NAMIBIA UNIVERSITY
OF SCIENCE AND TECHNOLOGY

ASSESSMENT OF PHENOTYPIC PROPERTIES OF THE RHESUS AND KELL BLOOD GROUP SYSTEMS AMONG BLOOD DONORS AND PREGNANT WOMEN IN NAMIBIA

By

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DECLARATION

I, Mary Nyaradzayi Mataranyika, hereby declare that the work contained in the thesis entitled **Assessment of Phenotypic Properties of the Rhesus and Kell Blood Group Systems Among Blood Donors and Pregnant Women in Namibia** is my own original work and that I have not previously in its entirety or in part submitted it at any University or other higher education institution for the award of a degree.

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DEDICATION

I would like to dedicate this work

to my granddaughter Mutsawashe Nicole Madzokere

ABSTRACT

Introduction

A determination of the phenotypic frequencies of blood group systems C, c, E, e and Kell among the Namibian blood donors and pregnant women and the prevalence of these antigens in Namibia was carried out. Two thousand nine hundred and eight six blood donor specimens and four hundred and eighty-four specimens from pregnant women were randomly selected for antigen characterisation. Donated specimens that were processed for grouping and Transfusion Transmissible Infections (TTIs) by NaMBTS and ready for discard were collected by the researcher for testing.

The main aim was to determine the prevalence of C, c, E, e and Kell antigens among the Namibia blood donors and pregnant women and advice NaMBTS and the Ministry of Health and Social Services (MoHSS) on the findings in order to improve the outcome of patients who will be receiving donated blood and the pregnant women. This was to lay the foundation for further studies to establish a database for typed donors in Namibia and then store blood that is antigen negative and reduce the risks of Haemolytic Disease of the Foetus and the New-born (HDFN) and Haemolytic Transfusion Reaction (HTR). In some cases, the survival of the red blood cells that will have been transfused is shortened by the effect of transfused antigens. This is the first time this research has been carried out in Namibia.

Methods

Reagents were supplied by Rapid labs (Ltd) 2016, Essex, United Kingdom and testing procedures were carried out according to the manufacturer's Instructions. Anti –E, Anti-e, Anti- Kell, Anti-C and Anti –c reagents were used to determine the corresponding human red cell antigens. Monoclonal human IgM antibodies were used for blood grouping using the agglutination test procedure. If red cells with a specific antigen were mixed with the corresponding reagent, agglutination was expected. If agglutination took place it meant that the test was positive for that particular antigen. If agglutination didn't take place it showed the absence of that particular antigen and the test was regarded as negative.

Results

The results that were obtained showed that all the specimens that were tested had an average prevalence level of 6.6 % for C, c, E, e and Kell antigens and the one that was a bit high (10.5%) was the e-antigen for both the donors and the Ante Natal Clinic patients (ANC) with 10.4% and 10.5% respectively. The K antigen had a prevalence of 8.7% on donors and 7.2 % on ante-natal patients. C-antigen had a prevalence of 6.0% on donors and 7.0% on ANC patients. E antigen had the same prevalence in both donors and ANC patients with 2.1 %. The Namibian Donor population compares well with other countries in the world.

Conclusion

The conclusion that can be derived from the results obtained is that the prevalence of e-antigens in the Namibia population is high and this is one of the antigens that is generally implicated in immunisation during pregnancy and blood transfusion reactions hence the need to add it on routine screening for antibodies. There is a need to screen antigens such as ein both donor specimens and antenatal patients and to have further research to see if there is a need to actually have banks with a specific database for typed donors.

ABBREVIATIONS

ABO	The major human blood group system
ANC	Ante Natal Care/Clinic
AfSBT	Africa Society of Blood Transfusion
AHTR	Acute Haemolytic Transfusion Reaction
ANC	Ante Natal Care/Clinic
BeST program	Better and Safer Transfusion program
CffDNA	cell-free foetal Deoxyribonucleic Acid
DNA	Deoxyribonucleic acid
EBV	Epstein Bar Virus
GACUB	Guidelines of Appropriate Clinical Use of Blood and Blood Products
HBsAg	Hepatitis B surface antigen
HCV	Hepatitis C Virus
HDFN	Haemolytic Disease of the Foetus and New-born
HIV	Human Immunodeficiency Virus
HTRs	Haemolytic Transfusion Reactions
Ig	Immunoglobulin
IgM	Immunoglobulin M
IT	Information Technology
MCA	Middle Cerebral Artery
MoHSS	Ministry of Health and Social Services
NaMBTS	Namibia Blood Transfusion Services
NAT	Nucleic Acid Testing
NUST	Namibia University of Science and Technology
QMS	Quality Management Systems
RCOG	Royal College of Obstetrics and Gynaecology
RCF	Relative Centrifugal Force
TTIs	Transfusion Transmissible Infections
WHO	World Health Organization

DEFINITION OF KEY TERMINOLOGIES

Alloantibodies   Antibodies that are produced after the body has been exposed to antigens from the same species that were not on the person's red blood cells.

Blood group antigens   These are immunogenic protein markers that are found on the surface of the red blood cells which are considered in blood grouping

Blood Donors   Persons who voluntarily have blood drawn and used for transfusions and/or made into biopharmaceutical medications.

Extended antigen typing This entails the extension of red blood cell typing to identify safe products for transfusion even including the use of DNA testing

Kell Blood Group System Is a group of antigens on the human red blood cell surface which are important determinants of blood type and are targets for autoimmune or alloimmune diseases which destroy red blood cells

Non-reactive/ non-typable Did not agglutinate with any of the antigens that were being used and therefore showed a very weak or negative result

Phenotypic Properties   Is the composite of an organism's observable characteristics or traits, such as its morphology, development, and biochemical or   physiological properties.

Pregnancy   Also known as gestation period. It is the time during which one or more offspring develops inside a woman

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CHAPTER ONE: INTRODUCTION

Namibia Blood Transfusion Service (NaMBTS) does not routinely screen for Rh antigens except Anti-D before transfusion and this study will indicate the level of allo-immunisation on recipients of blood to Rh antigens. Other countries like Cote d'Ivoire (Siransy, 2014) are also doing research in determining the prevalence of these antigens (C, c, Kell, E, e) in order to improve transfusion practices It has always been the aim of NaMBTS to improve the safety of the blood recipients as well as the safety of blood donors. The blood and blood components that NaMBTS has been processing has been of very high quality and reliable (Haemovigilance Report, 2014).

Knowing the Rh phenotypes will be important in the determination of the likelihood of Haemolytic Disease of the Foetus and New-born (HDFN) in cases of maternal antibody reactions and Haemolytic Transfusion Reactions (HTRs). This assessment will assist NAMBTS in identifying recipients likely to receive multiple red cell transfusions and then prepare phenotypically matched blood for them in order to prevent the production of atypical antibodies. The assessment will also check on antenatal specimens. There is a need for evidence-based research where antibody testing is essential especially in pregnancy as to give the necessary prediction on the potential risk for the development of the HDFN and also to prevent its occurrence wherever possible.

1.1 Background to the study

Very little information is available on the prevalence of the C, c, E, e and Kell antigens among expectant mothers and blood donors in Namibia. This is the first time a research of this type is being carried out in Namibia. The alloantibodies, which frequently develop and are encountered during compatibility testing, are primarily against antigens related to Rhesus (Rh) blood group systems including C, c, Kell, E and e and blood groups. Antibodies directed against these antigens are implicated in cases of Haemolytic Disease of the Foetus and the New-born (HDFN) and

Haemolytic Transfusion Reactions (HTRs) and are, therefore, regarded as clinically significant (Markroo, 2013).

Beside Rh Anti-D antigens the other common immunogens for triggering immune reactions is the Kell blood group. Anti-Kell is known to cause perinatal haemolytic diseases and transfusion reactions that can be severe or even be fatal. Although the clinical burden imposed on neonatal services by HDN has not yet disappeared but has been greatly reduced. The paediatricians who work with neonates continue to recognise a number of different presentations of neonatal haemolysis, many of which are considerably more discreet than the traditional "neonatal emergency" presentation of severe Rh D disease (Murray, 2007). It has also been noted that some ethnic groups show differences in the different antigens.

It has been estimated that about eighty percent (80%) of patients who are Rh D negative and have been exposed to D antigen positive red cells may develop anti-D IgG antibodies that may persist for the rest of their lives. This can cause HTR and HDFN as it is not practically feasible to match all the minor antigens before transfusion so as to avoid allo-immunisation. Though there are more than 50 Rh antigens, the five principal Rh antigens, i.e., D, C, c, E, and e are responsible for the majority of clinically significant antibodies and patients with alloantibodies must receive corresponding antigen negative blood (Gundrajukuppam ,2016).

1.2 Blood components

Blood is not alike even though it is made from the same basic elements. There are about eight (8) well known common blood groups (A+, A-, B+, B-, O+, O-, AB+, AB-). These blood groups are distinguished by the absence or presence of different antigens. If antigens are foreign to the body they are capable of causing an immune response. This is normally evident during blood transfusion especially with blood from an incompatible donor. Therefore, there is a need to cross match every blood that is meant for transfusion purposes in order to avoid any transfusion reactions (Daniels, 2013)

The absence or presence of two antigens A and B on the surface of red blood cells determines the four major blood groups A, B, AB and O.

Group A is determined by the presence of A antigen on red cells and the B antibodies in the plasma.

Group B is determined by the presence of B antigen on red cells and A antibodies in the plasma

Group AB has both A and B antigens on red cells but neither A nor B antibody in the plasma

Group O does not have the A nor B antigens on red cells (but both A and B antibody are in the plasma)

There are other very important antigens chief among them the Rh factor anti-D, which can be either present (+) or absent (−). Those patients who are Rh D-negative are given Rh-D negative blood and those patients who are Rh D-positive are also given Rh D-positive blood. It is also possible to give Rh D-negative blood to Rh positive patients without any harm. There is a total of thirty (30) different blood groups e.g. Lewis, MNS Rh, Duffy, Kidd, Diego etc.), which can potentially cause problems during blood transfusions if they are not handled well. Besides the ABO blood group systems, there is also the C, c, E, e and Kell blood groups which other countries have reported to cause transfusion reactions and have started testing them routinely (Avent & Reid, 2000).

1.3 Blood Safety

World Health Organisation (WHO) has advocated that all the blood that is going to be supplied to patients be upheld by 3 pillars: -

a) Blood should be collected from non- remunerated, voluntary, safe and regular donors

b) Provision of laboratory testing that is of very high quality

c) Clinicians should be careful in order to avoid unnecessary blood transfusions

Namibia through its National Blood Program has taken great strides in addressing all these WHO concerns. Each and every unit of donated blood is screened for Transfusion Transmissible Infections (TTI) markers- Human Immunodeficiency Virus (HIV) 1 and 2, Hepatitis B and C and Syphilis. Counselling at NaMBTS is done by professionals and those who test positive for markers of Transfusion Transmissible Infections (TTIs) are deferred permanently (Haemovigilance Report 2012). Health care workers including Clinicians are trained regularly so that they keep abreast

with policies of blood transfusion in Namibia. The Guidelines for Appropriate Clinical Use of Blood and Blood Products (GACUB) have been used as a training tool since its inception in 2006 in Namibia.

The other program that has been widely used for the training of health professionals by NaMBTS is the Better and Safer Transfusion (BeST) program. This was a new tool that was developed by Namibia in 2010 to train, audit, and monitor and evaluate the system that had been put in place through the GACUB. Extensive training on the transfusion reactions was carried out because the trend of increased transfusion reactions was already there.

Despite all these training efforts by the Blood Transfusion Services together with the Ministry of Health and Social Services, the trend of transfusion reactions kept on rising. An idea of making blood transfusion issues as part of the quarterly hospital meetings was suggested in order to address any blood transfusion issues including transfusion reactions. A decision was made to develop some guidelines in the form of MoHSS Standard Hospital Guidelines for blood transfusion in order to reduce the number of transfusion reactions. All this was done in order to reduce the number of deaths due to blood transfusion reactions (Haemovigilance, 2012).

1.4 Statement of the problem

Statistics recorded in the Haemovigilance Reports (HRs) of the Namibia Blood Transfusion Service show an increase in transfusion reactions (Table 1) The increase could also be caused by the way blood is being handled in hospitals and clinics. Misidentification of patients could be an issue as well. If blood administration procedures are not properly followed problems can arise with transfusion reactions. A training program, the Better and Safer Transfusion (BeST) program that I was part of was initiated in 2010 but not much change could be noted as seen on the increase of transfusion reactions. Because of this, it was decided to explore other reasons that could be causing the increase in transfusion reactions.

Table 1 below shows a steady increase in the reported cases of transfusion reactions from 2008 to 2015. The determination of these Rh antigens might help in giving us a direction as to the reason why there is an increase in transfusion reactions.

Table 1.1: Data from the Haemovigilance Reports of the Blood Transfusion Services of Namibia

Year	2008	2009	2010/11	2012	2013	2014	2015
Transfusion reactions	13	16	41	38	44	52	64
Total donations	20,537	22,089	38,916	24,141	28,143	29,599	31,810
Total TTI positives	522	602	882	529	285	358	720
Assumed ready for use units	20,015	21,487	38,034	23,612	27,858	29,241	31,090

Namibia Blood Transfusion Service does not routinely phenotype for all other Rh antigens except D before transfusion and this study will indicate the level of allo-immunisation on recipients of blood to Rh antigens. Other countries like South Africa are already phenotyping their blood routinely for C, c, Kell, E, and e. There is no literature to support the fact that this research has ever been carried out in Namibia.

Knowing the Rh phenotypes will be important in identifying phenotypically matched blood for recipients likely to receive multiple red cell transfusions. This is due to possibilities of haemolytic disease of the new-born in cases of maternal antibody, identifying compatible blood for recipients with atypical antibodies. This is in order to prevent the production of atypical antibodies and also screen antenatal specimens. Maternal allo-immunisation, which causes severe anaemia in neonates, is also a big cause of concern in Namibia (Haemovigilance report, 2014).

Besides D antigens, c antigens are the most common cause of severe red cell iso-immunisation. Perinatal mortality from anti-c is about one-tenth that of anti-D. Anti K (Kell) is the most common immune red cell antibody outside the ABO and Rh systems (Poole, 2007). Anti K impairs haematopoiesis (Vaughan, 2010) as well as causing haemolysis and peripheral sequestration and lysis of red blood cells. Foetal exchange transfusion has also been reported as occasionally

required because of iso-immunisation caused by Kell subgroups. All other antibodies to the Rh systems (C, E and e) are capable of causing severe haemolytic disease, when present in high enough titre (South Australia Department of Health, 2010).

CHAPTER TWO: LITERATURE REVIEW

2.1 Risk factors of transfusion reactions

Blood transfusion is a lifesaving process that has seen many a people gaining back their lives. Unfortunately, this does not come without any risks. There are times when the clinician has to weigh the gains against the risks. The risks include infections, blood transfusion reactions inclusive of non-immune and immune related reactions. Immune reactions normally happen when an allergic reaction takes place. It could also be caused by the body's immune system if not compatible with blood that is being transfused and thereby attacks the blood components. So much care and attention is being given in order to prevent these reactions but they still occur in rare occasions.

Depending on what will have caused the reactions some of them can be very severe and some can be mild and in some rare occasions they can even be fatal. Identifying a transfusion reaction on time can be key in resolving its further complications or prognosis. Anaphylactic shock can be caused by severe transfusion reactions. Allergic reactions like wheezing, itching and hives are symptoms of a mild reaction. Some patients require a closer blood match than that provided by the ABO positive/negative blood typing. That is why an African-American blood donation may be the best hope for the needs of patients with sickle cell disease, many of whom are of African descent (Tinegate, 2012). There is need though to do a research in order to establish any eveidence that could suggest that donors and receipents from the same background have reduced rate of transfusion reactions.

Haemovigilance Policy Document, 2015 for Namibia under the National Blood Programme looked at evaluating patient safety, and a system has been designed to capture both adverse recipient events (such as non-haemolytic or haemolytic transfusion reactions) and errors (such as the misidentification of patients, either during the collection of pre-transfusion blood samples or at the time of the actual transfusion).

The Namibian Haemovigilance system focuses on the improvement of procedures and on the prevention of adverse transfusion reactions. It is thus designed to provide data for the analysis and tracking of donors for the look back programs and deviations from standard operating procedures and transfusion errors.

2.2 Safety of Blood supply

The safety of the blood supply is dependent on several "layers of safety", including –
- The collection of blood from properly screened, voluntary, non-remunerated donors.
- The testing of all donated blood for transfusion-transmitted infections (including syphilis, hepatitis B and hepatitis C and HIV) using sensitive reagents and tests, and the routine quarantine of all blood products until infectious disease testing and final donor eligibility determination has been completed.
- Maintaining lists of deferred donors (persons either temporarily or permanently excluded from blood donation).
- The investigation of all blood and blood products affected by errors, accidents, or any other event that could jeopardize blood product safety(Gacub,2006)

Complications of transfusion can still occur despite these rigorous safeguards. The risk of error associated with the administration of a particular blood product to a particular patient is a persistent concern and needs to be closely monitored.

2.3 Adverse Transfusion reactions

A recent review article (Elsayid, 2017) classified adverse transfusion reactions as early (onset during or within hours of the transfusion) or late (onset days to months following transfusion) and provided estimates of reaction occurrence. Although the estimates varied considerably depending on the study, severe reactions have fatal event rates of between 1 per million and 1 per 8 million transfused components. Severe early reactions such as Acute Haemolytic Transfusion Reactions (AHTR) showed fatal events to be 1 for every 2.5 to 6 million blood recipients transfused. Febrile non-haemolytic reactions, while uncomfortable for the patient, are not usually associated with severe morbidity or mortality and are reported to occur in approximately 1 in 100 recipients transfused. This number is variable and depending upon the type of product administered and previous transfusions and pregnancies in the recipient.

Few countries in sub-Saharan Africa make systematic screening for antigens C, c, Kell, E, and e in the donors and recipients, thereby protecting transfused patients (Siransy, 2014). It is important that blood group antigen frequencies are determined in order to assess the risk of antibody formation so that those patients with red cell alloantibodies can be assisted. This normally happens with incompatible pregnancies and blood transfusion. The red blood cells that have antigens that are not present in the host blood circulation will evoke antibody reaction. If this happens, delayed or acute haemolytic transfusion reactions might occur as well as HDFN and may be fatal (Dean, 2005).

There is the need also for evidence-based research that antibody testing is essential in pregnancy. Almost 71 % of the alloantibodies that were identified by Markroo et al, 2014 were from the D, E, e, C, c and Kell and not Rh. They also concluded that it's very necessary to extend the antigen typing to the above and that complete matched blood is supplied to the recipients. If the identification is done as a routine, it makes it faster and easier to supply antigen specific blood and avoid unnecessary reactions or sensitizations (Markroo, 2014).

In South Africa, the most common blood type is O positive constituting 45% of the population and ethnic groups differ in the mix of these blood types. Latino-American people, for example, have a relatively high number of O's, while Asian people have a relatively high number of B's (Siransy, 2014). It is important that blood group frequencies are determined in order to assess the risk of antibody formation so that those patients with red cell alloantibodies can be assisted. It will be necessary to evaluate if there is any available blood that is devoid of the antigens that they react to.

There is need for evidence-based research where antibody testing is essential in pregnancy. The testing should be carried out at least at 16 weeks when the pregnant woman visits the ante-natal clinic and also at 28 weeks of gestation. Antibodies to the Red blood cell antigens that have the potential to cause neonatal risks can be identified at this stage. Some of the antibodies can even cause perinatal loss if they are not detected on time. Some of them can even cause jaundice which can lead to hyperbilirubinemia and anaemia (Strotmann, 2017).

With the new methods that have now been developed in foetal medicine, early diagnosis of these situations will improve the non – invasive new methods like the Middle Cerebral Artery (MCA) evaluation. The Doppler ultrasound scanning that is now available in checking on the progress of the foetus especially where anaemia is concerned will improve on the prognosis of the unborn foetus. According to the Royal College of Obstetrics and Gynaecology (RCOG) Green Top Guidelines, genotypes can now also be determined by the use of maternal blood samples' deoxyribonucleic acid (DNA). These new methods are not yet available in developing countries.

The use of serological methods is still being widely used. There are still errors that are being recorded in the use of the anti D immunoglobulins. There are even gaps that are not covered during the protocols that are being used during the screening of the samples. Hence the need to see the prevalence if these rare antibodies (Anti-C, Anti- c, Anti-E, Anti-e and Anti-Kell) in the maternal blood and detect the titre before the baby is born.

The recommendation will have an impact on the testing protocols in order to improve the well-being of the unborn baby and the mother. As the research is being done information on the women who have been previously given prophylactic Anti-D (RhoGam) should be recorded and the dosage should also be recorded. It is also necessary to have the information on any previous neonates/babies who will have been affected by the HDFN. Even though a general antibody screening test can be carried out it's possible that some of the antibodies might not be detected. The main aim of the cross-match is to make sure that the patient/ recipient receives ABO compatible blood (Scheffer, 2011). Some countries have already started pre-transfusion typing of all antibodies but Namibia types a few of the basic ones.

According to White et al, (2016) in THE United Kingdom, they have revised a guideline for the red cell antibody testing in expectant mothers. This document was meant to be used for the prediction of haemolytic disease of the new-born and where possible even prevent it. This prediction and testing are normally done at about twenty-eight (28) weeks of pregnancy or gestation in order to detect an antibody in the red cell that can be of clinical significance. Some of the foetal risks like jaundice, anaemia or even perinatal loss that is linked to the anti-K, anti-c and anti- D. RHD is routinely tested at NaMBTS.

Clinically significant antibodies might affect the new-born or the foetus and might also affect the provision of blood components that are compatible with the mother and even the new-born where necessary. According to Kahar and Patel 2014, only about 1% of pregnant women are known to have clinically significant antibodies. It has also been recommended that if any antibodies are detected, then further tests should be done to determine the concentration and even the specificity where possible. A titration method can be employed to determine the concentration and rule out the possibility of HDFN.

There are some instances where the identification of antibodies might require the paternal sample to be phenotyped as well. Doing this might give information that can be useful in the prediction of the foetus displaying any relevant antigen that might be problematic. This way might not be the best way of identifying these antibodies since it's not always the case that the partner is the father of the baby. In some severe cases, this might be complicated by the need to do a DNA to check on paternity which might even bring more complication if the supposed father's genotype does not match. In this case, it might be recommended to go straight to foetal genotyping and leave out paternal testing. This testing does not only safeguard the current baby but even future pregnancies. Partner testing is far much cheaper though than foetus testing.

According to Koelewijn et al. (2008), on work done in Netherlands, Anti-c and Anti-K and Anti-D are most implicated antibodies that cause haemolytic disease of the new-born and that needs antenatal intervention. Women who have had a previous history of HDFN should be referred to special hospitals or clinics even if their current antibody specificity has not been identified. Before any transfusion is carried out, additional antibody specificities should always be carried out.

It has also been noted that massive transfusion can also lead to a transfusion reaction. There is, therefore, a massive transfusion protocol that is available in Namibia so that when the reaction does happen they are confused with antibody transfusion reactions. The adult protocol differs from that for paediatrics. It's also possible that some Clinicians don't pay much attention to the protocols hence the death of even some patients. When reviews are being done, the first suspect is the antibody transfusion reactions and only after thorough tests are carried out will the actual cause be established.

According to Yu *et al* (2016), the alloimmune − mediated haemolytic transfusion reactions, together with the haemolytic disease of the new-born and foetuses are caused by alloantibodies directed at the red blood cells. The frequencies of the allo antibodies differ in different populations as well as different geographical areas. In a research carried out by Yu in Mainland China, it gave data on mediated haemolytic transfusion reactions which was limited. There is still need to carry further investigations on mediated haemolytic transfusion reactions to come up with valuable results. Their results showed that Rhesus anti (D) was the most common among all the antibodies that were investigated with about 98.94% followed by Anti-e with 92.28 %. Anti-C had 88.81 % and Anti-c with 58.43% and finally Anti-E had the least with 50.78%. Anti-Kell was found to be present in 100% of the donors.

Yu et al (2016) also mentioned the importance of having donor banks that are established from antigen typing. This will be established from known phenotypes from the population located in the Mainland. The establishment of donor banks can be worsened in places where the ethnic groups are diverse. This will also benefit patients who will have had multiple transfusions and also to avoid allo-immunisation in those who are susceptible like the pregnant women and the very young. Some conditions, by their nature require multiple transfusions like thalassemia, aplastic anaemia and sickle cell disease. It has also been noted that there is a variation of blood group antigens amongst ethnic and racial groups when different populations are considered for example: Europe, Africa, America and Asia.

Gundel (2018) in her presentation titled "Is it true that the majority of alloantibodies in Namibia are unidentified" presented at the 9TH Congress of Africa Society for Blood Transfusion (AfSBT), 19-22 June, Arusha, Tanzania looked at this scenario on a very objective manner. In her paper, she looked at proving the specificity of red cell antibodies. She also carried out a retrospective study on blood donor records, transfused patients and antenatal patients. The results that she found showed that unidentified antibodies were those ones that had no satisfactory conclusion using the roll-out method. The identified antibodies included those clinically significant antibodies to the known antigens e.g. D, C, c, E, e, Kell, Duffy and Kidd. Others were MNS, Lewis, and the P system. In-conclusive results also included different test results like Data Auto allo-antibody positive, full screen negative, cold and warm acting unidentified antibodies and Nucleic Acid Testing (NAT) positive unidentified antibodies.

The major causes of unidentifiable antibodies included autoantibodies masking alloantibodies, multiple antibodies, techniques and equipment used and the general incompetence of persons performing the tests. They then recommended the purchase of more complicated equipment and training of personnel and to minimise the use of emergency blood and the giving of Rh ABO group specific.

Markroo (2013) reported that when dealing with patients who have developed multiple alloantibodies, it is important to know the frequencies of various antigens. This is in order to give compatible blood to these type of patients. Many countries including Namibia still give blood that has been randomly cross-matched to patients.

With regards to maternal antibodies (White *et al,* 2016), a further determination of the specificities of identical antibodies should be carried out to determine their strength or concentration in order to determine the possibility of HDFN. Antibody titration is mostly used to check and determine those that are clinically significant like Anti-K besides the usual common ones like Anti –c and Anti-D. There is also a great chance of more than one antibody specificity could be selected. Issues of inclusion of paternity testing were also discussed by White et, al (2016) if antibodies have been identified in maternal blood that is capable of causing HDFN.

The father's sample can show the extent or likelihood of the foetus showing the red cell antigens that can cause HDFN and this might be used to counsel the couple on pregnancies in future. Complications will arise where the partner might not be the actual biological father. Where such complication could be foreseen it would be advisable to omit this altogether and use foetal genotyping which is non –invasive. This is also the same case where the pregnancy has been assisted with donated sperms because the father might not be found or be available farther testing. In more developed countries where cell-free foetal DNA (cffDNA) genotyping is available, it might be advised to proceed directly to that and omit the involvement of the father. The cffDNA can also be performed for RHC, RHE, RHc and RHD.

Finland, Netherlands and Denmark have introduced foetal typing of RHD as a routine to all pregnant women in order to minimise exposure of old and young women to blood products and

reduce the use of prophylaxis with Anti-D Immunoglobulin (Ig) with a reduction in medical costs. With regards to the antibodies that are mostly implicated in severe HDFN, it has been noted that mostly anti-D, anti-K and anti-c are the ones implicated in HDFN (Koelewijn *et al.*, 2008). A previous history of pregnancy with HDFN is generally regarded as risky and where possible referred to a specialist hospital for easy management. It is always advisable to carry transfusion on mothers and babies after a thorough check of additional antibody screening even if it is an emergency.

2.2 Barriers in recognition and reporting of Acute Transfusion Reactions

It was noted that in 2011, only about 20 blood transfusion reactions were reported and recorded in Namibia (Haemovigilance Report, 2011). This constituted about 0.1 % of all the transfused units from NaMBTS. In comparison, this showed that it was lower than the international records which stood at between 1-3%. It was then concluded by Basavaraju *et al* in 2013 that the chances of under reporting were very high and it was possible that the staff members were failing to recognise blood transfusion reactions. Therefore, there was need to estimate the actual prevalence of transfusion reactions, compare the reported statistics with the actual incidences that were found in the records and grade the transfusion reactions detected (Basavaraju, 2013).

A survey based on WHO guidelines (WHO, 2004) was designed to gather all the required information on transfusion practices in Namibia. One of the questions was specifically asking health care workers if they could identify the signs and symptoms of transfusion reactions such as transfusion – associated dyspnoea, transfusion associated circulatory overload, allergy, and sepsis due to bacterial contamination of donor unit, circulatory overload and many other signs and symptoms. Some gaps were found on the reporting competence and a retraining program was proposed and initiated (Basavaraju, 2013).

CHAPTER THREE: METHODOLOGY

3.1 Defining the scope of the Thesis

The thesis covered the assessment of phenotypic properties of the Rhesus and Kell blood group systems among expectant mothers and blood donors in Namibia. Only donated blood and blood from antenatal patients was assessed. Expectant mothers are routinely checked for the determination of their ABO blood group and Rh. The regions that were assessed were the Khomas, Omaheke and Hardap regions. They were not pre-selected but happened to be the regions that had blood collected during the assessment period. The age and sex were also not specific since donations were done by anyone from the ages of 16 to 65 years. Only five (5) parameters were assessed thus- C, c, K, E and e antigens.

3.2 Methodology

Reagents and procedures were according to Rapid labs Ltd insert 2016, Essex, United Kingdom Anti –E, Anti-e, Anti- Kell, Anti Cellano, Anti- C and Anti –c reagents were used to determine the corresponding human red cell antigens. Monoclonal human IgM antibodies were used as the blood grouping reagents. Agglutination was the basis of the test procedure. When red cells with a specific antigen are mixed with the corresponding reagent, agglutination was expected. If agglutination took place it meant that the test was positive for that particular antigen. If agglutination did not take place it showed the absence of that particular antigen and the test was regarded as negative.

These reagents had 0.1 % (w/v) bovine material and sodium azide. The porcine material is at times added to an Anti-e reagent since it is an accessory molecule in the recognition of foreign antigens. They can be used on microplates, slides and tubes. Any Medical Laboratory Technologist/ Technician trained in serology should be able to carry out the tests. The reagents can be used without any further dilution (Rapid labs Ltd insert 2016).

The reagents were stable at 2-8^0 C depending on the expiry date of the reagents. Positive and negative controls were used with each batch that was being tested. Even though these reagents were found to be negative from most infections and viruses e.g. HBsAg, HIV, EBV, HCV, they were regarded as potentially infectious and hence used with caution as if they were human specimens. If the reagents showed some turbidity, it was an indication of some bacterial contamination. If an abnormal consistency was seen, like agglutination, particles and fibrin formation the reagent was no longer fit for use and should, therefore,have been discarded. There was no need to do any special preparation of a patient before blood was collected. Microbial contamination and gross haemolysis are criteria for exclusion of testing using these reagents. If the specimens are not going to be tested immediately then they should be stored at 2-8^0 C. (Rapid labs Ltd insert, 2016)

These reagents had 0.1 % (w/v) bovine material and sodium azide. This experiment had the option of using microplates, tubes or slides but the researcher opted to use microplates because of the convenience of running multiple tests at the same time. The reagents that were used did not need any dilution.

3.3 Materials used in this study

Microplate test- Microplate, microplate shaker, centrifuge (100 rcf), isotonic saline and timer.
Test tube test- Test tubes,cetrifuge (1000 rcf),isotonic saline, 37^0 C incubator and timer.

3.4 Step by step testing protocols

Microplate Method
1. Using a microplate, one volume (25–50 μL) of each dilution of the blood group reagent (Anti –E, Anti-e, Anti- Kell, Anti- C and Anti –c) was added to one volume of 2–3% test red cells.
2. The contents of the wells were mixed using a microplate shaker and Incubated at 15–25°C for 15 minutes.
3. The microplate was centrifuged at 100g for 40 seconds. Gently dislodge the red cells from the bottom of the wells using a microplate shaker.
4. The reaction was read macroscopically

All wells that were showing a weak positive were confirmed using the test tube method.

Test-tube method.

1. In this method 3-5% of test red cells were suspended in isotonic saline.
2. This was then placed in an appropriately labelled test tube and one drop (40μl) of reagent was added to a drop (40μl) of the red cell suspension.
3. This was mixed well and centrifuged at 1000 Relative Centrifugal Force (RCF) for 20 seconds.
4. A gentle agitation was done to dislodge the red cells and macroscopically examined for agglutination.
5. All negative tubes were incubated at 370C for 5 minutes and re-examined. Incubation could enhance the reaction of rare phenotypes.
6. If no agglutination was noticed, then it meant that it was a true negative. Agglutination denoted a positive reaction.

3.5 Research questions

- What was the prevalence of C, c, E, e and Kell antigens among voluntary blood donors and expectant mothers in Namibia?
- Was there any particular pattern of the frequency of these antigens in terms of regions where the donors come from?
- Does Namibia need to routinely phenotype donor blood for C, c, E, e and Kell?

3.6 Research objectives

- To determine the prevalence of C, c, E, e and Kell antigens among voluntary blood donors and expectant mothers in Namibia.
- To extrapolate whether Namibia needs to routinely phenotype donor blood for C, c, E, e and Kell antigens
- To determine regional pattern(s) of these antigens.

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3.7 Study Design

The study was a quantitative study design and was also a descriptive cross- sectional study where laboratory procedures were used to characterise the antigens in the donated blood samples and samples from pregnant women.

3.8 Study Population

The population of regular blood donors is about 33,000 per year and that of pregnant women is about 13,000.

3.9 Sampling Method

Specimens were randomly selected from NAMBTS blood bank (2986 donors and 484 pregnant women) for antigen characterisation. Donors donate every day and from the regions as well so donated specimens were collected randomly for a fair distribution of results.

3.10 Sample size

$M = \underline{\pi \text{ plan-}(1\text{-}\pi \text{ plan}}$

$\qquad\qquad SE^2 \text{ plan}$

Prevalence

$\qquad SE (p) \sqrt{\underline{P (1\text{-}P)}}$

$\qquad\qquad m$

Plan = prevalence assumed

Plan = prevalence assumed =2.5% with a 0.005 margin error

Therefore, SE = SE= (0.01/ (2*1.96)) =0.02551

M=0.025(1-0.025)/0.02551^2=3745.56=3746 blood samples needed

Overall sample size= **3746**

Blood donations= 3000

Antenatal Patients samples= 746 was considered as a percentage for the ANC specimens.

ANC= 746√3746 = 19.9%

The study population has a proportion of 20%: 80% on the ANC: Blood donors.

The total number of blood samples that were projected to be carried out was 3746 using the assumed prevalence of 2, 5% (adopted from SA) but a total of 3470 was actually assessed. The researcher could not get the anticipated number of antenatal specimens because the volume of some of the specimens was not enough for processing.

3.11 Data Collection

Blood specimens were collected by the reseacher from NaMBTS to the Namibia University of Science and Technology (NUST) Laboratory. The specimens were collected in tubes covered with paraffin wax. Specimens were kept in the refrigerator at 2-$8°$C for 4 days. NaMBTS routinely test specimens that will have been collected from Khomas and other Regions and discarding them after 7 days. The researcher collected these specimens soon after they were tested for Transfusion Transsissible Infections (TTIs) and returned them to NaMBTS as soon as phenotyping was completed. This was done in order to make sure that the discard rules for blood were followed using bio safety units for incineration. Results were collected on a data collection tool (Appendix A). Laboratory and computer access was provided by NUST.

3.12 Data Analysis

Data on the antigen characterisation was collected and then tabulated for for calculations of prevalence as well as for regional distributions. The prevalence of the different antigens was assessed to determine the current situation on the phenotypes and direction for further research. Data collection and analysis was done over six (6) months. Data were presented in the form of tables, graphical displays and summary statistics. Data were analysed using the SPSS (version 23) data analysis software.

3.13 Reliability/ Validity

All quality control protocols as outlined in the inset were adhered to. Controls (normal and abnormal) were used during processing of specimens to ensure reliability and validity of results. The cold chain method for specimens was maintained using NAMBTS cold chain boxes which were used for boh collection and return of the specimens to NAMBTS. The specimens were stored at NUST refrigerators (2-8^0c) for 4 days.The specimens were tested within the 7 days optimum period required before discard.

3.14 Sampling technique

Random sampling

3.15 Limitations

The availability of ANC specimens was a problem. The researcher only ended up with 484 specimens instead of 746 projected specimens. This was due to the fact that most of the specimens were not collected in large enough volumes to run both tests for NaMBTS and for my research. The researcher could not foresee a few issues concerning ante natal patient's specimens. There was no need really to look into Ante Natal Clinic specimens differently since the outcome of the research was not going to be determined by whether one was expecting or not. If one had say Anti-K antigens the fact that one is expecting would not have changed that fact. These results were proved by almost the same results that were obtained on both the antenatal specimens and donor specimens.

There were problems in acquiring ANC specimens due to the fact that most of them came in very small quantities for ABO and Rhesus (Rh) blood grouping and therefore not enough was left to do phenotyping for some of the specimens. The other fact that made it very difficult for the researcher to get all the projected ANC specimens was the fact that unlike donor specimens which were tested for Transfusion Transmissible Infection like Hepatitis B and C, Syphilis and HIV 1 and 2, the ante natal clinic specimens were not tested for these potential infections. Even though it is well known that any laboratory specimen should be handled as potentially hazardous, more care needed to be given to these specimens. This was one of the main reason only a few about sixty-four percent (64%) of the total ANC specimens were collected.

3.16 Ethical Consideration

The study was approved by NUST ethical committee, the Ministry of Health and Social Services Research Ethics Committee and NAMBTS ethical committee. The Researcher's responsibilities were to make sure that all these approvals were obtained and that all ethical issues were attended to. The Researcher guarded against human rights violations of patients, and did not

allow or participate in any actions that led to violations of the rights of patients. The identity of patients was protected at all times e.g. the use of barcodes on specimens etc.

3.17 Significance/Contribution

It is hoped that recommendations from the study would improve the testing protocols at NaMBTS in order to improve the well-being of recipients of blood and blood products, unborn babies and their mothers. There is need to fill the gap on the prevalence of C, e, E, e and Kell in the Namibian donor population and expectant mothers which is currently not available and to inform the MoHSS and NaMBTS on the impact of the findings. Reviewing the way blood is tested in the country in order to minimize transfusion reactions and haemolytic diseases of the new-born in Namibia will be recommended if the need arises.

1. Filling the gap on the prevalence of C, e, E, e and Kell in the Namibian donor population.
2. Review the prevalence of C, e, E, e and Kell antigens on ante-natal specimens.
3. Advise the MoHSS and NaMBTS on the findings.
4. Review the way blood is tested in the country in order to minimise on transfusion reactions which are on the rise.
5. Review how the findings affect the likelihood of the haemolytic disease of the new-born in cases of maternal antibodies in Namibia.
6. There is need worldwide to see that the medical care of transfused patients is improved.

CHAPTER FOUR: RESULTS

The results that were obtained from the 3470 specimens that were processed were tabulated and graphically presented. The research proposal had indicated that a total of 3746 would be screened but due time, reagents and specimens' availability only a total of 3470 specimens were screened which was still a significate figure. This being the first research ever to be done in Namibia in this area this could be used as baseline results in future studies and this shows the prevalence of the Antigens in the Namibian population. The prevalence was also noted on the antenatal patients.

The research did not have a hypothesis since it did not compare the results with any other here in Namibia but just to assess the frequencies of the phenotypes hence there is no p-value. This was the first time this type of research has been carried out locally.

4.1 Frequencies of the different phenotypes

When all the data was in-cooperated into the statistical data sheet format and processed no data were missing.

From the results that came out, it showed that of the total 2986 specimens that were processed from the Namibia population of blood donors, 2009 representing 67.3 per cent were non-reactive to the five (5) panels of antigens that were used. Of that total 267 (8.7%) of them were reactive to Anti-K. A total of 179 specimens were reactive to Anti-C representing 6.0%. Anti-c was reactive to 163 specimens which were 5.5% of the total analysed specimens. Anti-E was reactive with 63 of the specimens representing 2.1%. Anti-e was reactive with 311 of the specimens representing 10.4%. This is how the prevalence of Anti-K, Anti-C, Anti-c, Anti-E and Anti-e is in the Namibian donor population according to this research. The graph below shows how this can be represented graphically.

Table 4.1: Is a representation of the frequencies distribution of the different phenotypes
(Kell, C, c, E and e) in the Namibia population N=2986

Phenotype	Frequency (n)	Percent (%)	Cumulative (%)
K	261	8.7	8.7
C	179	6	14.7
c	163	5.5	20.2
E	63	2.1	22.3
e	311	10.4	32.7
Non- Reactive	2009	67.3	100
Total	2986	100	
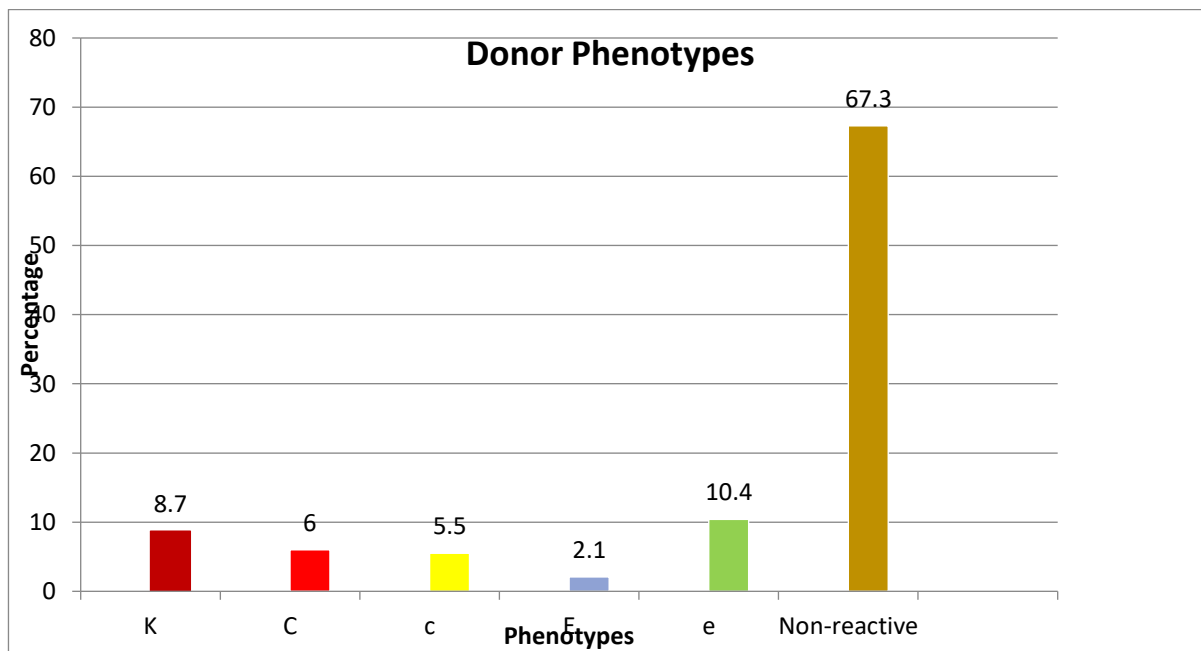
Figure 4. 1: Different phenotypes in the donor blood groups N=2986

Table 4.2: Frequency of (Kell, C, c, E and e) in the Namibian Regions- Khomas, Hardap and Omaheke.

Regions assessed

Region	Frequency (n)	Percent (%)	Cumulative Percent (%)
Khomas	2550	85.4	85.4
Hardap	330	11.1	96.5
Omaheke	106	3.5	100
Total	2986	100	

Most of the specimens came from the Khomas region and hence the higher volumes during the said period of experiment and graphically represented below. Khomas had 85.4% of the total specimens followed by Hardap with 11.1 % and finally Omaheke with 3.5 %
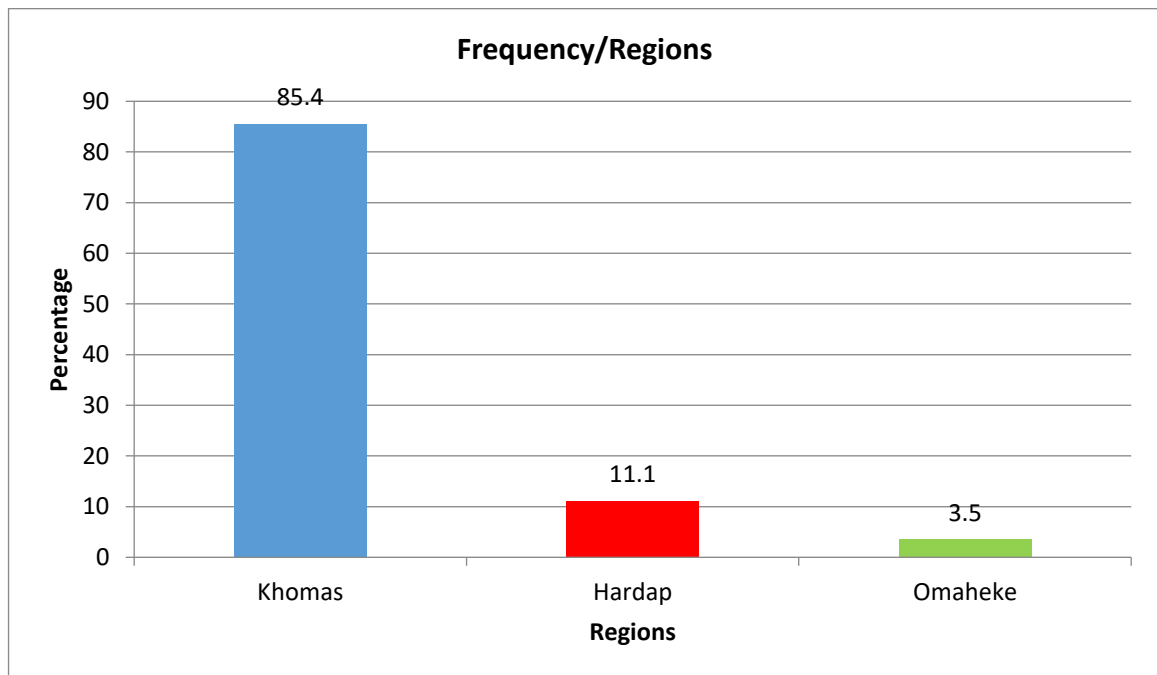
Figure 4.2: Frequency of phenotypes/ regions

The frequencies on age groups below <18 for the donors were 202 representing 6.8% of those specimens that were phenotyped. For those aged between 19-24, there were 783 (26.2 %). Age group between the ages of 25-44 had the highest number with 1341 (44.9%). The 45 to 64 age group had 603 specimens (20.2%). Very few above 65 donors came to donate blood representing 1.9 % of the total population that was phenotyped. Age is also shown below graphically.

Table 4.3: Frequency of the ages of donors

Age (Yrs.)	Frequency (n)	Age of donors Percent (%)	Cumulative percent
≤18	202	6.8	6.8
>18≤24	783	26.2	33
>24≤44	1341	44.9	77.9
>44≤65	603	20.2	98.1
>65	57	1.9	100
Total	2986	100	
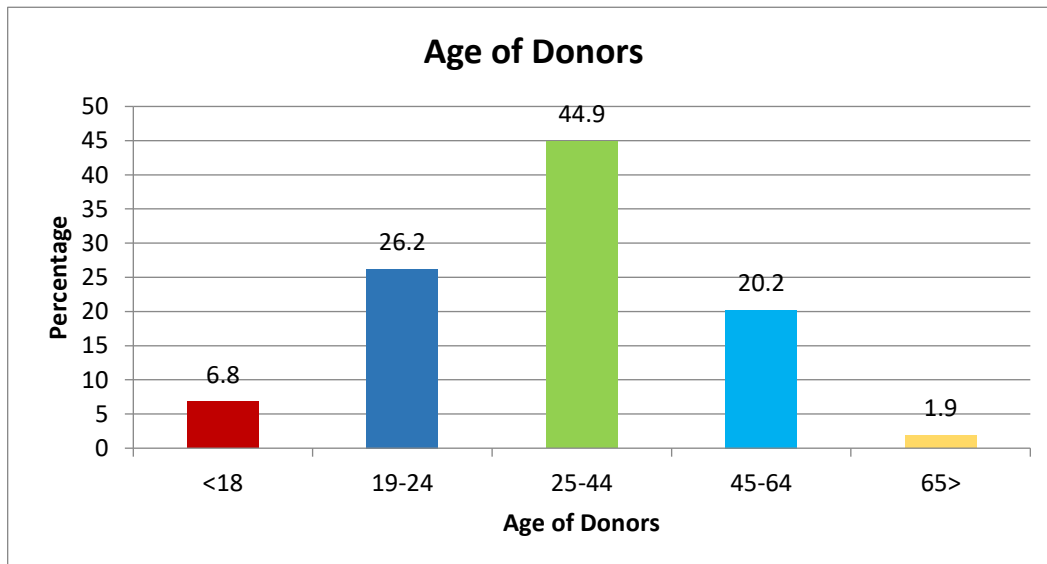
Figure 4.3: Graphical presentation of the ages of the donor participants

Table 4.4: Frequencies distribution according to gender of participants

Gender	Frequency (n)	Percent (%)	Cumulative Percent
Male	1583	53	53
Female	1403	47	100
Total	2986	100	

About 1583 (53%) males were phenotyped as compared to 1403 female (47%)
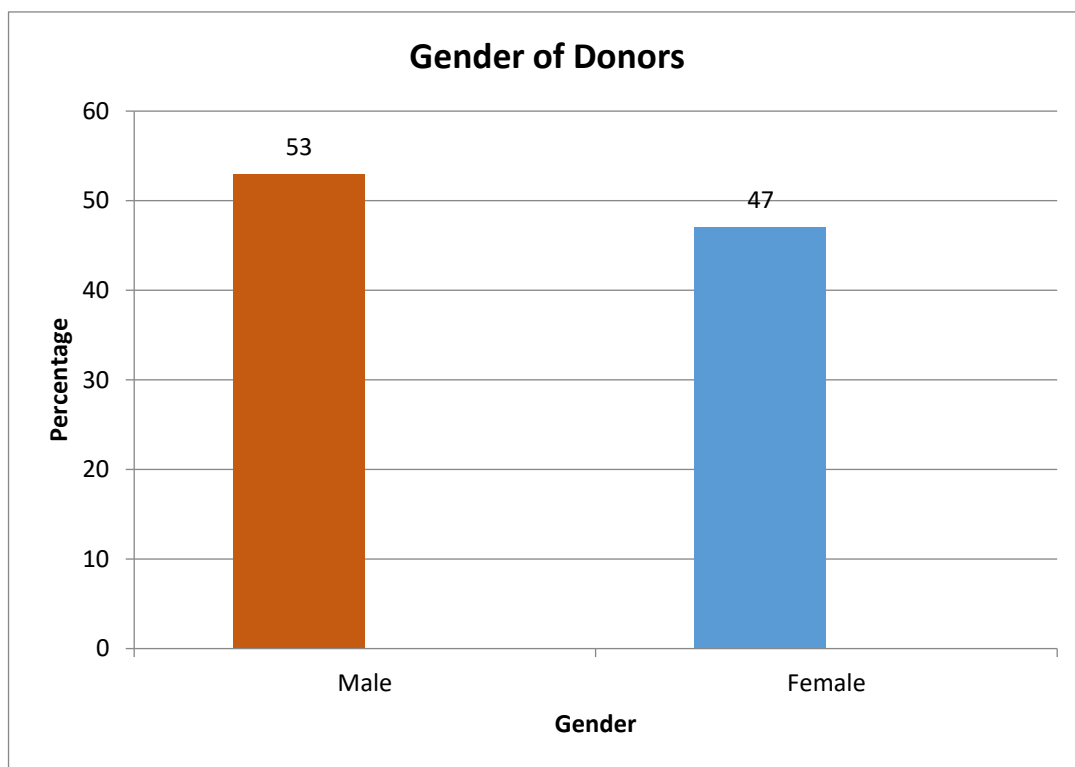
Figure 4.4: Graphical representations of the gender of donors as a percentage

Table 4.5: Cross tabulation of the gender of the donors with regards to the different phenotypes

| Gender | Phenotypes Frequency (n) | | | | | Non-reactive | Total |
	K	C	c	E	e		
Male	147(56.3%)	106(59.2%)	84(51.5%)	35(55.6%)	176(56.6%)	1035(51.5%)	1583(53%)
Female	114(43.7%)	73(40.8%)	79(48.5%)	28(44.4%)	135(43.4%)	974(48.5%)	1403(47%)
Total	261 (100%	179(100%)	163(100%)	63(100%)	311(100%)	2009(100%)	2986(100%)

The cross-tabulation was done to compare the reactives as with age. Males were more reactive to Anti-K with 147 as compared to females with 114 on the same parameter. In general, the males had higher rates in all the parameters when compared to the females as shown on the cross-tabulation above and on the graph below in the different categories.
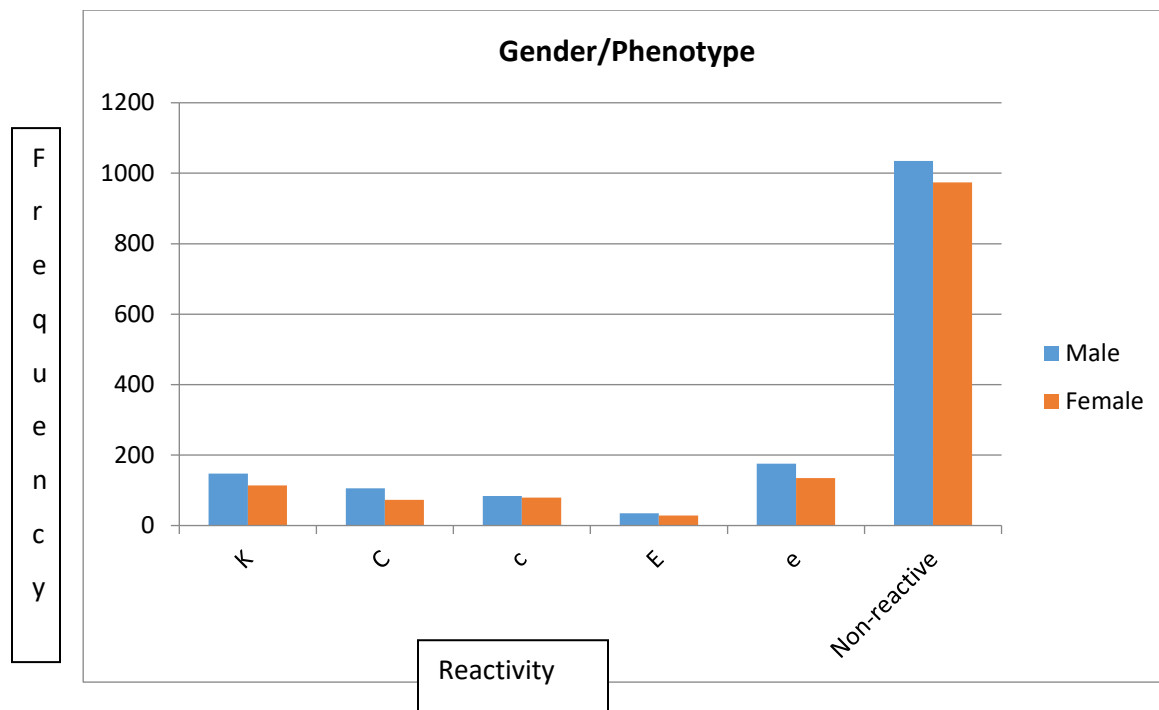
Figure 4.5: Cross tabulation in the different gender and in each category of the phenotypes

Table 4.6: Different phenotypes as compared to the regions where the blood donations came from.

Frequency Reactives (n)

Regions	K	C	c	E	e	Non-Reactive	Total
Khomas	221(84.7%)	154(86%)	141(86.5%)	52(82.5%)	258(83%)	1724(85.8%)	2550(85.4%)
Hardap	31(11.9%)	19(10.6%)	17(10.4%)	8(12.7%)	37(11.9%)	218(10.9%)	330(11.1%)
Omaheke	9(3.4%)	6 (3.4%)	5(3.1%)	3(4.8%)	16(5.1%)	67(3.3%)	106(3.5%)
Total	261(100%)	179(100%)	163(100%)	63(100%)	311(100%)	2009(100%)	2986(100%)

Figure 4.6: Graphic representation of the different phenotypes as compared to the regions

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Table 4.7: Different phenotypes according to the age of the donors

Age as compared to the Phenotypes

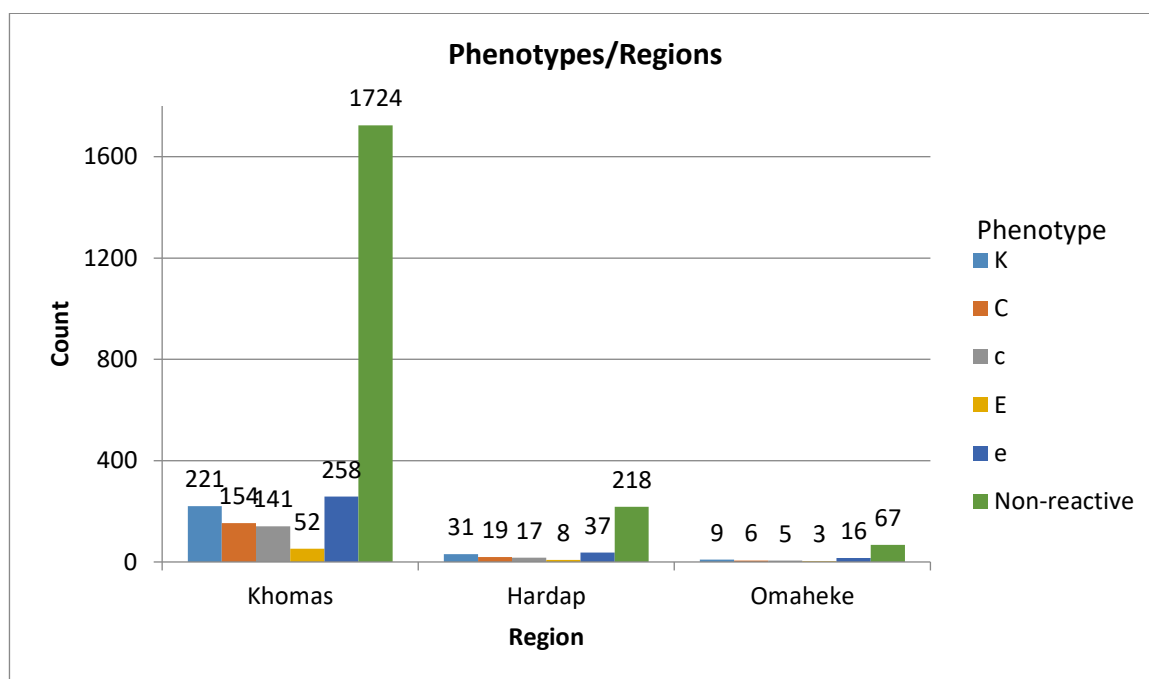| Age(Yrs)	Reactive K	C	c	E	e	Non-reactive	Total
≤18	**20**(8%)	**8**(4,5%)	**8**(4.9%)	**7**(11.1%)	**19**(6.1%)	**140**(7.0%)	**202**(6.8%)
>18≤24	**68**(26%)	**47**(26.2%)	**47**(28.8%)	**16**(25.4%)	**86**(27.6%)	**519**(25.8%)	**783**(26.2%)
>24≤44	**125**(48.9%)	**82**(45.8%)	**73**(44.8%)	**27**(42.8%)	**140**(45%)	**894**(44.5%)	**1341**(44.9%)
>44≤65	**44**(16.9%)	**41**(22.9%)	**32**(19.7%)	**10**(15.9%)	**60**(19.4%)	**416**(20.7%)	**603**(20.2%)
>65	**4**(0.2%)	**1**(0.6%)	**3**(1.8%)	**3**(4.8%)	**6**(1.9%)	**40**(2.0%)	**57**(1.9%)
Total	**261**(100%)	**179**(100%)	**163**(100%)	**63**(100%)	**311**(100%)	**2009**(100%)	**2986**(100%)
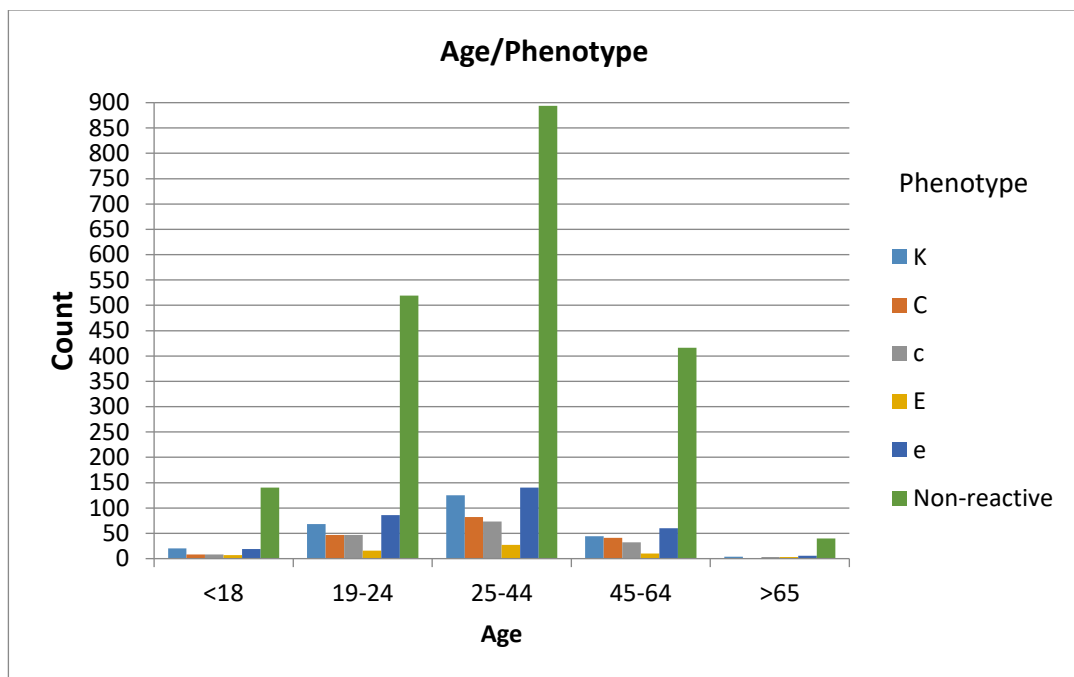
Figure 4. 7: **Cross tabulation of phenotypes according to the age of the donors**

The 25-44 age group had the highest volumes corresponding to the way they reacted to the antigens that were being used.

A total number of 484 specimens were collected for phenotyping. Of these 324 (66.9%) specimens were non-reactive. When the antenatal clinic specimens were phenotyped, they showed almost the same frequency as the donor specimens. Anti K was reactive to 35 specimens (7.2%), Anti-C, 34 (7.0%). Anti-c reacted to 30 (6.2) of the specimens. Anti –E reacted to 10 (2.1%) and finally Anti- e reacted o 51 (10.5%). These are also graphically represented below.

Table 4.8: ANC phenotypes that were assessed N=484

ANC PHENOTYPES

Phenotype	Frequency (n)	Percent (%)	Cumulative Percent(%)
K	35	7.2	7.2
C	34	7	14.3
c	30	6.2	20.5
E	10	2.1	22.5
e	51	10.5	33.1
Non -Reactive	324	66.9	100
Total	484	100	
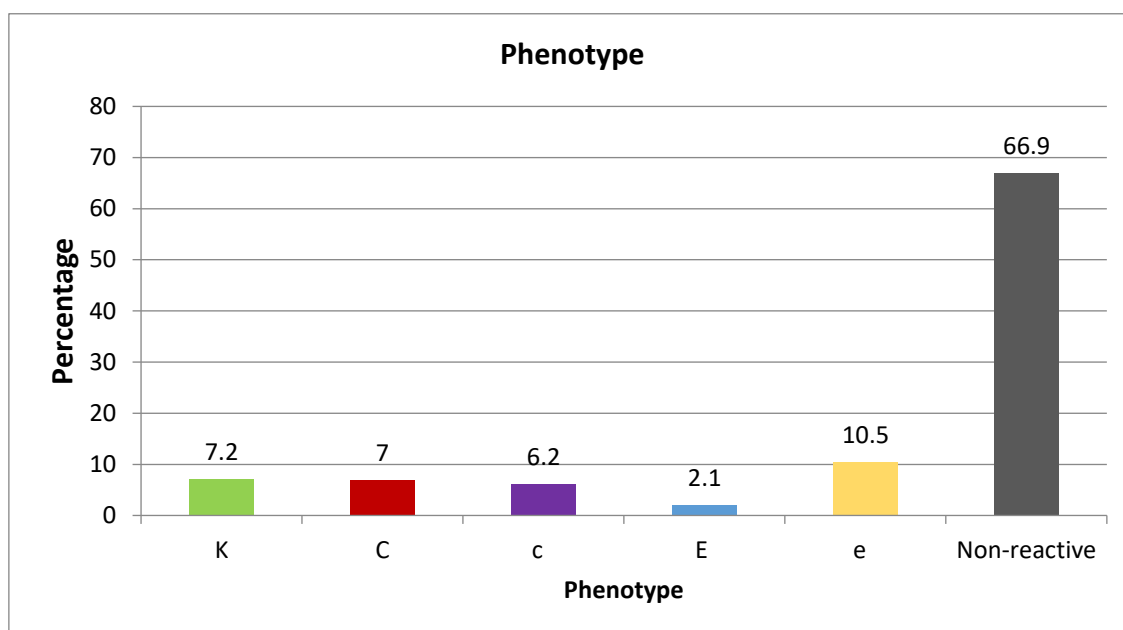
Figure 4. 8: ANC Graphical presentation of the phenotypes

Table 4.9: **F**requencies of reactivity as compared with age group −

Age(Yrs)	Frequency (n)	Percent (%)	Cumulative Percent(%)
≤18	51	10.5	10.5
>18≤24	246	50.8	61.4
>24≤44	183	37.8	99.2
>44≤65	4	0.8	100
>65	484	100	
Total			

The above frequencies show reaction as compared with age group. The higher the number of specimens on the age group, the higher the reactives as well.
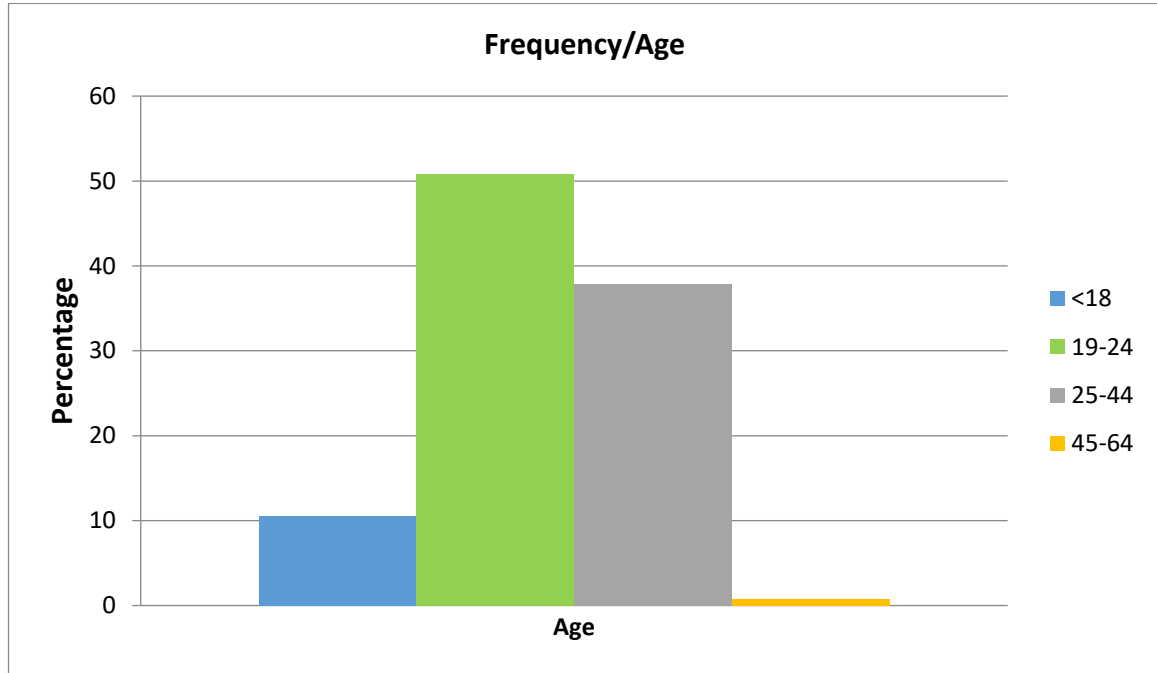
Figure 4. 9: Frequencies of reactivity as compared with age group

Table 4.10: Cross tabulation of age versus the Phenotypes in numbers

ANC AGE VESUS PHENOTYPES

Age	K	C	c	E	e	Non-Reactive	Total
≤18	4(11.2%)	4(11.8%)	3(22.0%)	1(10%)	4(7.8%)	35(10.8%)	51(10.6%)
>18≤24	20(57.2%)	16(47.1%)	17(56.7%)	4(40%)	27(52.9%)	162(50%)	246(50.8%)
>24≤44	10(28.7%)	14(41.1%)	10(3.3%)	5(50%)	20(39.3%)	124(38.3%)	183(37.8%)
>44≤65	1(2.9%)	0(%)	0(%)	0(%)	0(%)	3(0.9%)	4(0.8%)
Total	35(100%)	34(100%)	30(100%)	10(100%)	51(100%)	324(100%)	484(100%)
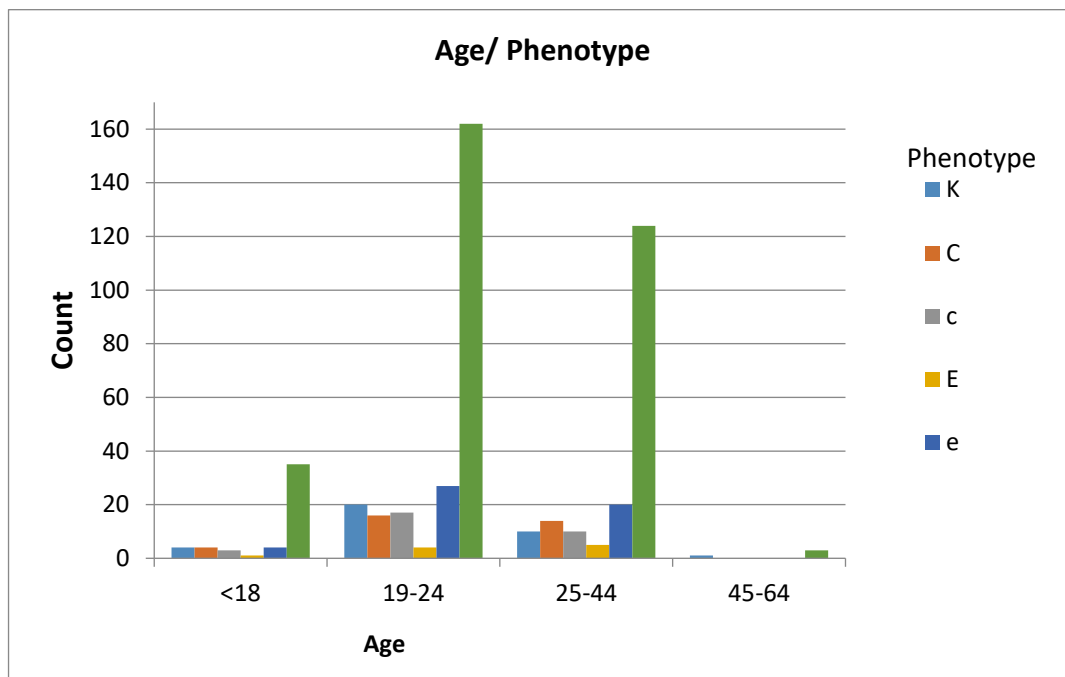
Figure 4. 10: Bar charts cross tabulation of age versus the Phenotypes in numbers (ANC)

CHAPTER FIVE: DISCUSSION

The information on the different blood group antigen prevalence is important in any population where there is a need to manage allo-immunisation. This is more so where there is need to manage patients who would be on dialysis, suffering from thalassaemia, those with sickle cell anaemia and even cancer patients. These groups of patients are likely to develop antibodies against some of the antigens that are frequent in a population. It is not possible to match most of these antigens before every transfusion. Knowing the prevalence of these antigens in a given population will assist in the safety of blood transfusion of such patients.

Unfortunately, the results that were found could not be compared with any other in Namibia since this was the first time this research has been carried out in Namibia. Comparing with other ethnic populations like India showed that different ethnic groups have different frequencies. Unfortunately, the screen cells that are mostly used to screen these antigen profiles are mostly commercially made from western countries whose antigenic profiling might be different from what the population needs. These are what is used to detect the antibodies in the patients as well.

Blood safety can be greatly improved by screening all the recipients of a blood donation. Namibia borders Angola on its northern borders and there is a lot of inter marriages amongst the populations there. Namibia is overwhelmed by patients from Angola that seek better medical services. These patients also do receive blood transfusions when the need arises and they are from a different ethnic group which might lead to problems.

The population size of Namibia is about 2.6 million using the latest 2018 demographic censorship that was done and NAMBTS collects an average of about 30,000 units per year so the tested volumes are significant as compared to the population. The blood samples collected were from both the Caucasian and Black Namibian population. The distinction was not made at the point of collection of specimens by the researcher if the donor was caucasian, black or coloured. Namibia does not differentiate on who gets whose blood (white, black or coloured) as long as it is

compatible. The only distinction that NaMBTS makes is the return of blood from malaria area back to the malaria area mainly because the clinicians there know well malaria symptoms if they should show any signs.

5.1 Conclusion

This research aimed to fill the gap on the prevalence of C, e, E, e and Kell antigens in the Namibian donor population which had never been done. The prevalence is shown in **Table 2** with the total 2986 specimens that were processed from the Namibia population of blood donors, 2009 representing 67.3 per cent were non-reactive to the five (5) panels of antigens that were used. Of that total 267 of them were reactive to Anti-K representing 8.7% of the total analysed specimens. A total of 179 specimens were reactive to Anti-C representing 6.0%. Anti-c was reactive to 163 specimens which were 5.5% of the total analysed specimens. Anti-E was reactive with 63 of the specimens representing 2.1%. Anti-e was reactive with 311 of the specimens representing 10.4%. The researcher succeeded in filling that gap.

The prevalence was also assessed on antenatal patients and of the total of 484 specimens that were phenotyped, 324 (66.9%) specimens were non-reactive. The ANC specimens showed almost similar frequency as the donor specimens. Anti K was reactive with 35 specimens (7.2%), Anti-C, 34 (7.0%). Anti-c reacted to 30 (6.2) of the specimens. Anti –E reacted to 10 (2.1%) and finally Anti- e reacted to 51 (10.5%).

This research will open gaps for further research on the need for Namibia to establish a database for typed donors in Namibia and then store blood that is antigen negative and reduce the risks of Haemolytic Disease of the Foetus and the New-born (HDFN) and Haemolytic Transfusion Reaction (HTR).

5.2 Recommendations

The findings will be communicated to NaMBTS and MoHSS and the following recommendation will be given

1. A review of the way blood is tested for transfusion in order to minimise on transfusion reactions.

2. Where necessary also review the protocols on maternal health using the available knowledge on the prevalence of maternal antibodies especially with regards to Anti-e which appeared quite high in this research.

3. Include Anti-e on the panel list for routine testing.

4. Further research needs to be carried out to check on the ethical prevalence of these antigens (Kell, C, c, E, and e) looking into other regions especially in the North and South and the VaHimbas including the San community.

5. There is need to reduce the blood transfusion reactions and safeguard maternal health

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Rapid labs insert revision 2, 2016

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Appendix A: Data collection Tool

Numbers	Phenotype	Gender	Region	Age			Key
1						Gender	
2						Male	1
3						Female	2
4							
5						Age	
6						16-24	1
7						25-45	2
8						46-65	3
9							
10						Regions	
11						Omaheke	1
12						Khomas	2
13						Hardarp	3
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Data collection tool							

Appendix B:SOP on Phenotyping Rhesus(C, c, E, e) and Kell blood groups

VERSION No	1.0
DATE OF ISSUE	15/06/2018
REVIEW INTERVAL	24 months
AUTHORISED BY	Mary N. Mataranyika
AUTHOR	Mary N. Mataranyika
COPY	Electronic Master copy
LOCATION OF COPIES	1. M.N.M Desktop 2. Students folder.

Document review history		
Review date	Reviewed by	Signature

1.0 INTRODUCTION

The alloantibodies, which frequently develop and are encountered during compatibility testing, are primarily against antigens related to Rhesus (Rh) blood group systems including C, c, E, e and Kell blood groups. Antibodies directed against these antigens can cause severe Transfusion Reactions and are implicated in cases of Haemolytic Transfusion Reactions (HTRs) and Haemolytic Disease of the Foetus and the New-born (HDFN), and are, therefore, regarded as clinically significant. The frequencies of these antigens vary in different populations.

1.1 Responsibility

It is the responsibility of the research team to ensure that the implementation of this procedure in conjunction with other basic laboratory procedures is abided by.

1.2 Definitions

- DAT   - Direct Antiglobulin Test
- EDTA - Ethylenediaminetetraacetic acid
- HTRs - Haemolytic Transfusion Reactions
- HDFN - Haemolytic Disease of the Foetus and the New-born
- MAP -   Mycobacterium Avian subspecies Para tuberculosis
- Rh   -   Rhesus.

1.3 Intended use

Anti-K, C, c E, and e are monoclonal human blood grouping reagents that are used for detecting the corresponding K, C, c, E, e human red cell antigens. These reagents are suitable for use by the test tube, slide or microplate techniques. These reagents are designed for use by operators trained in serological techniques.

1   Principle

When the reagent is used as recommended it causes agglutination (clumping) of red cell carrying the specific antigen (positive test). Lack of agglutination of the red cell demonstrates the absence of the specific antigen (negative test). The reagent has been optimized for use by the recommended techniques without further dilution or addition.

2   Composition

The reagents contain monoclonal human antibodies in a buffer solution with macromolecular chemical potentiators, sodium azide (0.1% w/v) and bovine material. In addition, anti e contains porcine material

3   Precautions

Treat all blood products as potentially infectious. The human donor or cell line used to prepare the reagent was tested and found to be negative for Anti-HIV, anti-HCV, Anti-HBsAg, Anti-EBV and MAP viruses. No known test can guarantee that products derived from human or animal sources are free from infectious agents.

Take appropriate care in the use and disposal of the product container and its contents. Turbidity may indicate bacterial contamination. Dispose of the product by overnight immersion in disinfectants at appropriate concentration or by autoclaving. These reagents contain sodium azide which might be toxic if ingested.

4   Controls and Advice

Testing of a positive and a negative control in parallel with each batch of tests is recommended. The test result must be considered invalid if the controls do not show the expected reactions. The use of reagent control in parallel with all tests done with this reagent is not required. Use of reagent control is recommended only in typing the red cells of patients known to have auto antibodies or protein abnormalities.

5   Storage and Stability

Store unopened and previously opened reagents at 2-8^{0}C until the expiry of the date on the label.

6   Specimen preparation

No specimen preparation of patients is needed before collection of specimen by any approved phlebotomy technique. Specimens should also be stored at 2-8^0 C. Specimen showing gross haemolysis or microbial contamination should not be tested with this reagent.

7   Material required for the Microplate technique

Microplate, microplate shaker, automatic microplate reader (optional) centrifuge (100rcf)

8   Microplate technique
- Prepare a 3-5% suspension of test red cells in isotonic saline.
- Add one drop (40µL) of either Anti-K, Anti-C, Anti-c, Anti-E or Anti-e reagent as per required test to appropriate test wells of a U-well microplate.

- Add an equal volume (40μL) of the suspension of test red cells to the appropriate test wells.

- Mix the contents of each well manually or with a microplate shaker.

- Incubate the microplate at room temperature for 15-20 minutes.

- Centrifuge at 100rcf for 40seconds.

- Re-suspend the red cells using the microplate shaker as in 4.

- Read tests macroscopically or with an automated reader. The use of an automated reader must be validated by the user.

9   Limitations

Red cells having a positive Direct Antiglobulin Test (DAT) may produce false positive results. The use of a monoclonal control reagent is recommended for the detections of such potentially false positive results.

10   Performance Characteristics

These reagents have been tested by each of the recommended techniques with donor, clinical and neonatal specimens collected in the anticoagulant EDTA and citrate. The sample population represented all major phenotypes. All tests showed 100% sensitivity and specificity.

Appendix C: NUST Registration form for research topics

NAMIBIA UNIVERSITY
OF SCIENCE AND TECHNOLOGY

13 Storch Street   T: +264 61 207 2118
Private Bag 13388   F: +264 61 207 9118
Windhoek   E: registrar@nust.na
NAMIBIA   W: www.nust.na

Office of the Registrar

REGISTRATION FORM FOR RESEARCH TOPICS
[Rule PG1.5]

All **registered** NUST students who are doing research are required to register their research topics as soon as their research proposals are approved by relevant authority

Surname	Mataranyika	S/N 217071716	
First name	Mary Nyaradzayi		Mrs.
Qualification:	Master of Sciences Degree		
Faculty:	Health and Applied Sciences		
Department	Health Sciences		
Supervisor 1 Name	Professor Sylvester Rodgers Moyo		Internal
Supervisor 2 Name	Dr. Innocent Maposa		Internal
Start Date	January 2017		

The registered Research Topic may be amended only with the approval of the relevant authority, i.e. either the department or the HDC.	
Research Topic ☐ Thesis	**Assessment of Phenotypic Properties of the Rhesus and Kell Blood Group Systems** **Among Donors and Pregnant Women in Namibia**

We hereby confirm the accuracy of the details above:

Student's signature

---21/11/2017---------------
Date

Academic Supervisor's Signature

---21/11/2017---------------
Date

Stamp

Appendix D: NUST ethical approval

namibia university
OF SCIENCE AND TECHNOLOGY

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NAMIBIA

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FACULTY OF HEALTH AND APPLIED SCIENCES

DECISION/FEEDBACK ON RESEARCH PROPOSAL ETHICAL CLEARANCE

Dear ~~Prof/Dr/Mr~~/Mrs/~~Other(s)~~: | Mary Mataranyika |

Student No (if applicable): 217071716

Research Topic:	Assessment of phenotypic properties of the Rhesus and Kell blood group systems among blood donors and pregnant women in Namibia
Supervisor (if applicable):	Prof SR Moyo
Co-supervisor(s): if applicable	Dr Innocent Maposa
Qualification registered for (if applicable):	Master of Health Sciences

Re: Ethical screening application No: | REC -0009/2017 |

The Research Ethics Screening Committee has reviewed your application for the above-mentioned research project. Based on the recommendation of the expert reviewer, the research as set out in the application is hereby:
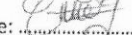
(Indicate with an X)

Approved: i.e. may proceed with the project		
Approved provisionally: subject to compliance with recommendation(s) listed below	X	
Not approved: Not to proceed with the project until compliance with recommendation(s) listed below and resubmit ethics application for consideration		
IS MINISTRY OF HEALTH & SOCIAL SERVICES (MoHSS) APPROVAL REQUIRED?	YES: X	NO:

It is important to note that as a researcher, you are expected to maintain ethical integrity of your research, strictly adhere to the ethical policy of NUST, and remain within the scope of your research proposal and supporting evidence as submitted to the REC. Should any aspect of your research change from the information as presented, which could have an impact or effect on any research participants/subjects/environment, you are to report this immediately to your supervisor or REC as applicable in writing. Failure to do so may result in withdrawal of approval. Kindly consult your supervisor or HoD if you need further clarification.

We wish you success in your research endeavour and are of the belief that it will have positive impact on your career as well as the development of NUST and the society in general.

Ethical issues that require compliance/ must be addressed		
No.	Ethical issues	Comment/recommendation
1.	Research involve characterisation of antigens in the donated blood samples	Require MoHSS/ NAMBTS approval and submit copy to FHAS-REC secretariat*.
2.		

NB: May attach additional page as required; * Failure to do so will invalidate research outcome

Full Name (reviewer): ...PROF YAPO G. ABOUA.. Signature: .... Date: 04/09/2017

Full Name: .... PROF OMOTAYO AWOFOLU... Signature: .... Date: 2|9|2017
Chair: Ethics Screening Committee

REPUBLIC OF NAMIBIA

Ministry of Health and Social Services

Private Bag 13198	Ministerial Building	Tel: 061 – 2032150
Windhoek	Harvey Street	Fax: 061 – 222558
Namibia	Windhoek	Email: shimenghipangelwa71@gmail.com

OFFICE OF THE PERMANENT SECRETARY

Ref: 17/3/3 **MM**
Enquiries: Mr. J. Nghipangelwa

Date: 16 October 2017

Ms. Mary Nyaradzayi Mataranyika
Namibia University of Science and Technology
Windhoek

Dear Ms. Mataranyika

Re: Assessment of Phenotypic Properties of the Rhesus and Kell Blood Grouping Systems among blood donners and pregnant women in Namibia.

1. Reference is made to your application to conduct the above-mentioned study.

2. The proposal has been evaluated and found to have merit.

3. **Kindly be informed that permission to conduct the study has been granted under the following conditions:**

3.1 The data to be collected must only be used for academic purposes;

3.2 No other data should be collected other than the data stated in the proposal;

3.3 Stipulated ethical considerations in the protocol related to the protection of Human Subjects' should be observed and adhered to, any violation thereof will lead to termination of the study at any stage;

3.4 A quarterly report to be submitted to the Ministry's Research Unit;

3.5 Preliminary findings to be submitted upon completion of the study;

Appendix E- Ethical approval MoHSS

3.6 Final report to be submitted upon completion of the study;

3.7 Separate permission should be sought from the Ministry of Health and Social Services for the publication of the findings.
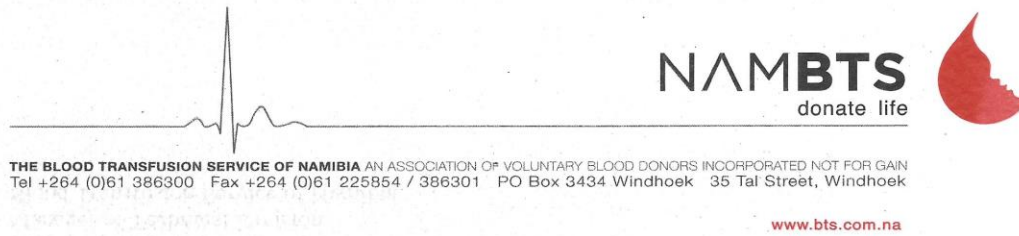
Yours sincerely,

Andreas Mwoombola (Dr.)
Permanent Secretary

"Your Health Our Concern"

Appendix F- NaMBTS approval

NAM**BTS**
donate life

THE BLOOD TRANSFUSION SERVICE OF NAMIBIA AN ASSOCIATION OF VOLUNTARY BLOOD DONORS INCORPORATED NOT FOR GAIN
Tel +264 (0)61 386300   Fax +264 (0)61 225854 / 386301   PO Box 3434 Windhoek   35 Tal Street, Windhoek

www.bts.com.na

25 October 2017

Prof S. R. Moyo
Director: Biomedical Sciences Programme and Research Supervisor
Department of health Sciences
School of Health and Applied Sciences
Namibia University of Science and Technology

Cc.   HOD Health Sciences
       Mrs Mary Mataranyika

Dear Prof

REF: Assessment of phenotypic properties of the Rh and Kell blood group systems among blood donors and pregnant women in Namibia

The above subject has reference:

Permission is hereby granted to the Masters of Health Sciences degree student and lecturers to use donor specimens to carry out the above mentioned study; under the following conditions:

1. No publications will be generated from the results without written permission from NAMBTS.
2. Data will be used for this study only.
3. NAMBTS will get a copy of the results of the study.

Trusting this is in order.

Kind regards,

Mr. Israel Chipare
Manager of Technical Division
Blood Transfusion Service of Namibia

Appendix G: Reagents, specimens and materials used in the research project