Frequency of Abo, Rhesus Phenotypes and Most Probable Genotypes and the Type of RHD Negative Variants among UAE Nationals in Al Ain District

Mariam Hamad Zaher Saeed Al Meqbali

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FREQUENCY OF ABO, RHESUS PHENOTYPES AND MOST PROBABLE GENOTYPES AND THE TYPE OF RHD NEGATIVE VARIANTS AMONG UAE NATIONALS IN AL AIN DISTRICT

Mariam Hamad Zaher Saeed Al Meqbali

This thesis is submitted in partial fulfilment of the requirements for the degree of Master of Science in Molecular Biology and Biotechnology

Under the Supervision of Dr. Rasheed Al Hammadi.

December 2015
Declaration of Original Work

J. Mariam Hamad Al Meqabli, the undersigned, a graduate student at the United Arab Emirates University (UAEU), and the author of this thesis entitled "Frequency of ABO, Rhesus phenotypes and most probable Genotypes and the type of RhD Negative variants among UAE Nationals in Al Ain District", hereby, solemnly declare that this thesis is my own original research work that has been done and prepared by me under the supervision of Dr. Rasheed Al Hammadi, Dr. Amina Ahmed, and Dr. Asma Al Menhali, in the College of Science at UAEU. This work has not previously been presented or published, or formed the basis for the award of any academic degree, diploma or a similar title at this or any other university. Any materials borrowed from other sources (whether published or unpublished) and relied upon or included in my thesis have been properly cited and acknowledged in accordance with appropriate academic conventions. I further declare that there is no potential conflict of interest with respect to the research, data collection, authorship, presentation and/or publication of this thesis.

Student’s Signature ____________________________ Date 29/1/16
Advisory Committee

1) Advisor: Dr. Rasheed Al Hammadi
   Title: Assistant Professor
   Department of Biology
   College of Science

2) Co-advisor: Dr. Amina Ahmed
   Title: Assistant Professor
   Department of Biology
   College of Science

3) Co-advisor: Dr. Asma Al Menhali
   Title: Assistant Professor
   Department of Biology
   College of Science
Approval of the Master Thesis

This Master Thesis is approved by the following Examining Committee Members:

1) Advisor (Committee Chair): Dr. Rasheed Al Hammadi
   Title: Associate Professor
   Department of Biology
   College of Science

Signature [Signature] Date [Signature]

2) Member: Dr. Salman Ashraf
   Title: Professor
   Department of Chemistry
   College of Science

Signature [Signature] Salman Ashraf Date 15/12/2015

3) Member: Dr. Amina Ahmed
   Title: Associate Professor
   Department of Biology
   College of Science

Signature [Signature] Date 15-12-2015

4) Member (External Examiner): Dr. Habiba Alsafar
   Title: Associate Professor
   Department of Biomedical Engineering
   Institution: Khalifa University Center for Biotechnology

Signature [Signature] Date 15-12-2015
This Master Thesis is accepted by:

Acting Dean of the College of Science: Dr. Ahmed Murad
Signature 
Date 24-1-2016

Dean of the College of the Graduate Studies: Professor Nagi T. Wakim
Signature 
Date 24.11.2016

Copy 6 of 8
Abstract

Knowledge of the frequencies and the phenotypes of ABO and rhesus system is very important for blood bank and transfusion services policies. The objective of this study was to determine the frequency of ABO, Rhesus phenotype and most probable genotypes in Al Ain area (UAE). The studied group consisted of 500 locals of both gender from different age groups (17-58 years old). ABO and Rh-hr phenotype reactivity were determined using conventional tube method and gel technology, molecular techniques were also applied. Our study shows the distribution of ABO phenotypes was as follows: O= 56%. A= 26.2%. B= 14.2% and AB= 3.6%. The distribution of Rh antigens was as follows: D= 90.4 %. C= 76.2%, E= 27.2%, c= 71.6% and e= 97.8%. And the distribution of most probable genotypes was as follows: R1 r= 29.8%. R1 R1= 28.4%. R1 R2= 16.8%. rr= 8.4%. R2 r= 8.2%. Ro r'=5%. R2 R2'= 2.2%, and r'r'= 1.2%. Our study showed that the most frequent antigen amongst five major antigens of Rh system was RhD while the least common was E antigen. We developed an innovative method that combines molecular analysis and serological testing of Rh genes to be applied in blood bank environment as it is considered as a first study done in the area. This approach could improve patient care and transfusion outcomes by reducing alloimmunization. The challenge lies in integrating such testing into blood bank environment, standardizing methods, obtaining Food and Drug Administration approval for labeling donor units, and enhancing information systems to incorporate and use this new information effectively.

Keywords: Frequency, ABO blood group, Rhesus system, phenotype, most probable genotype, UAE, Al Ain.
تحديد نسبة توزع الزمر الدمادية ومعامل التخثر من خلال الأنماط الظاهرية والجينية

الأكثر احتمالاً بين سكان منطقة العين

المنصب

هدف هذه الدراسة هو تحديد نسبة توزع الزمر الدمادية وعامل التخثر ABO و RhD من خلال تحديد الأنماط الظاهرية والجينية الأكثر احتمالابي سكان منطقة العين. وتألفت المجموعة المدروسة من 500 من السكان المحليين من الجنسين من مختلف الفئات العمرية. وقد تم كشف الأنماط الظاهرية للزمز الدمادية باستخدام الطريقة التقليدية التي تعتمد على الانزيمي المخبري وطريقة تكنولوجيا Gel، وطبقت أيضاً التقنيات الجزيئية.

وتظهر دراستنا أن توزيع الزمر الدمادية على النحو التالي: 56% ABO، 26.2% B، 3.6% AB، 9.8% C، 97.8% O و 71.6% D. وكان توزيع الأنماط الجينية الأكثر احتمالاً على النحو التالي: 4.4% Rh، 90% AB، 26.2% AB، 3.6% B، 9.8% C، 97.8% O و 71.6% D. والاحتمال احتمال أن الأكبر في الأنماط الظاهرية للعامل Rh هو الزمرة D والاقل شيوعاً هي الزمرة E.

من خلال هذه الدراسة فضننا بتطوير طريقة مبتكرة تجمع بين التحليل الجزيئي والاختبارات المصلية سعياً لتطبيقها في بنوك الدم في الدولة حيث تعتبر هذه أول دراسة أجريت في المنطقة. تطبيق هذه الطريقة ستساعد على تحسين طريقة علاج ورعاية المرضى عن طريق الحد من تفعيل المناعة الذاتية لدى المرضى. ويكمن التحدي هنا في دورالهذه الطريقة في بنوك الدم، وتحديد الأساليب والحصول على موافقة منظمة FDA الغذاء والدواء لتطبيقها من خلال وضع العلامات المائية على وحدات الدم وتعزيز نظام المعلومات لدعم واستخدام هذه المعلومات الجديدة على نحو فعال.

الكلمات الدالة: نسبة توزع، فصائل الدم، عامل الرمسي، الصفات الظاهرية، الصفات الجينية، دولة الإمارات، منطقة العين.
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Firstly:

All the praises, thanks and prayers are to Allah, who is the creator of everything, he is the sustainer and he is the one who has power over all.

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Finally:

I would like to express my special appreciation and thanks to the Al Ain Regional Blood bank Center for providing us with the samples.
Dedication

I loving dedicate my thesis to my beloved parents, sisters, and brothers who have never failed to give me a moral support and for giving me all I need and encouraging me to continue my higher education.

Moreover, I dedicate this thesis to my all supervisors Dr. Rasheed Al Hammadi, Dr. Amina Ahmed, and Dr. Asma Al Menhali who have really been there through the hard times.
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<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>AARBB</td>
<td>Al Ain Regional Blood Bank</td>
</tr>
<tr>
<td>EDTA</td>
<td>Ethylenediaminetetraacetic acid</td>
</tr>
<tr>
<td>FUC</td>
<td>Fucose</td>
</tr>
<tr>
<td>Gal</td>
<td>Galactose or Galactosamine</td>
</tr>
<tr>
<td>GalNAc</td>
<td>N-acetyl D-galactosamine</td>
</tr>
<tr>
<td>GCC</td>
<td>Gulf Cooperation Council</td>
</tr>
<tr>
<td>GLU</td>
<td>Glucosamine</td>
</tr>
<tr>
<td>HDN</td>
<td>Hemolytic Disease of Newborn</td>
</tr>
<tr>
<td>NAc</td>
<td>N-acetyl group</td>
</tr>
<tr>
<td>PCR</td>
<td>Polymerase Chain Reaction</td>
</tr>
<tr>
<td>PMPs</td>
<td>Paramagnetic particles</td>
</tr>
<tr>
<td>RBC</td>
<td>Red Blood Cell</td>
</tr>
<tr>
<td>RFLP</td>
<td>Restriction fragment length polymorphism</td>
</tr>
<tr>
<td>Rh</td>
<td>Rhesus group</td>
</tr>
<tr>
<td>SDS</td>
<td>Sodium dodecyl sulfate</td>
</tr>
<tr>
<td>SNP</td>
<td>Single Nucleotide polymorphism</td>
</tr>
<tr>
<td>UAE</td>
<td>United Arab Emirates</td>
</tr>
</tbody>
</table>
Chapter 1: Introduction

1 Literature review

1.1 ABO System

The ABO blood group system, which was the first human blood group system to be discovered by Landsteiner in 1901 (Landsteiner, K. (1900). Boskababy M., et al. (2005), & lyiola, O., et al. (2012)), remains the most important in transfusion practice. Landsteiner named the first two blood group antigens A and B using the first two letters of the alphabet, while the red blood cells (RBCs) not reacting with both antigens were called type C. Later in 1911 (Bashwari, L., et al. (2001)), Von Dungern and Hiszfeld used the letter O to name the RBCs not reacting with both anti-A and anti-B, and the term AB for RBCs reacting with both antigens. Therefore, there are four main groups as shown in table (1) (Mouhaus, H.A., et al. (2010), & Hoogi, E. (2008)).

<table>
<thead>
<tr>
<th>genotype</th>
<th>Blood type (phenotype)</th>
</tr>
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<tbody>
<tr>
<td>A/A</td>
<td>A</td>
</tr>
<tr>
<td>A/O</td>
<td>A</td>
</tr>
<tr>
<td>B/B</td>
<td>B</td>
</tr>
<tr>
<td>B/O</td>
<td>B</td>
</tr>
<tr>
<td>A/B</td>
<td>AB</td>
</tr>
<tr>
<td>O/O</td>
<td>O</td>
</tr>
</tbody>
</table>

Table 1: The four ABO blood types (phenotypes) and their possible six genotypes:

Determination of these four groups in humans depends on three allelic genes located near the tip of the long arm of chromosome 9 as shown in Figure 1 (Boskababy M., et al. (2005), & Nagariya, S. (2013)). Two alleles which one of them encodes an enzyme which produces the A substance, another B substance; and when both of these alleles are present in a heterozygote both carbohydrates are made. The
third allele, O, behaves essentially as a "null" allele, producing neither A nor B substance (John V. Dacie, et al. (1984)). The presence of A and B genes result in addition of sugars to the H antigen to produce the A and B antigens: N-acetyl D-galactosamine in the case of A gene and D-galactose in the case of the B gene as shown in figure (2) (Richard H. Walker. (1990), & Hosoi, E. (2008)).

Figure 1: Molecular location of ABO gene in chromosome 9. The ABO gene is located on the long (q) arm of chromosome 9 at position 34.2. as per http://ghr.nlm.nih.gov/gene/ABO

[\text{RBC}]---\text{O- GLU - GAL.} \quad \text{"stem" carbohydrate ("H" antigen)}
\begin{align*}
&\quad \text{N}\text{Ac} \quad \text{FUC} \\
&\text{[RBC]}---\text{O- GLU - GAL - GAL} \\
&\quad \text{N}\text{Ac} \quad \text{FUC} \\
&\text{[RBC]}---\text{O- GLU - GAL - GAL} \quad \text{substance B} \\
&\quad \text{N}\text{Ac} \quad \text{FUC} \quad \text{N}\text{Ac} \\
&\text{[RBC]}---\text{O- GLU - GAL - GAL} \quad \text{substance A}
\end{align*}

Figure 2: The structure of the blood group substances A and B which represent two modified forms of a stem carbohydrate present on red blood cells. (GLU: glucosamine, GAL: galactose or galactosamine, FUC: fucose and NAc represents an N-acetyl group).
In transfusion medicine determination of the antigens is very important because of the regular occurrence of the antibodies anti-A, and anti-B and anti-AB (table 2) reactive at 37°C, in persons whose red cells lack the corresponding antigens, so that if transfusion were to be given without regards to ABO groups, the transfusion reaction would occur and that maybe fatal (Klein, H.G., et al. (2014a)).

<table>
<thead>
<tr>
<th>Group</th>
<th>Subgroup</th>
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<th>Antibodies (agglutinins) in serum</th>
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<tbody>
<tr>
<td>O</td>
<td>-</td>
<td>None</td>
<td>Anti-A</td>
</tr>
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<td></td>
<td></td>
<td>Anti-A1</td>
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<td></td>
<td></td>
<td>Anti-A,B</td>
</tr>
<tr>
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<td>Anti-B</td>
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<td>A2</td>
<td>A</td>
<td></td>
</tr>
<tr>
<td>B</td>
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</tr>
<tr>
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<td>A1B</td>
<td>A + A1 + B</td>
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</tr>
<tr>
<td></td>
<td>A2B</td>
<td>A + B</td>
<td></td>
</tr>
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Table 2: Antigens and Antibodies in the ABO system

1.2 Molecular basis of A, B and AB blood groups

1.2.1 Structure and specificity of A and B-glycosyltransferases

The ABO gene comprises seven exons and encodes a polypeptide of 354 amino acids with a short amino terminal trans-membrane segment and a large globular cytoplasmic domain containing the catalytic site. The cytoplasmic domain is positioned in the lumen of the Golgi (Nagariya, S. (2013)). As mentioned earlier that the N-acetylle D-galactosamine alpha linked to the non-reducing end of oligosaccharide chains defines the A antigen, and that D-galactose alpha linked to the non-reducing end of oligosaccharide chains defines B antigens. (Morgan, W.T., et al. (2000)). The A-transferase catalysis the transfer of N-acetylle D-galactosamine (GalNAc) from UDP-GalNAc to H antigen acceptor, while the B-transferase transfer
D-galactose (Gal) from UDP-Gal to the H antigen acceptor. These two transferases differ by only four amino acid residues 176, 235, 266, and 268 in the A and B respectively (Patenaude, S.I., et al. (2002). & Nojavan, M., et al. (2012)) as shown in table (3). The transferase is organized in two subdomains; one subdomain comprises the amino terminal region and recognizes the UDP-sugar donor and the other carboxy terminal subdomain provides the acceptor binding site. Both subdomains are separated by a cleft containing the active site. Residues 266 and 268 are located in the active site of the enzyme and influence donor recognition. The B transferase residues Met266 and Ala268 are bigger than the corresponding residues in the A transferase and restrict the size of active site cleft to exclude UDP-Gal\(\beta\)Gal (Patenaude, S.I., et al. (2002)). Residues Gly235Ser and Leu266Met influence receptor binding (Letts, J.A., et al. (2006)). In the absence of donor or acceptor the enzyme adopt an open confirmation which leaves the donor and acceptor sites exposed to solvent. The closed confirmation is required for catalysis to occur. Binding of H antigen acceptor results in a closed confirmation in which the final nine carboxy terminal residues form hydrogen bonds with L-fucose residue of H antigen and UDP (Alfaro, J.A., et al. (2008)).

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>1874</th>
<th>87</th>
<th>156</th>
<th>176</th>
<th>235</th>
<th>266</th>
<th>268</th>
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<td>Pro</td>
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<td>Arg</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A2</td>
<td>Leu</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Deletion of C at nt 1061</td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>B</td>
<td>Gly</td>
<td>Gly</td>
<td>Met</td>
<td>Ala</td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>Cis AB</td>
<td>Leu</td>
<td>Ser</td>
<td>Met</td>
<td>Ala</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 3: Differences in amino acids (aa) between various allele of the ABO gene (Daniels. G. (2013))
ABO phenotype results from single nucleotide deletion or substitution in the penultimate codon changing the reading frame so that the A transferase produced has an extra 21 amino acids at the carboxy terminus (table 3), this affects the structure of the enzyme sufficiently to change its specificity and activity (Yazer, M.H., et al. (2008)). As an example, in the case of cis AB (a group AB father and a group O mother have an AB child), it would appear that substituting one or the other of the critical residues in the A or B transferase allows access to both UDP-Gal and UDP-GalNAc, but reduces enzyme activity, as the phenotype has weaker A and B antigen expression than normal (Patenaude, S.L., et al. (2002)).

1.2.2 Competition between A and B transferases

Subjects who have both A and B allele show diminished expression of both A and B antigens because A- and B- transferases act upon the same precursor substance (Daniels, G. (2013)). Thus, A1B red cells have less A than A1 cells and A2B cells less A than A2 cells. Similarly, B is slightly less strongly expressed in A1B than B cells. In some cases, red cells of genotype A1B may behave as A2B due to an interference with expression of A1 by a strong B allele. (Daniels, G. (2013)).

1.3 Molecular basis of blood group O

The absence of an active ABO glycosyltransferase results in the blood group O phenotype. Subject with O phenotype inherited an A1 transferase gene in which nucleotide 261 (delG) in exon 6 is deleted (Hosoi, E. (2008), & Daniels. G. (2001)). This nucleotide deletion changes the reading frame resulting in translation of an inactive protein with an altered amino acid sequence after amino acid 88 and terminating at amino acid 117 so that large catalytic domain is absent (Hosoi, E. (2008), & Daniels. G. (2001)). There are several rare O alleles resulting from different mutations which create a translated protein lacking glycosyltransferase
activity (Yazer, M.H., et al. (2008)). These rare O alleles create no clinical problems when conventional serological methods of ABO typing is employed, but provide considerable challenges for DNA-based methods of ABO typing because the mutation occur in an A1 or A2 transferase gene and so individuals could mistakenly be typed as A if the DNA based method employed is not configured to detect these variant O alleles (Klein, H.G., et al. (2014b), & Storry, R., et al. (2008))

1.4 Rh system

The Rhesus (Rh) blood group system which was discovered in late 1940's by Levine and Stetson (Agre, P., et al. (1991)) considered as the most polymorphic of human red blood group system, consisting of at least 51 independent antigens (Abou-Jabal, A., et al. (2003)) making it the most complex RBC system. This system considered as the second and next to the ABO, is the most clinically significant system in transfusion medicine. The clinical importance of the system is a consequence of the fact that the Rh-negative individuals are relatively easily stimulated to form immune anti-D antibodies through either pregnancy forming the hemolytic disease of newborn (HDN), which is most commonly the result of the destruction of infant's D-positive red cells by IgG anti-D antibodies (John V. Dacie et al. (1984)), or through transfusion of red cells to Rh-negative individuals. This ensured its continued use in clinical investigation.

1.4.1 Molecular biology of the Rh system

It was found that the biomedical studies of the Rh proteins could not be possible due to the hydrophobic nature of this protein, despite their clinical importance. For that, the molecular basis of most Rh antigens has been determined, and the structure of RH genes confirmed its polymorphic nature (Westhoff, C.M.
(2004)). Specifically, the conventional Rh antigens are encoded by two genes, RHD and RHCE, but numerous gene conversion events between them create hybrid genes. The resulting novel hybrid proteins containing regions of RhD joined to RhCE, or the converse, generate the myriad of different Rh antigens (Sharma, D.C., et al. (2013), & Avent, N.D., et al. (2000)).

1.4.2 Terminology

Current Rh terminology attempts to distinguish the genes and proteins from the antigens, which are referred to by the letter designations, D, C, c, E, and e. Capital letters and italics are used when referring to the RH genes, which include RHD, RHCE, and RHAG (Westhoff, C.M. (2007)). The different alleles of the RHCE gene are designated RHce, RHCe, and RHcE, according to which antigens they encode. The proteins are indicated as RhD and RhCE according to specific antigens they carry. Rh haplotypes are designated Dce, DCe, DeE, or ce, Ce, cE when referring to a specific CE haplotype (Hassan, F.M., et al. (2013)).

1.4.3 RH genes and Rh proteins

The expression of the Rh factors is controlled by two genes, the RHD and RHCE which lie in close proximity on chromosome 1 and encode 416 amino acids Rh proteins: one gene codes for D antigens and other gene for CcEe antigens in various combinations (Wanger, F.F., et al. (2000)) as shown in figure 3. The Rh genes are a source of significant diversity favored by the opposite orientation of RHD and RHCE genes. Each gene has ten exons that are 97% identical and encode proteins that differ by 32 to 35 amino acids. The RH genes are separated by a stretch of around 30 Kb which includes another gene (SMP1) (Wanger, F.F., et al. (2000)).
The D gene is flanked by two 9-Kb regions of homology denoted rhesus boxes. (figure 3)

**Figure 3:** Schematic diagram of RHCE and RHD genes and their proteins. The positions and orientations of the genes and the Rhesus boxes are indicated by open arrows and triangles, respectively. The exons are shown as vertical bars, and their exon numbers are indicated. The 2RH genes have opposite orientation, face each other with their 3′ ends, and are separated by about 30 000 bp. A third gene, SMP1, has the same orientation as RHD and is positioned between RHD and RHCE. The RHD gene is flanked on both sides by the 2 highly homologous Rhesus boxes, which are noted by (b). All exons are shorter than 200 bp, with the exception of the RHD and SMP1 3′ terminal exons.

### 1.4.4 Rh system nomenclature

The Rh system would become notorious for its complexity with numerous antigens and multiple nomenclatures defining it. Two methods of Rh notations have been described. The CDE notation of Fisher and Race described encoding of Rh antigens by three closely linked loci (Guzman, R.M.S., et al. (2010)). Recognizable haplotypes regarding this notation are CDe, cDE, cDe, CDE, cde, Cde, cdE and CdE.
The alternative Rh-hr notation of Wiener was based on the hypothesis of multiple alleles at a single locus. (Klein, H.G., et al. (2014c)). See table 4.

<table>
<thead>
<tr>
<th>Fisher</th>
<th>Short notations</th>
</tr>
</thead>
<tbody>
<tr>
<td>CDe</td>
<td>( R_1 )</td>
</tr>
<tr>
<td>cde</td>
<td>( r )</td>
</tr>
<tr>
<td>cDE</td>
<td>( R^2 )</td>
</tr>
<tr>
<td>cDe</td>
<td>( R^6 )</td>
</tr>
<tr>
<td>C°De</td>
<td>( R_{1w} )</td>
</tr>
<tr>
<td>cdE</td>
<td>( r'' )</td>
</tr>
<tr>
<td>Cde</td>
<td>( r' )</td>
</tr>
<tr>
<td>CDE</td>
<td>( R' )</td>
</tr>
<tr>
<td>CdE</td>
<td>( r^3 )</td>
</tr>
</tbody>
</table>

Table 4: Fisher nomenclature of Rh genes and the corresponding short notations (Rudmann. S.V. (2005)).

1.4.5 Variations of RhD

Some variant Rh phenotypes are caused by exchange of genetic materials between the two genes or from missense mutations. The Rh variants can weaken expression of the common antigens, produce partial antigens, generate low-prevalence antigens, and result in absence of high-prevalence antigen (Kappler-Gratias, S., et al. (2013)). These variants alleles encode Rh proteins with amino acid changes that cannot be distinguished serologically, but can be recognized by the immune system as foreign. Some patients are at risk for production of antibodies to both the RhCE and RhD proteins, a serious and potentially life-threatening complication. The challenge is to institute molecular screening of minority donors to
develop a registry of units that are genotyped for these variants. This is critical to meet the transfusion needs of these alloimmunized and often critically ill patients, especially as rare D-negative units are in very limited supply and are not always compatible.

1.4.5.1 D-Positive variants

Most individuals who are D-positive have identical RHD sequences, but approximately 2% have altered alleles that encode proteins with amino acid changes or have hybrid alleles encoding proteins with part of RhD fused to RhCE, or vice versa (Kappler-Gratias, S., et al. (2013)). These altered or hybrid proteins will result in forming weak D (reduced expression of D antigen) and/or partial D (altered D surface epitopes) (Flegel, W.A., et al. (2002)).

1.4.5.2 D-Negative variants

Antigens of the Rh blood group system are encoded by two homologous gene, RHD and RHCE, that produce two cell membrane proteins. D-negative red cells lack the D protein, and alloanti-D consists of antibodies to a variety of epitopes on the D polypeptide. There are three common genetic mechanisms responsible for D negative phenotype: deletion of RHD that results from chromosomal misalignment at meiosis and subsequent unequal crossing over between the rhesus boxes ((Flegel, W.A., et al. (2002) & Wanger, F.F., et al. (2000)) (Figure3), a pseudogene RHD containing a 37 bp insert and 1 or 2 stop codon, and a hybrid RHD-CE-Ds that probably produces an abnormal C antigen but does not produce a D antigens (Westhoff, C.M. (2007), & Touinssi, M., et al. (2009)). These three types are shown in figure (4) Knowledge of Rh phenotypes and variants in given population is relevant for better planning and
management of a blood bank: to find compatible blood for patients needing multiple
cross-transfusions, which would resolve the blood transfusion needs of recipients.

![Diagram](image)

**Figure (4):** Representation of the genomic organization of the D-positive haplotype and 3 D-negative haplotypes. White boxes indicate RHCE exon and black indicate RHD exon.

(Singleton, B.K., et al. (2000)).

### 1.5 Inheritance of red cell antigens

Two similar sets of chromosome (one set inherited from each parent) are present in all cells of the body except the germ cells. Each set is composed of 22 autosomes and one sex chromosome (the total number of chromosomes is 46). The sex chromosomes in the female are equal in size and are termed X chromosomes while in male there is one X chromosome and one much smaller chromosome, termed Y (Daniel D. Chiras. (2008)).

The segment of chromosomal DNA which determines a particular polypeptide is defining the gene, while the alternative forms of genes at a particular locus are termed alleles, for example A, B, and O (Talukder, S., & Das R. (2010)). An individual may be homozygous (e.g. AA) or heterozygous (e.g. AO) for all genes carried on autosomes. Whereas the term genotype is used for the sum of inherited alleles of a particular gene, for example AA, and AO phenotypes refers only to the recognizable product of the alleles (Hosoi, E. (2008) & Daniels, G. (2001)). As O has no serologically recognizable product, the genotypes AO and AA both have the
phenotype A. although O has no recognizable product, the DNA structure of this silent allele differs only slightly from that of A and B alleles (Daniels, G. (2001) & Mouhaus, H.A., et al. (2010)). However, in the Rh system, the gene D has no allele. At the corresponding position on the homologues chromosome, there is either another D gene or a D gene altered in such a way that D polypeptide cannot be made (Westhoff, C.M. (2007) & Touinssi, M., et al. (2009)).

Genes that are far apart on the same chromosome, for example Rh, or genes that are carried on different chromosomes are inherited independently and the antigens they determine are said to belong to different blood group system (Westhoff, C.M. (2007)). In some cases, more than one protein is produced from a single gene and thus one allele determines more than one polypeptide (Talukder, S., & Das, R. (2010)). Red cell antigens are either proteins or carbohydrates; the proteins (Rh) are the direct product of genes, but those that are carbohydrate (A) are determined indirectly by enzymes (glycosyltransferase) that are the gene products. These enzymes transfer the appropriate sugar, determining specificity on to a structure whose synthesis may be determined by one or more unrelated genes. In most cases there appears to be a simple correspondence between genes and antigens, so that if a person inherits a given gene the antigen can be detected on the red cells (Nagariya, S. (2013)).

1.6 Gene (allele) frequency

Gene frequency is simply the frequency of the allele in the population as a whole; the term allele frequency would be more precise but it is not generally used (Guzman, R.M.S., et al. (2010)). From a knowledge of the frequencies of the genes (allele)in a blood group system, phenotype frequencies are readily derived. In
different races the proportion of individuals belonging to a particular blood group varies widely. Both ABO and Rhesus blood groups varies in incidences in different population. There are many studies done all over the world to determine the frequency of ABO and rhesus phenotypes in different populations.

1.7 Frequencies of ABO and Rhesus in different populations:

With respect to studies in UAE, and to our knowledge, Taha. J.Y. (2012), has evaluated the frequency of Rh phenotypes and the most probable genotypes in Kalba region, UAE. She tested 661 samples randomly without considering the nationalities using tube methods, while Abou-Jabal. A et al (2003), studied a group of 1000 Jordanian donors using the same methods. Both of them concluded that, studying blood group phenotypes distribution is very important for blood bank and transfusion service policies. Also studying the Rh-hr phenotypes and genotype is of value in solving problem regarding incompatibilities, investigation of autoimmune hemolytic anemia, and in studies on the origins and movements of populations.

However, Bashawri. L., et al (2001), and Ghasemi. N., et al (2007), studied the frequency of ABO and Rh blood groups in the eastern region of Saudi Arabia and in middle school students of Yazd Province (Iran) respectively. Bashawri studied 5739 male potential blood donors during two different periods (1985-1989 and 1995-1999), while Ghasemi examined 2000 students using slide method considering the place and date of birth. They concluded that, the knowledge of the frequencies of the different blood group is very important for blood banks and transfusion services. And also it is important for clinical studies (disease associations) as well as for population genetics studies. In addition to that, Ghasemi found that, there is no relationship between ABO blood group and sex, place of birth and insemination season. They
also confirmed that ABO and Rh antigenic structure differs between regions and nations.

Determination of the distribution of ABO and Rh blood group in different region and nations helps in comparing the distribution trends of these blood groups and this what Jaff, M.S. (2010) did when they determined the distribution of ABO and Rhesus blood group in Kurds by studying group of total population of 53234 whose ABO and Rhesus blood groups were determined by standard methods during a period of about 5 years (2005-2009). He concluded that the blood group O is the commonest blood group, followed by A, B, and AB. By comparing what he found with other population he found that the distribution of ABO and Rh blood groups in Kurds has similar trends to the neighboring countries, and appears to be intermediate between eastern (Asian) and western European (Caucasian) data.

In addition of the above mentioned benefits, blood transfusion centers strive to provide blood products that are microbiologically safe and compatible with recipient blood characteristics. To achieve this, pre-transfusion screening at two levels is required. The first level involves blood cell antigens. A total of 339 blood group specificities including 297 in 33 systems have been recorded by the International Society Blood Transfusion (ISBT) (Reid, M., et al. (2012)). Because the ABO and Rh antigens can be immunologically significant due to presence of natural ABO antibodies and strong immunogenicity of RHD antigens, systematic ABO-RhdD typing is necessary. The second level of screening involves identification of Rh-Kell antigenic profiles and, to a lesser extent, Kidd, Duffy, and Ss phenotype (Siransy Bogui, L., et al. (2014)). This pre-screening is necessary depending on the patient, pathology, and risk of alloimmunisation.
In addition to ensuring transfusion safety, awareness of antigen frequency in a given population is important in defining product requirements and minimum stock levels. Given the high variability of red cell antigens, blood transfusion centers must determine antigen frequencies in the population that they serve. Consequently, the aim of this study was to determine the frequency of ABO, Rh blood group phenotype and most probable genotypes and the types of the RHD negative variants among UAE nationals in Al Ain area, which can be applied in practice for a rapid and efficient identification of blood donors and the immediate selection of needed compatible blood units. At the same time, this study will help in mapping a national status for future references through preparing database for the blood banks and hospitals blood transfusion centers. Also this study can decipher diversity or uniqueness of the UAE more specifically Al Ain populations structure.

1.8 Background of United Arab Emirates (UAE)

The United Arab Emirates (UAE) is part of the Gulf cooperation Council (GCC) which consists of six gulf countries including the kingdom of Bahrain, Kuwait, Qatar, kingdom of Saudi Arabia, and Oman. In addition the UAE is located in the eastern part of the Arabian Peninsula extend along part of the Gulf of Oman and the southern coast of the Arabian Gulf. The UAE is a constitutional federation of seven emirates: Abu Dhabi, Dubai, Sharjah, Ajman, Umm al-Qaiwain, Ras al-Khaimah and Fujairah. The federation was formally established on the second of December 1971. UAE occupies an area of 71023.6 Sq.Km. along the south-eastern tip of the Arabian Peninsula. As shown in figure (5), Qatar lies to the west, Saudi Arabia to the south and west, and Oman to the north and east. The UAE lies between latitude 22.35°, 2.25° north, and longitude 51.31°, 57.1° east. (UAE statistical center, 2010). Furthermore, the capital and the largest city of the federation in the UAE is Abu
Dhabi, which accounts for 87 percent of the UAE's total area. Abu Dhabi consists of several cities and Al Ain one of them.

![Map of United Arab Emirates](http://www.nationsonline.org/oneworld/map/united_arab_emirates_map.htm)

**Figure 5:** Political and Administrative Map of United Arab Emirates

1.9 **Background of Al Ain Area**

Al Ain is the second largest city in the Emirate of Abu Dhabi and the fourth largest city in the United Arab Emirates. With a population of 568,221 (2010), it is located approximately 160 kilometres east of the capital Abu Dhabi and about 120 kilometres south of Dubai. Al Ain has a higher proportion of Emirati nationals than elsewhere in the country, but the majority of its residents are expatriates particularly from the Indian sub-continent. Many people are from Bangladesh, Pakistan and some from Afghanistan. There are fewer other expatriates than in the larger centers of Abu Dhabi and Dubai. (Al Ain municipality site).
1.10 Frequency of the donors in the population

The group from the population who are qualified to be blood donors consists of healthy adults aged 17-65 years. The highest frequency of donation in the world corresponds to about 10% of the population eligible to give blood donating once per year. (Hassig, A. (1991) & Linden, J.V., et al. (1988)). The number of units collected per Al Ain population during the 3 years (May 2012- July 2015) is shown in table 5. Losses from outdated red cells accounted for the same period was 570 (1% from total collection) (AARBB) of the supply but given the fact that red cells can be transferred only to compatible recipients, the number of usable units outdated appears to be extremely low (AARBB statistics 2015).

<table>
<thead>
<tr>
<th>Nationality</th>
<th>May-Dec 2012</th>
<th>Jan-Dec 2013</th>
<th>Jan-Dec 2014</th>
<th>Jan-July 2015</th>
</tr>
</thead>
<tbody>
<tr>
<td>Other Nationality</td>
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<td>12267</td>
<td>6784</td>
</tr>
<tr>
<td>UAE Nationality</td>
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<td>4507</td>
<td>5054</td>
<td>2503</td>
</tr>
<tr>
<td>Total</td>
<td>9702</td>
<td>16069</td>
<td>17321</td>
<td>9287</td>
</tr>
</tbody>
</table>

Table 5: number of whole blood units collected in Al Ain Regional blood Bank during three years (May 2012 – July 2015).

1.11 Blood utilization and shortages

Despite the constant rise in collection (table 5), blood collectors report frequent shortages and emergency appeals for blood are disturbingly common. Some 15,000 units of red cells are collected annually in AARBB and the numbers continue to rise. (AARBB 2015 statistics). With the current shelf life, the blood supply more closely resembles a pipeline than a bank or reservoir. A few days of under collection can have a devastating effect on supply. There are little general agreements about what constitutes a shortage. Measures of postponed surgery and transfusion, as well as increased rates of RhD-Positive transfusions to RhD-Negative recipients provide
some indication of shortage at the treatment level (Westhoff, C.M., et al. (2008)). Blood utilization may require to reach steady-state or higher to cover the shortage in blood cells through controlling the patterns of donation and usage. Ordinarily, more blood is donated by younger age groups, whereas more is used by the elderly (cobain, T., et al. (2007)) variation in RBC use per capita among countries can be explained largely by the age distribution differences of the populations rather than by the different national treatment standards. (Ali, A., et al. (2009)). Substantial resources are required to be invested in donor recruitment and retention or methods need to be adopted to serve the changing population demographic and reject the blood shortages to become a way of life. These methods may include determination of the frequency of the most significant red cell antigens (ABO & Rh) to ensure safe transfusion to recipients. Awareness of antigens frequency in a given population is important in defining product requirement and minimum stock requirement to overcome blood shortages. So in this study, we determined the frequency of ABO, Rhesus phenotype and most probable genotypes and the types of RhD negative variant among UAE nationals in Al Ain area.
1.12 Objectives

Since there is no data available on the ABO and Rh frequencies in Al Ain area, this study will be of value in solving problems regarding blood transfusion incompatibilities. Also, the determination of Rh(D) antigens will help in avoiding the risk of forming hemolytic disease of newborns or for neonatal Allo-immune thrombocytopenia through determining the paternal allele inherited by the fetus. Moreover, Blood bank usually has a problem of ever-changing stock position and it is very difficult to predict the incidence of a particular blood group at a particular time. The main objective of our present study is to assess the distribution of blood groups in Al Ain area, that will help in determining the stock of blood and blood components needed, so that there will be no shortages or expiry issues of these products. Additionally, this study will help in saving the time and the money needed in collecting, processing, and testing of these products. Results from this study can help in mapping a national status for future references. Moreover, it will help in preparing database for the blood bank and also create awareness as who is exposed to which of the diseases. Besides this, the knowledge of the blood groups and genotypes in regards to the health of an individual is important and useful for medical diagnosis, genetic information, genetic counseling, forensic medicine needs, and also for the general wellbeing of individuals.
Chapter 2: Methods

2.1 Study approval

This study was approved by the Al Ain Medical District Human Research Ethical committee which is an accredited organization of Federal Wide Assurance (FWA) and compliant with ICH/GCP standards.

2.2 Blood samples collection

Random blood samples were selected from local (UAE national) male and female (440 male, and 60 females) donors from different age groups (17 – 58 years old) who donated blood at Al Ain Regional Blood Bank (AARBB). The selection of these samples depend in the report generated from eProgessa the laboratory information system used in AARBB. This report showed the gender, age, the place of birth and other information. All individual investigated were from Al Ain area and the consent form of all donors was obtained before donation.

2.3 Serology typing

2.3.1 ABO phenotyping

Forward and reverse ABO grouping was performed by conventional tube method and by gel technology. For forward ABO grouping, commercially available monoclonal blood group antisera i.e Anti-A, Anti-B, and Anti-AB were used while for reverse grouping, the A1 and B cells prepared in 5% suspension were used. All these reagents were purchased from Bio Rad. Gel technology also was used by performing the test in the ABO forward and reverse grouping using Bio Rad gel card technology.
2.3.2 RhD typing

RhD typing was done by tube method as well as by gel method using monoclonal/ polyclonal Anti-D (Rh₀ & Rh₁) and by using Bio Rad gel card respectively.

2.3.3 Rh phenotyping

For detection of status of rest of the major antigens of Rh system apart from Antigen D i.e. Antigens C, c, E & e specific monoclonal antisera (Bio Rad) were used and test was performed by tube method as well as by gel method. False positive and false negative results were strictly avoided by taking quality control measures at each step.

2.3.3.1 Determination of most probable Rh genotypes

By using the five available antisera either by tube method or by gel cards for the five major antigens of Rh group, phenotype of the donor is reflected in the results. Determination of exact genotype is not possible without testing parents and other family members or by RNA testing. For this reason most probable genotype is determined from gene frequency estimate depending in the phenotype results.

Following steps are taken to determine the possible genotypes from the individual’s phenotype:

1. If D is positive, the number of possible genotypes is one less than the number of positive reactions.
2. If D is negative, the number of possible genotypes is two less than the number of positive reactions.

3. One haplotype is inherited from each parent (for example, DCE/dce is one genotype having both the DCE and the dce haplotypes).

4. **Most probable genotype from the possible genotypes was calculated as shown in the following example:**

If donor's phenotype is D+, C+, E-, c+, e+, then we have 4 positive reactions and the D is positive. We should have 3 possible genotypes. When D is positive, we don't know if the donor inherited D from 1 parent or both parents. So we immediately have 2 possibilities:

a. **One genotype** where D was inherited from both parents: D??/D??

b. **One genotype** where D was inherited from only 1 parent: D??/d??

Next look at alleles C and c. If only one is present, it must be in both haplotypes. If both are present assign one allele to one haplotype, and the other allele to the remaining haplotype. In this example, we have both C and c present, so C will assign to one haplotype and c to the other. (DCE/Dc? Or DCe/dc?). Repeat this process to E and e. and because only e is present, it must be in both haplotypes (DCE/Dce = R1R1r, or DCe/dce = R1r). Fisher & Race Rh nomenclature was used in this study. (see table 4).
2.4 Molecular analysis for the determination of RhD negative variants

2.4.1 DNA Extraction

All the DNA extracted process were performed according to manufacturer's protocol. The first step of analyzing the genomic DNA is to isolate DNA from tissue or cells using a combination of physical and chemical methods. Many different platforms and technologies are available. In the current study the genomic DNA of 45 RhD negative samples were extracted from 200 µl aliquots of EDTA whole blood either on the Maxwell™ 16 instrument to perform automated isolation of genomic DNA from whole blood using Maxwell™ 16 DNA purification kit Cat# AS1010 according to manufacturer's instructions. This instrument purifies samples using paramagnetic particles (PMPs), which provide a mobile solid phase that optimizes capture, washing and elution of the genomic DNA. The magnetic particle handlers transport the PMPs through purification reagents in the prefilled cartridge and mix during processing. This instrument can process up to 16 samples in 30-40 minutes. The other method used to extract the genomic DNA from whole blood was the manual procedure using QIAamp DNA Mini and Blood Mini Kit. The basic principle of this kit can be described in four stages: the first stage is to lyse the red blood cells with buffer AL, physical agitation in the presence of proteinase K. buffer AL contains sodium dodecyl sulfate (SDS) detergent that disrupt cell membrane and dissociates protein DNA complex. Proteinase K solution. meanwhile, degrades proteins including DNA scaffolding proteins and other protein debris. The second stage is to isolate DNA from the cell by adding Ethanol (96-100%). Ethanol is used to precipitate DNA out of fluid suspension. Therefore, it increases DNA affinity to bind to the QIAamp Mini spin column for further elution of DNA through the spin
column. The third stage is DNA purification. The purification step uses wash buffer (AW1 and AW2). Each of the buffer has a different ethanol concentration. AW1 being the more concentrated buffer followed by AW2. This ensure the DNA remains bound to the spin column. The buffers act to dissolve and remove cellular debris and contaminants that are not bound to the spin column matrix. The result of this stage is to leave clean and purified DNA bound to the column. The final stage of the DNA extraction is elution. In this step buffer AE is used. The buffer AE will increase the yield by up to 15% and suitable for long term storage of DNA, since DNA stored in water is subject to acid hydrolysis. A 200 μl sample of whole blood using both protocol typically yields 6 μg of DNA (30ng/μl) with an A260 / A280 ratio of 1.7-1.9.

2.4.2 Quantitative measurement of extracted genomic DNA

DNA quantification is critical for molecular analysis. There are several methods used to establish the concentration of DNA in solution. The most common method of DNA quantification is spectrophotometric quantification. In this study DNA was quantified by using Nano Drop 2000 UV-Vis spectrophotometer. This instrument measures the concentration and purity of the extracted DNA using only 1μl and the results obtained in less than 15 seconds from sample pipetting to wiping the pedestal clean. Each sample was run 3 times to make sure of the quality of the results obtained and the concentration average was calculated.

2.4.3 Qualitative determination of extracted DNA

The extracted genomic DNA samples were analyzed by electrophoresis on a 1% agarose gel (1.5g agarose, 150ml 1X TBE and 8μl EtBr. The voltage=100). Then the gel was analyzed under the UV light and the graphs were taken. Electrophoretic
separation is based on the principle that a charged particle will migrate towards one of the electrodes when placed in an electrical field. The speed and direction a charged particle moves can be determined by its net charge, size, shape and molecular weight as well as external factors such as the composition of the buffer, the voltage used among other variables.

2.4.4 Amplification process using T100™ Thermal Cycler (Bio Rad)

The PCR allows selective amplification of a determined portion of DNA in vitro. The technique was made possible by the discovery of Taq polymerase, which is stable at high temperatures. In this study PCR was performed using T100™ Thermal Cycler (Bio Rad) to amplify and detection of the RH gene of the RhD negative samples, which is based on the diagnostic amplification of a DNA sequence present in the Rh-positive individuals and absent in Rh-negative.

2.4.4.1 Primer selection

Because D and CcEe genes are highly homologous, it is possible to design primers that anneal to a region of the D gene and also to a region of the CcEe gene, see figure 4. In that way, all DNA samples provide a template for amplification. Primer D1 for a sequence located in exon 4 (position nt 521-540), and primer D2 for a sequence located in exon 5 (position nt 670-651), were used to distinguish the RHCE and RHD genes as shown in table 6 which were selected according to Simsek et al. The bands resulting from the PCR amplification are shown in figure 6.
2.4.4.2 Selection of the appropriate genomic DNA volume

According to the results obtained from the gel electrophoresis (genomic DNA) the appropriate volume for each samples (45 samples) were determined to prepare the final PCR reaction of 25µl to be run.
2.4.4.3 Preparation of PCR reaction

The positive RHD samples which used as a control samples was carried out with 2 μl genomic DNA, 12.5 μl TAQ PCR Master Mix, 1.5 μl of each forward and reverse primer (10pM) and 7.5 μl DNA free water in a 25 μl reaction volume as shown in table 7. The other PCR reactions were prepared with different genomic DNA volumes as shown in tables 8, 9 & 10.

<table>
<thead>
<tr>
<th></th>
<th>Volume μl</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNA</td>
<td>2</td>
</tr>
<tr>
<td>FD1</td>
<td>1.5</td>
</tr>
<tr>
<td>RD2</td>
<td>1.5</td>
</tr>
<tr>
<td>PCR Master Mix</td>
<td>12.5</td>
</tr>
<tr>
<td>H2O DNA free water</td>
<td>7.5</td>
</tr>
<tr>
<td>Total volume</td>
<td>25</td>
</tr>
</tbody>
</table>

Table 7: First PCR reaction for QC samples and the other remaining DNA samples
### Table 8: Second PCR reaction for samples # 17, 26, & 29

<table>
<thead>
<tr>
<th>Volume µl</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>DNA</td>
<td>9</td>
</tr>
<tr>
<td>FD1</td>
<td>1.5</td>
</tr>
<tr>
<td>RD2</td>
<td>1.5</td>
</tr>
<tr>
<td>PCR Master Mix</td>
<td>12.5</td>
</tr>
<tr>
<td>H2O DNA free water</td>
<td>0.5</td>
</tr>
<tr>
<td>Total volume</td>
<td>25</td>
</tr>
</tbody>
</table>

### Table 9: Third PCR reaction for samples # 28 & 30

<table>
<thead>
<tr>
<th>Volume µl</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>DNA</td>
<td>5</td>
</tr>
<tr>
<td>FD1</td>
<td>1.5</td>
</tr>
<tr>
<td>RD2</td>
<td>1.5</td>
</tr>
<tr>
<td>PCR Master Mix</td>
<td>12.5</td>
</tr>
<tr>
<td>H2O DNA free water</td>
<td>4.5</td>
</tr>
<tr>
<td>Total volume</td>
<td>25</td>
</tr>
</tbody>
</table>
### Table 10: Fourth PCR reaction for samples # 11 & 12

<table>
<thead>
<tr>
<th></th>
<th>Volume µl</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNA</td>
<td>1</td>
</tr>
<tr>
<td>FD1</td>
<td>1.5</td>
</tr>
<tr>
<td>RD2</td>
<td>1.5</td>
</tr>
<tr>
<td>PCR Master Mix</td>
<td>12.5</td>
</tr>
<tr>
<td>H2O DNA free water</td>
<td>8.5</td>
</tr>
<tr>
<td>Total volume</td>
<td>25</td>
</tr>
</tbody>
</table>

**2.4.4.4 Thermal cycle parameters for T100™ Thermal Cycler (Bio Rad)**

All PCR reactions consisted of hot-start cycle at 95°C for 5 minutes, followed by 35 cycles of PCR in T100™ Thermal Cycler. Each cycle should be done at different conditions including 95°C for 45 seconds, 64°C for 1 minute and 72°C for 1.5 minutes, followed by final extension at 72°C for 5 minutes as shown in table 11.

<table>
<thead>
<tr>
<th>Cycle</th>
<th>Stage</th>
<th>Temp.</th>
<th>Duration</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>once</td>
<td>Hot-start</td>
<td>95°C</td>
<td>5 minutes</td>
<td>Reconfiguration of enzyme</td>
</tr>
<tr>
<td>35</td>
<td>Denaturation</td>
<td>95°C</td>
<td>45 seconds</td>
<td>dsDNA split to ssDNA</td>
</tr>
<tr>
<td></td>
<td>Annealing</td>
<td>64°C</td>
<td>1 minute</td>
<td>primer anneal to binding site</td>
</tr>
<tr>
<td></td>
<td>Extension</td>
<td>72°C</td>
<td>1:30 minutes</td>
<td>primer extend by enzyme</td>
</tr>
<tr>
<td>once</td>
<td>Post-</td>
<td>72°C</td>
<td>5 minutes</td>
<td>All strands extend to include extra adenine base</td>
</tr>
<tr>
<td></td>
<td>extension</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>once</td>
<td>Hold</td>
<td>4°C</td>
<td>∞</td>
<td>Temp. decrease to donate end of process</td>
</tr>
</tbody>
</table>

**Table 11: PCR thermal cycles parameters**
2.4.5 Detection of amplified PCR products

The PCR products were analyzed by electrophoresis on 1.2% agarose gel. TrackIt Tm 1Kb plus DNA ladder Cat. No. 10488-085 was used as a marker to size linear double-stranded DNA fragments from 100bp-12kb. Then the gel was analyzed under the UV light and the graphs were taken.

2.4.6 PCR products sequencing

The unpurified PCR products were packed and sent to Macrogen Korea that served over 10 years in sequencing field using cutting edge technology and delivering fast reliable results. They use high throughput Applied Biosystems 3730XL sequencers. The Applied Biosystems 3730XL DNA analyzer are automated, high throughput capillary electrophoresis systems used for analyzing fluorescently labeled DNA fragments. (for more detail refer to Applied Biosystems 3730/3730XL DNA analyzer sequencing chemistry guide).
2.4.6.1 Run Sample overview

Below is a diagram of the DNA Analyzer run cycle.

![DNA analyzer run cycle diagram]

**Figure 7:** DNA analyzer run cycle

2.4.6.2 Sample Packing Requirements:

The below table No.12 shows sample packing requirements to be shipped to Macrogen Korea.

<table>
<thead>
<tr>
<th>Template</th>
<th>Sample requirements</th>
<th>Primer requirements</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unpurified PCR products</td>
<td>100ng/μl Minimum volume of 30 μl</td>
<td>10pmole/μl = 60ng/μl</td>
</tr>
</tbody>
</table>

**Table 12:** samples packing requirements
2.4.7 Sequencing results analyzes

The NCBI database was used to obtain the two nucleotide sequences of exon 4 to exon 5 of both RHCE (accession ID: 6006) and RHD (accession ID: 6007) gene. These obtained sequences were compared to the following accession numbers: intron 4 RHCE: Y10604, and intron 4 RHD: Y10605 to recognize the size differences between intron 4 of RHD and RHCE.

The sequences of the tested samples were aligned to the wild type sequences of RHD and RHCE using MUSCLE and T-coffee programs. And the results were analyzed.

2.4.8 Statistical analyzes:

Frequency of ABO and the Rh phenotype and most probable genotypes for all studied group, and RHD-negative variants screening among RHD-negative donors were assessed using dependent sample T-test where $P < 0.05$ Indicates a significant difference using SPSS Program.
Chapter 3: Results

3.1 Serological analysis

Random Blood samples were selected from UAE local male and female (440 males & 60 females) donors from different age groups (17-58 years old) who donated blood at Al Ain Regional Blood Bank (AARBB). These samples (total of 500 samples) were tested for ABO forward and reverse grouping using conventional tube method and DiaMed Gel Technology. The Rh antigens (D, C, c, E & e) were typed using also the same methods. And by analyzing the results obtained the frequencies of ABO and Rh phenotypes and most probable genotypes were determined.

3.1.1 ABO phenotype frequency

The striking feature of the ABO system in Al Ain population studied here (total studied population) was the predominance of the phenotype O (56%), followed by A (26.2%), B (14.2%), and AB (3.6%) as shown in table 13 & figure 8. The frequency of ABO blood groups in a total of 500 male and female donor population was compared. The percentage of A, B, AB and O blood groups among studied male subjects were 26.5, 14.5, 3.8, and 55% respectively (table 13 & Figure 9). For the females, the percentages of ABO blood groups were 25, 11.6, 1.6, and 61.6% for A, B, AB, and O groups respectively (table 13 & figure 10). There was no significant difference in the distribution of ABO blood groups between male and female subjects.
<table>
<thead>
<tr>
<th>Blood Group</th>
<th>Male</th>
<th>Female</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number</td>
<td>%</td>
<td>Number</td>
</tr>
<tr>
<td>A</td>
<td>117</td>
<td>26.5</td>
<td>15</td>
</tr>
<tr>
<td>B</td>
<td>64</td>
<td>14.5</td>
<td>7</td>
</tr>
<tr>
<td>AB</td>
<td>17</td>
<td>3.8</td>
<td>1</td>
</tr>
<tr>
<td>O</td>
<td>242</td>
<td>55</td>
<td>37</td>
</tr>
</tbody>
</table>

Table 13: Distribution of different ABO blood groups among male, female and total studied population.

Distribution of ABO in the Total Studied Population

Figure 8: Distribution of ABO in the total studied population

Distribution Of ABO between Male Subjects

Figure 9: Distribution of ABO between male subjects
Figure 10: Distribution of ABO between female subjects

3.1.2 Distribution of RhD (positive & negative) phenotypes

The percentage of Rh positive and Rh negative blood groups among studied male subjects were 91.1 and 8.9% respectively as shown in table 14 & figure 11. For studied female, the percentage of Rh blood groups were 85 and 15% for Rh positive and Rh negative groups respectively (table 14 & figure 11). There was no significant difference in distribution of Rh blood groups between male and female subjects. The distribution of Rh positive and Rh negative groups among total studied population were 90.4, and 9.6% respectively as shown in table 14 & figure 11.

<table>
<thead>
<tr>
<th>Blood Group</th>
<th>Male</th>
<th>Female</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number</td>
<td>%</td>
<td>Number</td>
</tr>
<tr>
<td>Rh-positive</td>
<td>401</td>
<td>91.1</td>
<td>51</td>
</tr>
<tr>
<td>Rh-negative</td>
<td>39</td>
<td>8.9</td>
<td>9</td>
</tr>
</tbody>
</table>

Table 14: Distribution of different Rh phenotypes among male, female, and total studied population
3.1.3 Distribution of Rh antigens

Regarding Rh antigens, table 15 shows the distribution of Rhesus antigens in the total population studied which are as follows: D = 90.4%, C = 76.2%, E = 27.2%, c = 71.6% and e = 97.8%. These antigens were compared to each according to its reactivity with each antiserum as shown in figure 12. The genotype frequency was based on the most probable genotype for a given phenotype. For that, the distribution of most probable genotypes as follows: R₁ r = 29.8%, R₁ R₁ = 28.4%, R₁ R₂ = 16.8%, rr = 8.4%, R₂ r = 8.2%, R₁ r = 5%, R₂ R₂ = 2.2%, and r'r = 1.2% as shown in table 15.
<table>
<thead>
<tr>
<th>D</th>
<th>rh' C</th>
<th>rh'' E</th>
<th>hr' c</th>
<th>hr'' e</th>
<th>Wiener</th>
<th>Fisher-Race</th>
<th>Wiener</th>
<th>Fisher-Race</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pos</td>
<td>Pos</td>
<td>Pos</td>
<td>Pos</td>
<td>Pos</td>
<td>Rh0-pos</td>
<td>D-pos</td>
<td>Rh0-pos</td>
<td>D-pos</td>
<td>90.4</td>
</tr>
<tr>
<td>Pos</td>
<td>Pos</td>
<td>Pos</td>
<td>Pos</td>
<td>Pos</td>
<td>rh' - pos</td>
<td>C-pos</td>
<td>rh' - pos</td>
<td>C-pos</td>
<td>76.2</td>
</tr>
<tr>
<td>Pos</td>
<td>Pos</td>
<td>Pos</td>
<td>Pos</td>
<td>Pos</td>
<td>rh'' - pos</td>
<td>E-pos</td>
<td>rh'' - pos</td>
<td>E-pos</td>
<td>27.2</td>
</tr>
<tr>
<td>Pos</td>
<td>Pos</td>
<td>Pos</td>
<td>Pos</td>
<td>Pos</td>
<td>hr' - pos</td>
<td>c-pos</td>
<td>hr' - pos</td>
<td>c-pos</td>
<td>71.6</td>
</tr>
<tr>
<td>Pos</td>
<td>Pos</td>
<td>Pos</td>
<td>Pos</td>
<td>Pos</td>
<td>hr'' - pos</td>
<td>e-pos</td>
<td>hr'' - pos</td>
<td>e-pos</td>
<td>97.8</td>
</tr>
<tr>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
<td>Pos</td>
<td>Pos</td>
<td>rh rh</td>
<td>ccddee</td>
<td>rr</td>
<td>cde/cde</td>
<td>8.4</td>
</tr>
<tr>
<td>Neg</td>
<td>Neg</td>
<td>Pos</td>
<td>Neg</td>
<td>Pos</td>
<td>rh rh</td>
<td>ccdDe</td>
<td>r'r</td>
<td>Cdc/cdE</td>
<td>1.2</td>
</tr>
<tr>
<td>Neg</td>
<td>Pos</td>
<td>Pos</td>
<td>Pos</td>
<td>Pos</td>
<td>rh rh</td>
<td>CcdEe</td>
<td>r'r</td>
<td>Cde/cdE</td>
<td>0</td>
</tr>
<tr>
<td>Neg</td>
<td>Pos</td>
<td>Neg</td>
<td>Neg</td>
<td>Pos</td>
<td>Rh h</td>
<td>CcDeE</td>
<td>r'r</td>
<td>Cde/cdE</td>
<td>0</td>
</tr>
<tr>
<td>Pos</td>
<td>Neg</td>
<td>Neg</td>
<td>Pos</td>
<td>Pos</td>
<td>Rho r</td>
<td>ccdE</td>
<td>r'r</td>
<td>Cde/cdE</td>
<td>5</td>
</tr>
<tr>
<td>Pos</td>
<td>Neg</td>
<td>Pos</td>
<td>Pos</td>
<td>Pos</td>
<td>Rho r</td>
<td>CcDe</td>
<td>r'r</td>
<td>Cde/cdE</td>
<td>29.8</td>
</tr>
<tr>
<td>Pos</td>
<td>Neg</td>
<td>Pos</td>
<td>Pos</td>
<td>Pos</td>
<td>Rho r</td>
<td>CcDeE</td>
<td>r'r</td>
<td>Ccd/cdc</td>
<td>8.2</td>
</tr>
<tr>
<td>Pos</td>
<td>Pos</td>
<td>Neg</td>
<td>Neg</td>
<td>Pos</td>
<td>Rho r</td>
<td>CcDeE</td>
<td>r'r</td>
<td>Cde/cdE</td>
<td>28.4</td>
</tr>
<tr>
<td>Pos</td>
<td>Neg</td>
<td>Pos</td>
<td>Pos</td>
<td>Pos</td>
<td>Rho r</td>
<td>CcDeE</td>
<td>r'r</td>
<td>Cde/cdE</td>
<td>2.2</td>
</tr>
<tr>
<td>Pos</td>
<td>Pos</td>
<td>Pos</td>
<td>Pos</td>
<td>Pos</td>
<td>Rho r</td>
<td>CcDeE</td>
<td>r'r</td>
<td>Cde/cdE</td>
<td>16.8</td>
</tr>
<tr>
<td>Pos</td>
<td>Pos</td>
<td>Pos</td>
<td>Neg</td>
<td>Pos</td>
<td>Rho r</td>
<td>CcDeE</td>
<td>r'r</td>
<td>Cde/cdE</td>
<td>0</td>
</tr>
<tr>
<td>Pos</td>
<td>Pos</td>
<td>Pos</td>
<td>Pos</td>
<td>Neg</td>
<td>Rho r</td>
<td>CcDeE</td>
<td>r'r</td>
<td>Cde/cdE</td>
<td>0</td>
</tr>
</tbody>
</table>

Table 15: Distribution of Rh phenotypes and most probable genotypes

Distribution of Rh phenotypes

Figure 12: Distribution of Rh antigens
3.2 Comparison of the distribution of different blood groups between the present and other studies

Distribution of the ABO and Rh blood groups obtained in the present study were significantly different with those of several other studies (P < 0.05 to P < 0.001 for ABO groups and P < 0.05 to P < 0.01 for Rh groups) as shown in tables 16, 17, 18 and figure 13.

<table>
<thead>
<tr>
<th>Population</th>
<th>References</th>
<th>A%</th>
<th>B%</th>
<th>AB%</th>
<th>O%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asia</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Al Ain (UAE)</td>
<td>present study</td>
<td>26.4</td>
<td>14.2</td>
<td>3.6</td>
<td>56</td>
</tr>
<tr>
<td>Kalba (UAE)</td>
<td>Taha, J.Y. (2012)</td>
<td>24.2</td>
<td>22.8</td>
<td>4.7</td>
<td>48.4</td>
</tr>
<tr>
<td>Kuwait</td>
<td>Al-Bustan, S., et al. (2002)</td>
<td>16.2</td>
<td>14.2</td>
<td>2.6</td>
<td>66.7</td>
</tr>
<tr>
<td>Kurds (Iraq)</td>
<td>Jaff, M. S. (2010)</td>
<td>32.4</td>
<td>23.8</td>
<td>6.5</td>
<td>37.16</td>
</tr>
<tr>
<td>India</td>
<td>Agrawal, A., et al. (2014)</td>
<td>22.8</td>
<td>32.26</td>
<td>7.74</td>
<td>37.12</td>
</tr>
<tr>
<td>India</td>
<td>Khattak, I.D., et al. (2008)</td>
<td>18.8</td>
<td>32.5</td>
<td>9.9</td>
<td>38.7</td>
</tr>
<tr>
<td>Pakistan</td>
<td>Khattak, I.D., et al. (2008)</td>
<td>27.9</td>
<td>32.4</td>
<td>10.5</td>
<td>29.1</td>
</tr>
<tr>
<td>Europe</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Britain</td>
<td>Khattak, I.D., et al. (2008)</td>
<td>41.7</td>
<td>8.6</td>
<td>3.0</td>
<td>46.7</td>
</tr>
<tr>
<td>Hungary</td>
<td>Tauszik, T. (1995)</td>
<td>27.6</td>
<td>12.18</td>
<td>4.2</td>
<td>55.9</td>
</tr>
<tr>
<td>Turkey</td>
<td>Akbaş, F., et al. (2003)</td>
<td>12.2</td>
<td>12.1</td>
<td>0.8</td>
<td>73.9</td>
</tr>
<tr>
<td>French</td>
<td>Borghese, B., et al. (2014)</td>
<td>47.5</td>
<td>10.3</td>
<td>3.0</td>
<td>38.9</td>
</tr>
<tr>
<td>Africa</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nairobi</td>
<td>Lyko J., et al. (1992)</td>
<td>15.8</td>
<td>12.6</td>
<td>2.39</td>
<td>69</td>
</tr>
<tr>
<td>Sudan</td>
<td>Khalil, I.A., et al. (1988)</td>
<td>18.14</td>
<td>12.3</td>
<td>2.68</td>
<td>66.8</td>
</tr>
<tr>
<td>Guinea</td>
<td>Loua, A., et al. (2007)</td>
<td>22.5</td>
<td>23.8</td>
<td>4.72</td>
<td>48.8</td>
</tr>
</tbody>
</table>

Table 16: Comparison of distribution of different ABO blood groups in the present study with those of other studies:
<table>
<thead>
<tr>
<th>Population</th>
<th>References</th>
<th>Rh + (%)</th>
<th>Rh - (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asia</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Al Ain (UAE)</td>
<td>present study</td>
<td>90.4</td>
<td>9.6</td>
</tr>
<tr>
<td>Kalba (UAE)</td>
<td>Taha, J.Y. (2012)</td>
<td>91.1</td>
<td>8.9</td>
</tr>
<tr>
<td>Jordan</td>
<td>Abou-Jabal, A., et al. (2003)</td>
<td>87.2</td>
<td>12.8</td>
</tr>
<tr>
<td>Kurds (Iraq)</td>
<td>Jaff, M. S. (2010)</td>
<td>91.7</td>
<td>8.27</td>
</tr>
<tr>
<td>India</td>
<td>Agrawal, A., et al. (2014)</td>
<td>94.61</td>
<td>5.39</td>
</tr>
<tr>
<td>India</td>
<td>Khattak, I.D., et al. (2008)</td>
<td>94.45</td>
<td>5.5</td>
</tr>
<tr>
<td>Pakistan</td>
<td>Khan, M., et al. (2009)</td>
<td>89.5</td>
<td>10.5</td>
</tr>
<tr>
<td>Europe</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Britain</td>
<td>Khattak, I.D., et al. (2008)</td>
<td>83</td>
<td>17</td>
</tr>
<tr>
<td>Germany</td>
<td>Akbas, F., et al. (2003)</td>
<td>95</td>
<td>5</td>
</tr>
<tr>
<td>French</td>
<td>Borghese, B., et al. (2014)</td>
<td>76.9</td>
<td>23.1</td>
</tr>
<tr>
<td>Africa</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>North America</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>USA</td>
<td>Khattak, I.D., et al. (2008)</td>
<td>85</td>
<td>15</td>
</tr>
</tbody>
</table>

Table 17: Comparison of distribution of RhD phenotypes in the present study with those of other studies
### Table 18: Comparison of distribution of different Rh antigens in the present study with those of other studies

<table>
<thead>
<tr>
<th>Population</th>
<th>References</th>
<th>D%</th>
<th>E%</th>
<th>C%</th>
<th>E%</th>
<th>E%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Al Ain (UAE)</td>
<td>present study</td>
<td>90.4</td>
<td>27.2</td>
<td>76.2</td>
<td>97.8</td>
<td></td>
</tr>
<tr>
<td>Kalba (UAE)</td>
<td>Taha, J.Y. (2012)</td>
<td>91.1</td>
<td>21.0</td>
<td>73.2</td>
<td>97.3</td>
<td></td>
</tr>
<tr>
<td>Jordan</td>
<td>Abou-Jabal, A., et al. (2003)</td>
<td>87.2</td>
<td>30.2</td>
<td>67.9</td>
<td>96.6</td>
<td></td>
</tr>
<tr>
<td>Saudi Arabia</td>
<td>Hassan, F.M., et al. (2013)</td>
<td>93.1</td>
<td>13.8</td>
<td>21.9</td>
<td>99.8</td>
<td></td>
</tr>
<tr>
<td>West Africa</td>
<td>Siransy Bogui, L., et al. (2014)</td>
<td>92.9</td>
<td>13.8</td>
<td>21.9</td>
<td>99.8</td>
<td></td>
</tr>
</tbody>
</table>

**Figure 13:** Comparison of distribution of different Rh antigens in the present study with those of other studies

### 3.3 Comparison of distribution of Rh phenotype and most probable genotypes between the present and other studies

The distribution of the most probable genotypes was obtained and found as follows:  \( R_1 r = 29.8\% \),  \( R_1 R_1 = 28.4\% \),  \( R_1 R_2 = 16.8\% \),  \( rr = 8.4\% \),  \( R_2 r = 8.2\% \),  \( R_0 \) \( r = 5\% \),  \( R_2 R_2 = 2.2\% \), and  \( r'r = 1.2\% \) as shown in table 19 that can be compared with other studies.
<table>
<thead>
<tr>
<th>phenotypic Designation</th>
<th>Most Probable Genotype</th>
<th>% Present study</th>
<th>% Kalba UAE</th>
<th>% Saudi Arabia</th>
<th>% West Africa</th>
<th>% Jurdan</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wiener Fisher-Race</td>
<td>Wiener Fisher-Race</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rh0-pos</td>
<td>D-pos</td>
<td>90.4</td>
<td>91.1</td>
<td>93.1</td>
<td>92.93</td>
<td>87.2</td>
</tr>
<tr>
<td>rh' - pos</td>
<td>C-pos</td>
<td>76.2</td>
<td>73.2</td>
<td>62.3</td>
<td>21.97</td>
<td>67.9</td>
</tr>
<tr>
<td>rh'' - pos</td>
<td>E-pos</td>
<td>27.2</td>
<td>21.0</td>
<td>42.7</td>
<td>13.82</td>
<td>30.2</td>
</tr>
<tr>
<td>hr' - pos</td>
<td>c-pos</td>
<td>71.6</td>
<td>71.0</td>
<td>41.7</td>
<td>99.85</td>
<td>80.3</td>
</tr>
<tr>
<td>hr'' - pos</td>
<td>e-pos</td>
<td>97.8</td>
<td>97.3</td>
<td>91.0</td>
<td>99.85</td>
<td>9.6</td>
</tr>
<tr>
<td>rh rh</td>
<td>ccddee</td>
<td>8.4</td>
<td>7.3</td>
<td>5.6</td>
<td>10.4</td>
<td></td>
</tr>
<tr>
<td>rh' rh</td>
<td>Ccde</td>
<td>1.2</td>
<td>1.2</td>
<td>1.3</td>
<td>0.9</td>
<td></td>
</tr>
<tr>
<td>rh'' rh</td>
<td>ccdEe</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td>rh' rh''</td>
<td>Ccde</td>
<td>0</td>
<td>0.1</td>
<td>0</td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td>rh' rh'</td>
<td>CCdee</td>
<td>0</td>
<td>0.6</td>
<td>1.7</td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td>Rhorh</td>
<td>ccDe</td>
<td>5</td>
<td>10.9</td>
<td>30.8</td>
<td>60.5</td>
<td></td>
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<tr>
<td>Rh1 rh</td>
<td>CcDe</td>
<td>29.8</td>
<td>30.9</td>
<td>9.5</td>
<td>18.5</td>
<td>33.3</td>
</tr>
<tr>
<td>Rh2 rh</td>
<td>ccDe</td>
<td>8.2</td>
<td>6.7</td>
<td>11.8</td>
<td>12.2</td>
<td></td>
</tr>
<tr>
<td>Rh1 Rh1</td>
<td>CcDe</td>
<td>28.4</td>
<td>28.1</td>
<td>8.3</td>
<td>18.9</td>
<td></td>
</tr>
<tr>
<td>Rh2 Rh3</td>
<td>CcDe</td>
<td>2.2</td>
<td>1.8</td>
<td></td>
<td>3.2</td>
<td></td>
</tr>
<tr>
<td>Rh1 Rh3</td>
<td>CcDe</td>
<td>16.8</td>
<td>11.5</td>
<td>30.8</td>
<td>1.8</td>
<td>13.3</td>
</tr>
<tr>
<td>Rh2 Rh3</td>
<td>CcDe</td>
<td>0</td>
<td>0.6</td>
<td>13.7</td>
<td>0.3</td>
<td></td>
</tr>
</tbody>
</table>

Table 19: Comparison of distribution of Rh phenotype and most probable genotypes between the present study and those of other studies.
3.4 Molecular analysis

3.4.1 Molecular analysis of the extracted genomic DNA

A total of 48 samples out of 500 found to be RhD negative that correspond to 9.6% for RhD negative phenotype. The cde phenotype was counted for 40 samples (8.4%) and Cee phenotype found in 5 samples only (1.2%). In our study, the DNA of 45 samples were extracted (3 samples were lost), and the quality of the extracted DNA was assessed either by Nanodrop or by gel electrophoresis. The captured gel pictures are shown in figures 14.

![Figure 14: Determination of the quality of extracted DNA of the tested samples.](image)
3.4.2 Detection of RHD gene by PCR Products analysis

For detecting the presence or absence of the RHD gene, the PCR amplification for intron 4 of the RHD and RHCE genes was performed by primers D1/D2 as shown in figure 6. In that way, all DNA samples provide a template for amplification. Different annealing temperature (58, 61, and 64°C) were used, and the best was 64°C. Rh-positive PCR products (quality control samples) showed two-bands of different sizes that correspond to 600bp (D gene) and 1200bp (CcEe gene), while Rh-negative samples showed a single band corresponding to the fragment of the CcEe gene as shown in figure 15.

![PCR products](image)

**Figure 15:** Quality Control PCR products: (+): indicate RhD positive, (-) indicate Rh negative

Intron 4 of the RHCE gene was amplified in all the 45 tested samples (RhD-negative). These samples gave the 1200 bp bands with different band intensity as shown in figure 16.
3.4.3 Sequencing of Intron 4 of the RHD and RHCE as PCR products

The RHCE and RHD genes are composed of 10 exons. A deletion of 600 bp in intron 4 of the RHD gene was identified. Huang showed all sequences of intron 4 in the RHD gene, and indicated the existence of the human \textit{Alu} repeat in intron 4 of RHCE gene (Huang, C. (1996)). Avent, ND et al. demonstrated the \textit{Alu} element similar to Hung's report in addition to all sequences of intron 4 in both RHD and RHCE genes (Avent, N.D., et al. (1997)). To identify intron 4 in both the RHD and RHCE genes, we carried out PCR amplification with Primers D1/D2. The nucleotide sequences of intron 4 of RHCE and RHD genes are shown in figure 17. It was
confirmed that the difference in size between intron fragments of RHD and RHCE genes derives from a deletion, and that the non-deleted sequence of RHD gene is identical to the RHCE gene except for three nucleotides (Huang, C. (1996) & Avent, N.D., et al. (1997)). The sequence results were compared with the wild type sequence of the exon 4 through exon 5 of RHD and RHCE gene.

**Figure 17:** The nucleotide sequences of intron 4 of the RHCE (upper) and RHD (lower) genes. The RHCE and RHD genes consist of 1,075 and 426 nucleotides, respectively. The black letters demonstrate the common region of the RHCE and RHD genes. The white letters show the specific region of the RHCE gene. The letters surrounded with an oblong frame indicate the nucleotide substitutions between the RHCE and RHD genes. The broken line indicates the deleted region of the RHD gene.
The wild type sequences were aligned with the DNA sequences obtained and we found that these sequences were more close to the RHCE gene than the RHD gene and this confirm the results obtained from the PCR products gel electrophoresis (1200 bp bands correspond to the CeEe gene). Figure 18 shows one samples as an example.

**Figure 18**: Sequence alignment of the RHCE, RHD and the sequence obtained for one of the experimental samples.
Chapter 4: Discussion

4.1 Distribution of ABO Phenotypes

A gene for the specification of antigens A or B or type O determines the blood type. There are DNA differences, or polymorphisms, that determine the function of glycosyltransferase, resulting in different ABO blood types. Distribution of ABO phenotypes and their percentages among 500 local donors of Al Ain district were determined. Data obtained by serological ABO testing observed in Al Ain population are given in table 11. There was no significant difference in the distribution of ABO blood groups between male and female subjects, this is due to the fact that blood group are autosomal and the frequencies are not different in the two genders (Taha, J.Y. (2012)).

A comparison of the distribution of ABO and Rh blood groups in Al Ain population obtained in the present study with those of several other studies showed significant difference in the distribution of both ABO and Rh blood group antigens. In our study the ABO blood groups showed that the blood group O was most prevalent followed by group A, B, and AB. This arrangement in the distribution of ABO groups is similar to that found in Saudi Arabia and Kuwait. In contrast, the commonest groups in India and Pakistan are the blood group O and B, while in Europe the commonest blood groups are O and A. In addition, the distribution of different blood groups in the present study showed slight difference in the percentage compared to that of an only document regarding the distribution of blood groups in UAE, Kalba region (Taha, J.Y. (2012)).
These findings confirm that the distribution of blood group in different areas of the world varies which is perhaps due to genetic differences between different populations. Therefore, it is necessary to determine the blood groups of different geographical areas.

Research on ABO group system has been of immense interest, due to its medical importance in different diseases. The ABO blood group system is not only important in blood transfusions, cardiovascular disease, organ transplantation, erythroblastosis in neonates, but also one of the strongest predictors of national suicide rate and a genetic marker of obesity (Mollison PL (1979), Hein, H., et al. (2005)). The genetic history of a person can be known by studying the blood groups (Sokolov, R. (1993)). And this and other facts confirm the importance of determination of the prevalence of ABO groups in different populations to meet transfusion medicine and other field's requirements.

4.2 Distribution of Rh phenotypes

The studied group consisted of 500 samples selected from both genders (440 males and 60 females) and from different age groups. In Rhesus system, our study shows prevalence of Rh positive was 90.4%, while only 9.6% was Rh negative. These figures are similar to other studies carried in other Asian and African countries. These figures can be also compared to that of Europe which shows significant difference in the distribution of Rh negative phenotype as shown in table 16. This suggests that the expected frequency of Rh iso-immunization would be lower in our population than that encountered in the Europe countries. But regarding the distribution of Rh negative among female subjects it showed higher percentage
compared to the male, this can increase the Percentage of HDN in RhD negative pregnant women.

The distribution of the most probable genotypes that was obtained from the phenotypes has been showed in table 18. It was found that there were only 8 Rh-hr most probable genotypes characterized Al Ain population, while the other most Rh probable genotypes which are: Rz R1, Rz R2, r'r', r'r'', and r"r not found in this studied population. This variation can be compared to thirteen Rh phenotypic groups of various frequencies which were recorded by Taha, J.Y. in Kalba region, UAE, and Abou-Jabal, A., et al. 2003 in Jordan.

The distribution of Rh phenotype and most probable genotypes widely range among different races and religions. Our study showed that the most frequent antigens amongst five major antigens of Rh system was RhD while the least common was E antigen. And regarding the Rh phenotype and most probable genotype it was found that the most common in Rh positive samples were CCDee and DCe/DCe (R1 R1) respectively. While in Rh negative samples it was cde/cde (rr).

Rh blood group system has vital role in population genetics study and more importantly in transfusion practices. Knowledge of Rh phenotypes in a given population is relevant for better planning and management of blood banks (Al-Sheikh, I., et al. (1998)). Patients with sickle cell disease, thalassemia, etc, who need multiple blood transfusion are not few in our region, they may develop allo-antibodies against Rh antigens. And to solve this we need to know Rh phenotypes of the donors and recipients. Here we recommend that such identification procedures prior to transfusion is most vital in transfusion practice. Such study can be applied in practice for rapid and efficient identification of blood donors and immediate
selection of needed compatible blood units through forming donor/patients registry data system.

4.3 Determination of the Rh negative Variants

Two genes which are the RHD and RHCE located in the close proximity on chromosome 1 encode the Rh proteins, the RhD and RhCE. The genes are 97 percent identical, each has 10 exons and encodes proteins that differ by 32 to 35 amino acids. The large number of amino acid differences explains why exposure to RhD can result in an immune response in an RhD-negative individuals. These two genes can encode different variants that can't be identified serologically, and importantly they are clinically significant and have caused transfusion fatalities (Chou, S.T et al, 2010).

The distribution of D-negative individuals worldwide attract the early attention of population biologists, who attempt to make predictions of human origins and migrations based on Rh phenotype (Cavalli-Sforza, LL. (1991)). It is now obvious that the D-negative phenotype has arisen numerous times on different genetic backgrounds. There are three known mechanisms: which are: the entire deletion of the RHD gene that occurred on a Dce haplotype; that is, the RHCE allele carried with the deletion is ce. The other variants can be a raised due to the RHD that is not expressed because of a premature stop codon, nucleotide insertions, point mutations, or RHD/CE hybrids (Huang, C. (1996) & Avent, N.D., et al. (1997)). D-negative phenotypes in African and Asian persons are often caused by inactive or silent RHD, rather that RHD deletion (Chang, J.G.., et al. (1998) & Shao, C., et al. (2002)). In D-negative African black persons, 66% have RHD genes that contain a 37-bp insertion, which results in a premature stop codon, and 15% carry a hybrid RHD-CE-D linked
to ce. termed Cce, characterized by expression of weak C and no D antigen (Singleton, B.K., et al. (2000)).

The frequency of RhD-negative phenotype varies widely in different parts of the world. Our study showed that all of the RhD negative donors have the Cce /ce phenotypes. The RhCce positive phenotypes accounted for 1.2%, and Rhce positive accounted for 8.4% of Al Ain studied RhD-negative subjects. Based on PCR genotyping all of the D negative cases in our study showed deletion of intron 4; however, that doesn't mean there are no other genetic variation. Additionally, our sequence results showed (figure 18) that most of the PCR product sequences match to the RHCE gene rather than to RHD gene. This genetic analysis was confirmed via phenotypic analysis that these samples were RhD negative.

This pilot study confirmed not only that the RhD-negative phenotype were D-negative but also they were RhD negative genotypically where PCR product sequences showed that the DNA samples were D negative with similarity to RHCE phenotype. However, the results obtained can't be used to determine the type of RhD-negative variants. Molecular assays for blood group genes currently rely on polymerase chain reaction (PCR) amplification to generate sufficient material for analysis. One of the most common approaches uses oligonucleotide primers that are sequence specific, with amplifications indicating the presence or absence of an allele. Another common method is PCR restriction fragment length polymorphism (RFLP) with primers designed to amplify the region containing an allele single nucleotide polymorphism (SNP) followed by digestion with a restriction enzyme to detect the SNP. The size of the products, as determined by electrophoresis, indicates the presence or absence of the SNP. (Klein, H. G., et al. (2014d))
Molecular testing shall be combined with serological testing to improve transfusion safety and outcomes. And the challenge is to develop automated platform that sample several regions of the gene for unequivocal interpretation.
Chapter 5: Conclusion

In a conclusion, this study provide serologic frequencies of ABO and Rh phenotypes and most probable genotypes in order to ensure safe blood transfusion in Al Ain area. And it will be considered as a first study in this area up to our knowledge, which would be of great value for the UAE specially Al Ain area as it provides a reference figure about ABO and Rh-hr antigens which would be applied in blood transfusion practices.

The studied group consisted of 500 samples selected from both gender (440 males and 60 females) and from different age groups. It was found that the blood group O was predominant followed by A, B, AB. And regarding the Rh phenotypes it was found that there were only 8 Rh-hr most probable genotypes characterized Al Ain population which are as follows: R1 r, R1 R1, R1 R2, rr, R2 r, R0 r, R2 R2, and r'r while the other most Rh probable genotypes which are: Rz R1. Rz R2, r'r', r'r", and r'r not found in this studied population. This is variation can be compared to thirteen Rh phenotypic groups of various frequencies which were recorded by Taha, J.Y. in Kalba region, UAE.

Tests to determine an individual's D status would distinguish those RBCs that lacks, or have altered D epitopes and at risk of immunization to conventional D form those who carry mutations that reduce expression levels of D and are not at risk of producing anti-D. Unfortunately, serologic reagents cannot discriminate between these RBCs. These limitation suggest that molecular testing to determine D status should be commonplace in the future. RH molecular testing is an important tool that, when combined with serologic methods can resolve discrepancies in results, aid the management of hemolytic disease of newborns and improve transfusion safety and
outcomes for patients. The application of molecular genotyping to transfusion medicine has the potential to dramatically change blood bank testing by enabling the electronic selection of donor units that have been antigen-matched for recipients at multiple blood group loci. This approach should improve patient care and transfusion outcomes by reducing alloimmunization. The challenge lies in integrating such testing into blood bank environment, standardizing methods, obtaining Food and Drug Administration approval for labeling donor units, and enhancing information systems to incorporate and use this new information effectively.
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