC membranes including exposure of phosphatidylserine is a phospholipid at the extracellular face of the RBC membrane and is associated with the macrophage clearance) leading to macrophage clearance of sickled erythrocytes. We report on a case demonstrating that DHTR may occur as a result of PS exposure on antigen-matched RBC, resulting in macrophage clearance and hemolysis without detection of autoantibodies or allo antibodies .Patients may present with fever and anaemia usually 2 to 14 days after transfusion of a red cell component, jaundice, high bilirubin, high LDH, reticulocytosis, spherocytosis, positive antibody screen and a positive Direct Antiglobulin Test (in case of sensitize RBC) (Settyet al, 2002)

Haemolytic disease of the new born

Rh antibodies are capable of causing hemolytic transfusion reaction with extra vascular hemolysis. They may also result in severe haemolytic disease of newborn (HDN) when a Rh negative mother carries a Rh positive fetus, as the Rh agglutinogen from the fetus trigger an immune response from the mother, producing Rh agglutinins and causing the blood of the fetus to agglutinate (coagulate). Symptoms in the newborn usually include an abnormally large liver or spleen, anemia or low red blood cell count, and in serious cases, swelling of the skin or internal organs. Treatment of the mother with Rh-immunoglobulin during term may help decrease the complement cascade and therefore lower the severity of the fetus's symptoms (Farlax 2015)

Updated studies related to frequency of Rh antigens

WM Shahata et al (2012) studied the frequency of blood group and Rhesus antigens among two hundred male blood donors attending the Central Blood Bank, Sudan, observed.

The frequency percentage of ABO blood phenotypes in the total samples were as follows: O(51.5%), A (29.5%), B (16%), and AB (6%); whereas the frequency percentage of Rh antigens were D (93%), e (79.5%), c (68.5%), C, (27%), E (18.5%). and were concluded that the frequency of the Rh antigens can be shown in this order D > e > c > C > E (WM Shahata et al, 2012). Malik A. El fadni et al (2014), studied phenotypes, haplotypes and probable genotypes of thousand un- related individual from ten major Sudanese tribes, Red blood cells were tested for common Rhesus antigens using Particle gel immune diffusion and slide agglutination techniques.

The phenotypes, haplotypes and most probable genotypes were determined. Similarities between different Sudanese populations were calculated using Jaccard's coefficient of similarities. Phenotypic data obtained was referred to as alleles, haplotypes, genotypes based on reasonable assumptions that every Rh blood group antigen represents a gene that is always expressed and has a Mendelian dominant mode of inheritance.

The ē, and D were the most common antigens/alleles with frequencies of 98.4%, 93.8% and 90.7% respectively. The most prevalent genotype was with a frequency of 44.2%, while and genotypes were detected with lower frequencies of 21.7% and 10.9% respectively. The CdE and C E genotypes were the least common. In conclusion, ē, and the D antigens/alleles were the most common among the major Sudanese tribes. The C and the E antigens were the least expressed (Malik A. Elfadni et al, 2010). Younis (2011), tested two hundred and thirty-two blood samples (110 M and 122 F) from Gaza city in Palestine against monoclonal IgM anti-C, anti-c, anti-E, anti-e and a blend of monoclonal/polyclonal IgM/IgG anti-D.

Concluded the most frequent Rh antigen in the total sample was e, while the least frequent was E. The order of the combined Rh allele frequencies in both M and F was CDe>cDe>cde>CdE>cDE>Cde> CDE (Younis Abed EL-Wahhab Skaik 2011). Jeremiah et al (2005), within the various ethnic nationalities in the south-south region of Nigeria were studied Rh antigens and phenotype frequencies, resulted to that the frequencies of the Rh antigens within the nationalities were c (100%), e (96.38%), D (96.38%), E (15.22%), and C (3.62%) for the Ibibios; c (100%), e (95.60%), D (96.70%), E (21.98%), and C (0%) for the Efiks; and c (100%), e (94.29%), D (91.43%), E (28.57%), and C (2.86%) for the Ibos. The overall frequencies of the Rh antigens in these 720 individuals were c (100%), e (95.56%), D (94.44%), E (18.89%), and C (2.78%).

Forty (5.56%) were found to be D-, while all were found to possess the c antigen. The most frequently occurring Rh phenotype was Dccee, with a frequency of 73.61 percent. The alternative allele, C, did not appear in homozygous form (CC) in the population tested

(Jeremiah ZA et al, 2005). Jenan.Y.Taha (2012),was investigated Rh antigen and Phenotype frequency in Kalba region, UAE, concluded that most frequently occurring antigenwas e (97.3%), followed by D (91.1%), C (73.2%), c 470 (71%) and E (21%) (Jenan Y Taha 2012).

MATERIAL AND METHODS

Study area and duration

Gezira state is situated between blue and white Nile in the east-central region of the country and is boarded to north of Khartoum state; south Sennar state :east of the Gadarif state; and west of the White Nile state. The area approximately is 27.549.2 square kilometers with population 3.575.280 inhabitants (Sudan.gov.sd.2012). The state contains localities and Wad Medani is a capital of the state. This study conducted in central blood bank in Wad Medani (during June) which is reference blood bank in the Gezira state and admitted different blood donors coming from all over Gezira state with different ethnic groups.

Study design

This is descriptive cross sectional study hospital based, conducted to determine the frequency of Rh antigens (D, C, c, E, e) among blood donors attending to Wad Medani Central Blood Bank.

Study population

Donors attending to Wad Medani central blood bank.

Inclusion criteria

Sudanese blood donors.

Sampling method

Blood samples were collected randomly by (simple random sampling technique from suitable blood donors.

Sample size

132 blood samples was collected from blood donors.

Sample collection

2.5 ml of blood was collected from blood donors under a septic condition in Ethylenediamine tetra-acetic acid (EDTA) containers, mixed well and labeled.

Data collection tools

The data was collected by using a questionnaire. A questionnaire will be designed to include all needed information.

Laboratory analysis

Materials

Slides
Cotton
70% alcohol
Syringes
EDTA blood container
Pasteur pipettes
Stop watch

Reagents

Anti-C, anti-c, anti-E, anti-e are ready to use reagents prepared from cell culture supernatant of respective humanhybridoma cell lines (Eryclone.Goa. India)
IgM –IgG anti-D.

Laboratory procedures

Slide test: was performed for each blood sample
One drop of anti –sera reagent was placed on clean glass slide. Then one drop of whole blood was added.
Mixed well over an area of 2.5cm.
Slide was rocked gently back and forth.
Agglutination was observed within 2 minutes.

Interpretation of result

According to company the result was considered positive when agglutination was observed; it was indicated to the presence of corresponding antigen.

No agglutination is a negative result and indicated the absence of corresponding antigen.

Data analysis

All data collection from practical and questionnaires survey was entered in Microsoft office excel. Then the result analyzed by Statistical Package for Social Sciences (SPSS) program version 20, across tab correlation was done. Statistical analysis: The results were analyzed using statistical software package of social sciences (SPSS) version 17 and descriptive data were expressed as means.

Ethical clearance

Ethical clearance was obtained from the University of Gezira ethical committee and blood bank authority. Verbal informed consent was obtained from all donors.

RESULT

One hundred thirty two of fit blood donors attending to Central Blood in Wad Medani were recruited in this study from different ethnic groups. The middle tribes (Kawahla, Agalien, SHokriaa, Masalamiah, Arakien, KHawalda, Rofaien, Magarba) considered the majority of study population (51%), followed by northern tribes (Jaalien, Danagla, Budirria), Westerntribes (Bargo, Tama, Foor, Nuba, Miseria), African tribes (Hawsa, Falata), eastern tribes (BaniAmer) table (1).

Table (1) frequencies of study population according to ethnic groups (N=132)

tribes		Frequency	Percent	Valid Percent	Cumulative Percent
	Middle tribes	68	51.0	51.0	51.0
	North tribes	29	22.0	22.0	68.9
	East tribes	1	.8	.8	69.7
	West tribes	14	10.6	10.6	79.6
	African tribes	20	15	15	93.5
	Total	132	100.0	100.0	

Frequency of ABO blood group among blood donors

The frequency distribution of ABO group is shown in table -2. 73 (55%), 31(23%), 26 (20%) and 2 (2%), frequencies were reported for O, A, B and AB, respectively. Over the period of this study, we observed an overall trend of ABO blood group was O >A> B> AB in blood donors. That mean O was the most encountered blood group while group AB was the least group encountered among blood donors.

Table (2) distribution of ABO blood group among blood donors.

Blood group		Frequency	Percent	Valid Percent	Cumulative Percent
	A	31	23.5	23.5	23.5
	B	26	19.7	19.7	43.2
	AB	2	1.5	1.5	44.7
	O	73	55.3	55.3	100.0
	Total	132	100.0	100.0	

Frequency of Rh blood group among blood donors

Frequency of Rh blood groups depicted in tables (3 through7). Rh e was the most phenotype observed in this study (100%) table (3), followed by Rh c phenotype (93%) table (4), Rh D (92%) table (5), Rh C (43%) table (6) and Rh E phenotype (16%) table (7) respectively.

Table (3) frequency of Rh e phenotype.

Rh e		Frequency	Percent	Valid Percent	Cumulative Percent
	positive	132	100.0	100.0	100.0

Table (4) frequency of Rh c phenotype.

Rhc		Frequency	Percent	Valid Percent	Cumulative Percent
	Positive	123	93.1	93.1	93.1
	Negative	9	6.8	6.8	6.8
	Total	132	100.0	100.0	100.0

Table (5) frequency of Rh D phenotype.

RhD		Frequency	Percent	Valid Percent	Cumulative Percent
	positive	122	92.4	92.4	92.4
	negative	10	7.6	7.6	7.6
	Total	132	100.0	100.0	100.0

Table (6) frequency of Rh C phenotype.

RhC		Frequency	Percent	Valid Percent	Cumulative Percent
	positive	57	43.1	43.1	43.1
	Negative	75	56.9	56.9	56.9
	Total	132	100.0	100.0	100.0

Table (7) frequency of RhE phenotype.

RhE		Frequency	Percent	Valid Percent	Cumulative Percent
	Positive	21	16	16	16
	Negative	111	84	84	84
	Total	132	100.0	100.0	100

Frequency of coexisistance Rh Cc and Rh Ee

About 50 (38%) of blood donors were represented both C and c phenotype table (8)

Table (8) frequency of Rh Cc phenotype.

Rh Cc		Frequency	Percent	Valid Percent	Cumulative Percent
	Positive	50	37.8	37.8	37.8
	Negative	82	62.2	62.2	62.2
	Total	132	100.0	100.0	100.0

Mild number of donors 21(17%) were have both E and e phenotypes table (9)

Table (9) frequency of Rh Ee phenotype.

RhEe		Frequency	Percent	Valid Percent	Cumulative Percent
	Positive	21	16.7	16.7	16.7
	Negative	110	83.3	83.3	100.0
	Total	132	100.0	100.0	

Correlation between the Rh antigen frequencies and ethnic group showed that

Rh e phenotype are dominant in all the studied population table (10), while Rh E represented the lowest one, has high frequency in northern tribes (20.6%) and low frequency in western tribe tribes Table (11).

Table (10) correlation between Rh e and tribes.

Tribes		Total	Rhe	Rh e
			Positive	Negative
	Middle tribes	68	68	0
	North tribes	29	29	0
	East tribes	1	1	0
	West tribes	14	14	0
	African tribes	20	20	0
Total		132	132	0

Table (11) correlation between RhE and tribes.

Tribes		Total	RhE	
			Positive	Negative
	Middle tribes	68	12	56
	North tribes	29	6	23
	East tribes	1	0	1
	West tribes	14	1	13
	African tribes	20	2	18
Total		132	21	111

Rh D phenotype was observed with high percentage in all ethnic groups .table (12)

Table (12) correlation between Rh D and tribes.

Tribes		Total	RhD	
			Positive	Negative
	Middle tribe	68	63	5
	North tribe	29	28	1
	East tribe	1	1	0
	West tribe	14	11	3
	African tribe	20	19	1
Total		132	122	10

Rh c phenotype also was highly expressed in all tribes especially in African and western one table (13).

Table (13) correlation between Rh c and tribes.

Tribes		Total	Rhc	
			Positive	Negative
Total	Middle tribe	68	60	8
	North tribe	29	28	1
	East tribe	1	1	0
	West tribe	14	14	0
	African tribe	20	20	0
		132	123	9

While northern and middle tribes, were represented Rh C with higher frequency than others. Table (14)

Table (14) correlation between RhC and tribes.

Tribes		Total	RhC	
			Positive	Negative
	Middle tribes	68	36	32
	North tribes	29	13	16
	East tribes	1	1	0
	West tribes	14	3	11
	African tribes	20	4	16
Total		132	57	75

Frequency of Cc among blood donors

In case of Cc and Ee phenotypes expression, both were observed in both middle and north tribes slightly higher than others tribes, Cc phenotype had high expression than Rh Ee phenotype tables (15),(16).

Tribes		Total	RhCc		C (−) ve c (+) ve	C (−)ve c(−) ve
			C (+)ve c (+)ve	C (+)ve c (−)ve		
	Middle tribes	68	30	6	30	2
	North tribes	29	12	1	16	0
	East tribes	1	1	0	0	0
	West tribes	14	3	0	11	0
	African tribes	20	4	0	16	0
Total		132	50	7	73	2

Table (16) correlation between RhEe and tribes.

Tribes		Total	RhEe		E (−) ve e (+) ve	E (−) ve e (−) ve
			E (+)ve e (+) ve	E (+) ve e (−) ve		
	Middle tribes	68	12	0	56	0
	North tribes	29	6	0	23	0
	East tribes	1	0	0	1	0
	West tribes	14	1	0	13	0
	African tribes	20	2	0	18	0
Total		132	21	0	111	0

DISCUSSION

From their discovery until now Rh blood group system remain the second important blood group after ABO blood group system, ABO antibodies are mostly immunoglobulin M, can be able to activate complement, which in conjugation with the high density of ABO antigens sites on RBCs, is responsible for severe, life threatening acute haemolytic transfusion reaction in case of ABO incompatible transfusion. In contrast to ABO antibodies Rh antibodies are IgG antibodies which are un able to activate the complement system, in case of in compatible Rh antigen blood transfusion may be resulted for delayed hemolytic reaction, or sensitization (the antibodies that product from miss match Rh transfusion coat the RBC,s containing corresponding antigens and make sever reaction in second transfusion with RBC,s contain same antigen, Rh antibodies considered the first causes for hemolytic disease of new born (HDN). This study was done in Wad Madani which is the capital of Gezira state. The Gezira state represents mixture of races and tribes from inside and outside Sudan. In the current study, socio demographic characteristic of the study population indicated that the middle tribes considered the majority of study population (51%) followed by northern tribes (22%), Western tribes (13%) and African tribes (13%), eastern tribes (1%). Laboratory analysis conducted in this study revealed that the percentage of ABO blood phenotypes in the total samples were as follows: O (55%), A (23%), B (20%), and AB (2%), this result are identical with world ABO distribution. This agreed with Malik *et al*, 2010, Younis 2011, Shahata *et al*, 2013, Jeremiah 2005. The frequency of the Rh antigens can be shown in this order e >c >D >C >E, this agreed with Malik *et al* 2010.

Rh e antigen has highest prevalence (100%) in all study population, this agree with Malik A. 2010,Younis 2011, Jenan 2010, they considered Rh e had highest prevalence when compared with other Rh antigens with percentages 98.4%, 97%, 97.3% respectively while disagree with Shahata *et al*, 2013, who considered Rh e prevalence is second one after Rh D phenotype with 79.5 percent also disagree with Jeremiah 2005 with 95.6% prevalence classified as the second phenotype after Rh c phenotype.

Rh c classified as second phenotype in prevalence among the study population (93%), this percentage near to those obtained by Malik et al 2010(90.7%) and Younis 2011(80%), higher when comber with Jenan 2010 (71%) and Shahata *et al*, 2013(68.5%), slightly reduced than Jeremiah ZA 2005 (100%). Rh D prevalence considered the third in prevalence (92%), this agreed with Younis 2011 (92%), slightly lower when compared with Jeremiah 2005 (96.7%), Malik *et al* 2010 (93%) and Shahata et al, 2013 (93%), while was shown slight increase when compared with Jenan 2010(91%). Rh C and RhE which represented the lower prevalence 43%, 16% respectively. This slightly agreed to Shahata *et al*, 2013 C (27%), E (18.5%). Rh C prevalence slightly reduced when compared with Jenan 2010 (73%) and Younis 2011 (69%). While disagreed to Jeremiah 2005 0%. Rh E prevalence slightly increased in Jeremiah 2005(21%), Jenan 2010 (21%) and Younis 2011 (38%) studies. In this study some results were similar to results obtain in African (Nigeria) and other one obtained in Arabian (Kalba- United Arab Emirates and Gaza – Palestine). This may be due to the fact that Sudanese individual are mixture of African and Arabian races.

CONCLUSIONS

1. This study showed that the most frequent Rh antigen in Gezira region is (e) antigen.
2. Knowledge of blood group phenotype distribution is very important for Blood Banks and transfusion services policies.
3. Furthermore, to study a significant institution regarding the need for blood group prevalence which is not only important for transfusion medicine but also for organ transplantation and genetic researches.

Recommendation

1. Rh e and Rh c should be add to screening blood group test (ABO and Rh D).
2. Area wide survey for distribution Rh antigens with large sample size is highly recommended.
3. Other study including other antigenic blood group like Duffy, MNSs and Kell.

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