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Blood Groups ABO and Rh System Among Paternity and Kinship Cases of Iraqi Medical Legal Directorate

Ahmed Kadhim Mohammed

PhD Forensic molecular genetics, Expert in Forensic Sciences, Medical Legal Directorate, Ministry of Health, Baghdad / Iraq

***Corresponding author**

Ahmed Kadhim Mohammed, PhD Forensic molecular genetics, Expert in Forensic Sciences, Medical Legal Directorate, Ministry of Health, Baghdad Iraq. Tel: +964-07901707159.

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Abstract

ABO and Rh are the most important blood group systems with various distributions reported for different populations. Traditional ABO blood group and Rh serology is based on the immunoreactivity of antisera with the carbohydrate A, B and H antigens on Red Blood Cells (RBC). Due to its medical importance in relation to different diseases, pursuing a line of investigation on the ABO and Rh blood group systems has been of significance for years. It is well known that these blood group systems are of great importance in blood transfusion and organ transplantation. Furthermore, the susceptibility to several diseases has been associated with the ABO phenotype, but such correlation remains controversial. Progress in the molecular biology of the ABO system has recognized the molecular basis of the red blood cell antigens and has provided a genetic model for ABO polymorphism at the molecular level. The ABH antigens are not primary gene product, but they are the enzymatic reaction product of enzymes called glycosyltransferases. After the ABO system, the Rh (Rhesus) blood group system is regarded as the second most important blood group system, as some of the severe hemolytic transfusion reactions and most hemolytic disease of the fetus and new born (HDFN) cases are associated with antibodies to the Rh group antigens.

Objectives: *To determine ABO blood group and Rh factor phenotype antigens frequency, distribution, and document among paternity cases of Iraqi Medical Legal Directorate / Clinical Forensic Department / paternity and kinship division.*

Materials and Methods: *The material and methods were used ABO and Rh kits for detection phenotype antigens of this blood types, the principle of test is one step immunological reaction. Total samples (1997) were referred to medical Legal Directorate (MLD). Out of the total participants there were 1815 (98.4%) of samples complete information for inclusion criteria. the sample were used for estimation of phenotypic distribution of ABO, Rh. ABO blood group serology was performed at MLD, Paternity and Kinship Division using commercially available monoclonal Anti-A, Anti-B, reagents according to the national blood bank standard operating procedures. The Method principle is based on antigen antibody reaction (agglutination) some tests were confirmed under microscope examination to see weak reaction.*

Results: *The most common blood group was O (52.3%) and the least common was AB (3.18%).97.7% of the blood participants were Rh Positive and the rest were Rh Negative. Most participants were young adults, representing the age group of 19 – 29. Most participants were male (88.1%).*

Conclusion: *Blood group O and Rh+ factor was dominant in both sexes, Up-to-date knowledge of the distribution of blood types in a local setting is critical to the functioning of any national health service. To date, there has been a lack of data in Iraqi population.*

Keywords: ABO System and Rh System, Blood Phenotypes

Introduction

In 1900, Karl Landsteiner of the University of Vienna identified that red blood cells contain antigens on their surfaces, and that blood plasma contains antibodies targeted to particular antigens [1, 2]. Experiments with blood transfusions have been carried out for hundreds of years. ABO system it is the ONLY system that the reciprocal antibodies are consistently and predictably present in the sera of people who have had no exposure to human red cells. Many patients have died, and it was not until 1901, when the Austrian Karl Landsteiner discovered human blood groups, that blood transfusions became safer. He found that mixing blood from two individuals can lead to blood clumping.

The clumped RBC (Red Blood Cells) can crack and cause toxic reactions, this can be fatal [3].

Clumping was an immunological reaction which occurs when the receiver of a blood transfusion has antibodies against the donor blood cells. The blood group antigen is of clinical importance in blood transfusion, organ transplantation, autoimmune haemolytic anaemia, fetomaternal blood group incompatibility, paternity identification, anthropologic investigation and forensic medicine. There are 29 systems for blood grouping classification one of these is the ABO system [4, 5]. The majority of cell types investigated in human have A, B, and O antigen originally found on the surfaces of red cells, but later they were also found on surface of various types of cells as well as in secretions [6]. This include platelets, lung tissues, intestinal mucosa, mucous cells, epidermis, nervous receptors and vascular endothelium [7-10].

The knowledge of the distribution of Rhesus antigen in a population is critical in managing a transfusion service in areas such as antenatal serology, paternity testing as well as selecting compatible blood and blood products. Even after Karl Landsteiner's discovery in 1900, transfusion reactions were still prevalent [1]. It was not until 1940 when Landsteiner and Weiner discovered the Rh factor that transfusion medicine involved less risk. Immunogenicity of the Rh factor along with A, B antigens made it mandatory for pre-transfusion testing currently there are more than 50 antigens in the Rh blood group system but the principal Rh antigens of medical interest are D, C, E, c and e [11]. A person with Rhesus antigen is referred to as Rhesus positive while individuals lacking the antigen are Rhesus negative. When a Rhesus negative person is exposed to Rhesus positive blood, antibodies will be produced, which cause potentially fatal hemolytic reactions [12, 13].

Genotyping enables the identification of both maternally and paternally derived alleles. Due to large number of alleles that could give similar phenotype but differ in genomic structure, there are several reasons for determining genotyping for blood group. These reasons include forensic medicine and paternity testing and linkage analysis [14-17].

Several new strategies have been employed to detect ABO genotype and other genetic markers by PCR and DNA typing. Restriction fragment length polymorphism (RFLP) method has taken advantage of altered restriction enzyme recognition sites caused by nucleotide substitution in the ABO locus of the A, B, or O alleles. This method used to differentiate between B and O alleles by PCR-RFLP [18-21].

In 1940, Landsteiner and Wiener found that sera from rabbits (and later guinea pigs) immunized with RBCs from rhesus monkeys red cells agglutinated 85% of human RBC samples [22]. Initially, it was thought that the animal and human antibodies identified a common factor, Rh, on the surface of rhesus and human RBCs [23].

Material and Methods

From first of Jan 2009 to end of Dec 2011 Samples were analyzed from all referred persons to Medical Legal Directorate / Clinical Forensic Department / paternity and kinship division. Conventional blood group (ABO and Rh) were done for selected 1815 participants that had inclusion criteria; blood samples collected in EDTA tubes. The BioTech kit, for ABO, Rh were used. Slid method was done for this purpose [24]. The weak antigen may be need exam under microscope. The difference in amount of antigen expressed on the red cell membrane between a homozygous and a heterozygous can often be detected serologically and is termed the dosage effect. The result of agglutination classified to about four grades according to density of agglutination [25].

Interpretation of results

Positive: Agglutination indicates positive reactions to respective group or Rhesus factor.
Negative: No Agglutination indicates negative reactions to respective group or Rhesus factor

Setting and Study Design

Descriptive and cross section study, conducted in Iraqi Medical Legal Directorate / Clinical Forensic Investigation / Paternity and Kinship Division, which serves as the referral center for all Iraqi province's paternity and kinship cases. From first of Jan 2009 to end of Dec 2011 in a total population of 1997 participants, from which a selected sample of 1815 individuals between the ages 18 to 65 were obtained because have all inclusion criteria.

Results

A total of 1997 participants out of the total participants, there was complete information for 1815. Findings show that phenotype O is the most common with a frequency of 51.18, followed by phenotype A (37%), B (8.66%) and AB (3.14%). Additionally, it was found that 91.33% of the individuals were Rh + and 8.67% were Rh -.

Ethical Consideration

This study was approved form scientific and ethical council in the Iraqi medical legal directorate(MLD) / Ministry of Health (MOH). This research was conducted based on Article 2 of the Iraqi Forensic Medicine Law of 2013.

Case Definition Inclusion and Exclusion Criteria

Any evidence referred officially from police offices and investigation bureaus to our clinical forensic department / paternity and kinship investigating during the period of the study were included in this study. The inclusion criteria for this study were participants between the ages of 18 – 65, with a personal weight above 50 kg, and who met the haemoglobin cut of criteria. All participants were required to have a haemoglobin level of at least 12.0 g/dL for females and 13.0 g/dL for males as per world Health organization (WHO) standards. A total of 1997 participants out of 1815 met inclusion criteria. 1815 participants (98.4%) had the complete information required for analysis in this study.

Sampling

During the period of the implementation totally (1977) whole blood samples were investigation for ABO and Rh systems phe-

notype antigens detection.

Outcome

Blood samples collected in EDTA tubes. The BioTech kit for ABO , Rh were used. Slid method was done for this purpose the weak antigens may be need exam under microscope. The difference in amount of antigen expressed on the red cell membrane between a homozygous and a heterozygous can often be detected serologically and is termed the dosage effect. The result of agglutination classified to about four grades according to density of agglutination.

Statistical Analysis

The database was examined for errors using range and logical data cleaning methods, and inconsistencies were remedied. An expert statistical advice was sought for. Statistical analyses were done using SPSS version 21 computer software (statistical package for social sciences) in association with Microsoft Excel 2013.

Result

A total of 1997 participants during the period, out of the total participants, there was complete information for 1815 (98.4%) blood donors. As shown in Table 1, there were more male participants (88%, n = 1597) as compared with female participants (12%, n = 218). The age distribution of the participants was 43% (n = 773), 29% (n = 530), and 28% (n = 508) for the age groups of 18–29, 30–39 and 40–65 respectively.

Table 1: Gender and Age Distribution of the Participants

Characteristic	Frequency (n)	Percentage %
Sex		
Female	218	12
Male	1597	88
Age (years)		
18 - 29	773	43
30 – 39	530	29
40 - 65	508	28

The most common blood type among the participants was blood group O (52%, n = 949), followed by blood group A (26%, n = 465), blood group B (19%, n = 342) and blood group AB (3%, n = 59%). As shown in Table 2, 98% (n = 1773) of participants were Rhesus positive while 2% (n = 42) were Rh negative.

Table 2: Blood Type and Rh Antigens of the Participants

Blood Type	Frequency (N %)
A	465 (26)
B	342 (19)
O	949 (52)
AB	59 (3)
Ph Antigens	
Rh negative	42(2)
Rh positive	1773(98)

In both sexes, blood group O was the commonest being 52% (n = 830) among male and 55% (n = 119) among female participants, the least common blood group was blood group AB representing 3% of male participants and 1.8% of female participants.

There was no difference between the blood type and the sex ($\chi 2$ = 3.7021, P value 0.895). Except for blood group AB, all the other blood groups had Rh-negative antigens in the donated blood. The prevalence of Rh-negative antigens was 3% (n =13), 2% (n=8) and 2% (n=21) for blood group A , B and O respectively. Table 3 represent the p value between Rh and blood group there were no difference in Rh between different blood group (X2=1.923, P value 0.712).

Table 3: Distribution of Rh Antigens Per ABO Blood Group Among Participants

ABO System	Rh-ve (%)	Rh +ve (%)
A	13(3)	452(97)
B	8(2)	334(98)
O	21(2)	928(98)
AB	-	59(100)

ABO and Rh system with E, e, C, c, are all somatic genes traits, therefore no effected to gender, birth place, age, and race on those traits, never effected to general ratio of all blood group because no effected to environment on expression of gene to this trait , this called pure genetic trait which controlled by gene only rather than other traits which controlled by gene and environment like tall and skin color .The gene of ABO is independent to RH gene each one of them had specific sequence on DNA (gene), therefore each of them inherited separated for them, but there some neighbourhood (unblocked genes) between RH gene and E, e, C, c genes therefore there were high inherited significant between them.

Discussion

This study determined the distribution of ABO and Rh antigens among blood of participants the current study showed that the majority of participants were male, which is consistent with other studies in Asia and in most regions globally. The inheritance of ABO and Rh blood groups follows the Mendelian rules. ABO blood group genes are located on the ninth chromosome and the Rh gene is located on the first chromosome. The frequency of ABO and Rh blood groups vary according to ethnic groups. One contributing factor might be that women do not meet cut-of values for haemoglobin given normal menses, menorrhagia, prenatal iron deficiency anemia and postnatal blood loss [26]. Older individuals may suffer from medical conditions such as ischemic heart disease, diabetes mellitus, malignancy and hypertension hence negatively impacting their ability to be well enough to inclusion criteria. In Kenya, Uganda, Mauritania and Ethiopia similar studies also showed the predominant blood group to be O and the least prevalent to be AB [27]. These trends, in keeping with other studies, may suggest that blood group AB is the least dominant while O is the most dominant overall across the continent. However, there is regional variability; some studies show that in Western and Central Africa, the most predominant group was B while in Eastern and Southern countries, blood group O dominated. In Pakistan, as Asian Population one study showed that blood group B is the most dominant while in Nepal it was blood group B. In Britain the most predominant blood group is O and the least is AB. These regional differences may be explained by genetic mapping and the varying origins of diverse ethnic groups.

In Iraq there are two specific studies for ABO system one at phenotypic level which revealed that: blood group O is the most common (35.7%) followed by B (28.3%), A (26.2%) and AB (9.8%). Rh D blood grouping was also evaluated and showed that; Rh D positive blood were (92%), while Rh D negative blood were (8%) [28]. The second study at molecular level which revealed the thirty samples show 14 (A)blood group, 13 of them are Heterozygous AO and one is Homozygous AA. The 14 samples are blood group B, 13 of them are Heterozygous BO and one of them is Homozygous BB. One sample for AB, O blood groups this type have only Homozygous phase and each gene are dominant in AB type so called co-dominant. In O type the gene is recessive and expressed only in Homozygous type only whereas the A and B genes are dominant over O [29].

Individuals who are Rhesus negative in our study were only 2.3% in contrast to other studies, which showed a range between 5 and 17%. Our study showed a slightly lower prevalence of Rh-positive blood donors in comparison to other studies in the African continent as well as in comparison with global trends. Given the number of participants in this study, however, this cannot be said to be statistically significant. Globally we share the same blood group types however clearly there are some geographic, regional, and ethnic differences. As well, the growing literature investigating the association of blood groups with the pathogenesis of cancer requires locally specific information on Rh distribution among other factors [30-39].

Recommendation

The study was conducted in MOH/MLD Clinical Forensic Department / Paternity and Kinship Division the results should not be generalized to Iraqi Population as a whole, this cross-section study selects specific samples, therefore meta study should be done in the future to improve these results.

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