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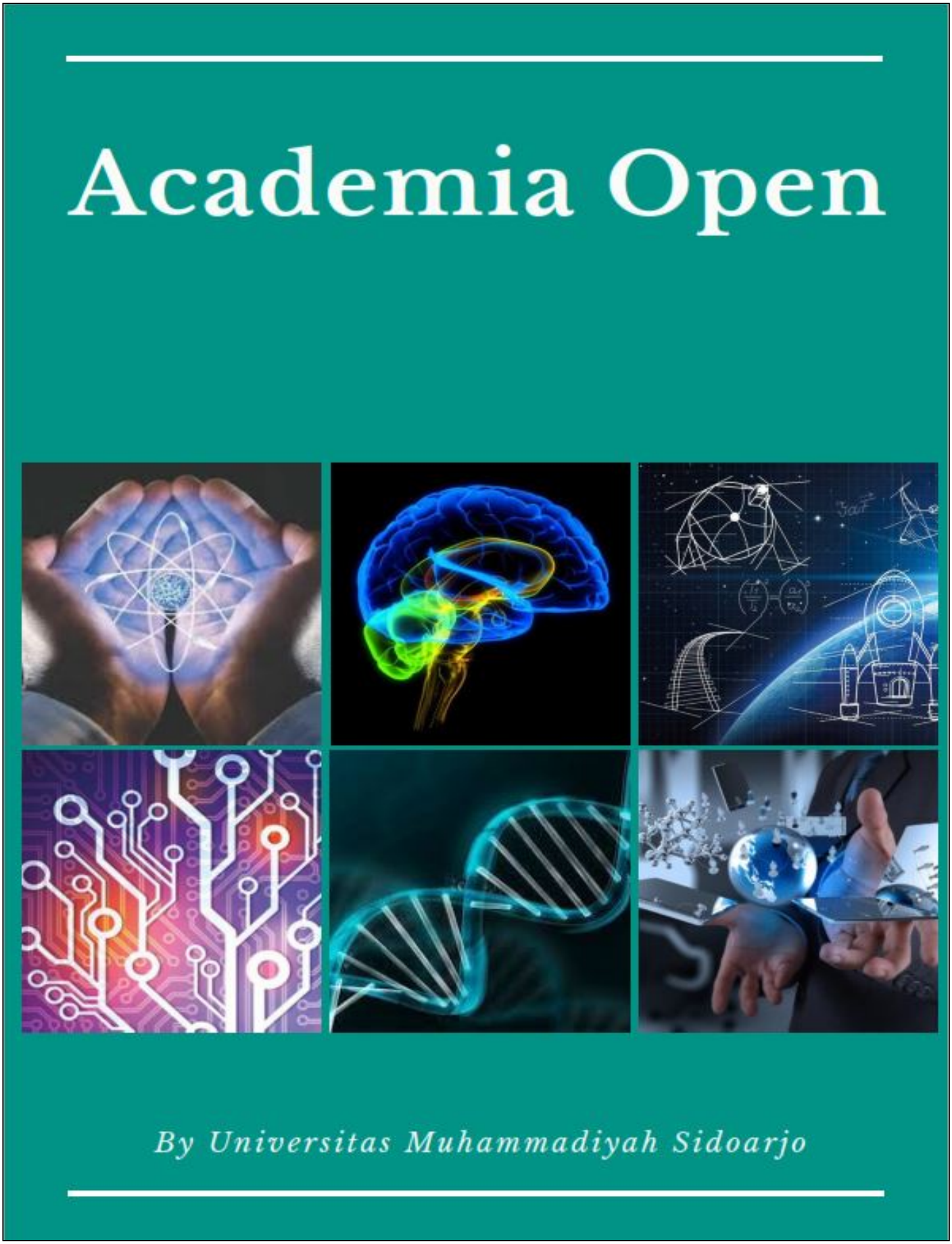

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Prevalence of a Subtype in Iraqi Donations of National Blood Transfusion Center

Prevalensi Subtipe pada Donasi Pusat Transfusi Darah Nasional Irak

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Abstract

General Background: The ABO blood group system is essential in blood transfusion, with subtypes of A and B groups influencing clinical outcomes. **Specific Background:** Subtypes A1 and A2 differ in the amount of antigen on red blood cells, impacting blood typing accuracy. **Knowledge Gap:** The prevalence of A2 and A2B subtypes in Iraqi donors remains underexplored, and their detection in routine screening can be challenging. **Aims:** This study aimed to determine the prevalence of A2 and A2B subtypes among Iraqi blood donors and evaluate the necessity of Anti-A1 reagent in accurate subtype identification. **Results:** In 2022, type O blood donors were the most prevalent, followed by B, A, and AB. A routine screening identified 0% A2 and 0.7% A2B subtypes, but 5% and 14.2% were A2B. **Novelty:** This study highlights the limitations of routine serological testing in detecting A subtypes, demonstrating that the use of Anti-A1 reagent significantly improves accuracy. **Implications:** Given the low rate of discrepancy between forward and reverse grouping, the Anti-A1 reagent should be routinely used for detecting A subtypes in clinical settings. Additionally, molecular techniques may be required to distinguish between rarer A subtypes such as A3, Ax, and Am.

Highlights:

1. ABO subtypes A1 and A2 are vital for precise blood transfusion typing.
2. Routine tests miss A2; Anti-A1 reagent ensures accurate subtype detection.
3. Molecular techniques help identify rarer subtypes like A3 and Ax.

Keywords: ABO system, blood subtypes, Iraqi donors, Anti-A1 reagent, blood typing

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Introduction

The first scientist who discovered the ABO system was Karl Landsteiner in 1901 when he drew blood from himself and his associates [14]. Therefore, he was the first one to perform Forward and Reverse grouping, which is considered the most frequent test in blood banking and both should be performed in all donors and patients[6].

ABO system is characterized by the presence of several carbohydrate antigens, which are located in different places of the body mainly in the surface of red blood cells (RBC) in addition to being present in smaller quantities in different cell lines and tissue in the body. Depending on the presence or absence of these antigens, individuals are divided into four types which can be either A, B, AB, or O [17].

In addition to the A and B antigens, this system is characterized by the presence of naturally occurring antibodies (isohemagglutinin) to the antigens they don't have. For instance, the individual with type A blood has antibodies against the B antigen and so with the rest of the other types of this system [26].

Each of the ABO system antigens and antibodies affects the blood transfusion process. Incompatible ABO blood type between donors and recipient (patient) can cause a potentially life-threatening immune reaction. Therefore, it is important to match the blood type of the donor with that of the recipient before performing a blood transfusion [23]. In addition to the main types of this system (A, B, O, AB), several weak A and B phenotypes belong to the ABO system that differ in the number of antigens present in the cell surface, they have lesser amount carried on red cells[1].

Historically the first time noted that there is more than one antigen belonging to A blood type was by the scientist Von Dungern in 1911. he noted that there were two different antigens in the blood type A depending on how A antigens of the red blood cell reacted with anti A and anti-A1 reagent. Clinically, the two main subtypes were A1 and A2. the differences between these subtypes in the number of antigens epitopes on the cell surface of RBC. A1 has approximately five times more than A2. The prevalence of this subgroup among individuals is different by region and race, for example, on European ancestry A1 represented 80% among people, while A2 is considered the second most common subtype 20% [1], [22].

Based on the study of the enzyme, there is quantitative and qualitative differences in the expression of an antigen between A1 and A2 and the reason for these differences belongs to the glycosyltransferase activity, which shows that the A1 enzyme is more active than A2 by 5-to-10-time active A2. So, when the suspension of RBC and group A are mixed with anti-A and anti-A1 reagents, the result of reactions will be different reactions depending on the specific antigenic properties of the red blood cells being tested. it is worth mentioning that when using anti-A monoclonal reagents probably there are no differences in agglutination degree between A1 and A2, both show strong agglutination. One way to distinguish A1 from A2 by using Anti A1 lectin, which is agglutinated with A1 red cells but not with A2 because is diluted to a level that should not agglutinated [2,3]. In addition to A2, there are several weakest subtypes of A which include (A3, Ax, Am, and Ael), this weak A subtype usually is recognized by apparent discrepancy between Forward (red cell) and Reverse (serum or plasma) grouping[3,4,5].

Aim of the study: to determine the prevalence of A subtypes (A2 and A2B) In the Iraqi donors and to compare the percentage of these subtypes that distinguish as a part of routine work (by the discrepancy between forward and reverse grouping or weak degree of agglutination in the (forward grouping) and the true percent of this subtypes by using Anti A1 reagent.

Methods

This study was a one-year retrospective analysis of blood groups of donors in the National Blood Transfusion Center (NBTC) in Baghdad, Iraq, which included all Iraqi donors who donated blood during the year 2022. All of them were screened for virus infections and tested for ABO and Rh typing. Blood samples were collected from the venous of all donors at the time of donation in the anticoagulant (EDTA) tubes. All blood samples were typed for ABO grouping by using column agglutination technique (gel card).

If the result of gel card mixed field or there is any discrepancy between Forward and Reverse grouping or if the score of reaction between antibody and antigen (in the Forward grouping) is equal or less than +2, the samples were re-tested a second time by using tube method from the same sample and new sample. The tube method includes forward grouping (cell grouping) using commercially available antisera (Rapid lab) and Reverse (serum) grouping by using A1 and B cells which are prepared manually in the grouping laboratory as a part of routine work utilizing the standard test. in addition to the foreword and Reverse grouping mentioned above, the samples were tested by using the reagent Anti A1 lectin to ensure if the donors have a subtype of A (A2 or A2B) by observing the agglutination between RBC suspension and Anti A1 reagent if there is agglutination the interpretation will be A type while in the absence of agglutination the sample will be consider A2 subtype. confusing

A second group of donors included in this study is 1154 blood samples that interpretation as A1 and A1B type,

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which mean not suspected as subtypes are included in order to compare the result of A2 subtype obtained from routine work and the realistic percentage of this subtype in Iraqi donors. All this group was tested by using Anti A1 reagent regardless of the degree of agglutination observed by using monoclonal A reagent,

Result and Discussion

The current study included all Iraqi donors who donated blood during the year 2022 in the national blood transfusion center in Baghdad, Iraq. The results of the statistical analysis showed that there were highly significant differences in p-value <0.0001. The total number of Iraqi donors reached 216714, the most common type among donors was O type (35%) followed by B (29.1%), A (27.1%) and AB type (8.8%) respectively. As shown in Table 1 and Figure 1.

Blood type	Numbers	Percentage
A blood type	59482	27.1%
B blood type	63073	29.1%
O blood type	75938	35%
AB blood type	18221	8.8%
Total donors	216714	100%

The chi-square statistic is 27.984. The *p*-value is < 0.0001. The result is significant at $p < .05$.

Figure 1. *Statistical analysis of ABO system distribution Iraqi donors*

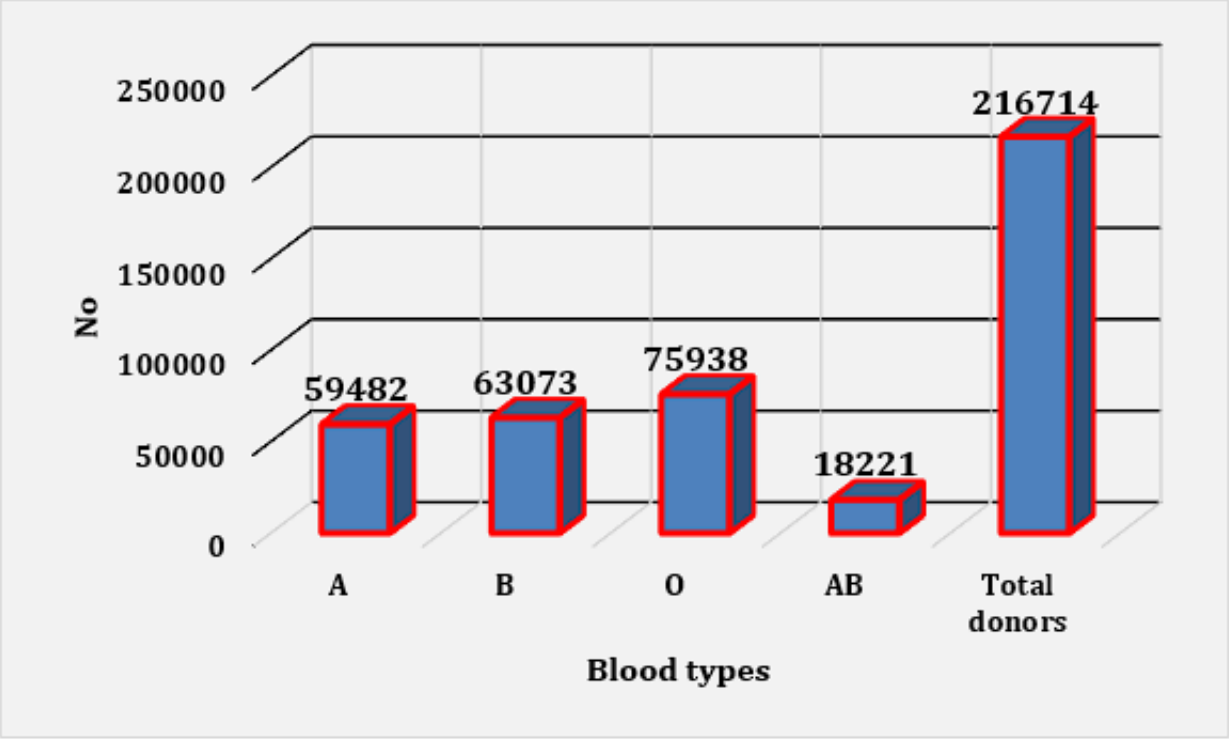

Figure 2. *Distribution of blood types in Iraqi blood donors*

Regarding the Rh factor, the majority of donors were Rh D positive in this study 90.3% while the rest donors were

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Rh D negative 9.7%. also, for the distribution of the Rh D with the ABO type, the sequence is the same from the most prevalent (O type) to the least (AB type), The results of the statistical analysis showed that there were highly significant differences in p-value <0.0001, as shown in Table 2.

ABO blood type	Rh D positive	Percentage	Rh D negative	Percentage
A	53644	24.50%	5838	2.60%
B	57221	26.40%	5852	2.70%
O	68340	31.50%	7598	3.50%
AB	16476	8%	1745	0.80%
Total numbers	195681	90.30%	21033	9.70%
The chi-square statistic is 39.325. The *p*-value is < 0.0001. The result is significant at $p < .05$.				

Figure 3. *Statistical analysis of Rh D distribution Iraqi donors*

In addition to this distribution, the A blood type is classified into subtypes A1 and A2 depending on the discrepancy between the Forward and Reverse test which was very low among Iraqi donors (only 3 cases in A-type and 4 cases in AB type or by the degree of agglutination on the Forward test (equal or less than +2) will be re-tested by Anti A1 lectin. The numbers and percentages of A subtype that were detected as a part of routine work from A and AB are 3 (0%) and 124 (0.7%), respectively. While in the second group which included 1154 samples (577 A type + 577 AB type) and is distinguished from the first group by using Anti A1 reagent to all samples, the numbers and percentage of A2 and A2B subtypes were 29 (5%) and 82 (14.2%), respectively as mentioned in table 3.

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Test groups	A blood type		AB blood type		
	A1	A2	A1B	A2B	Total numbers
G1	59479 100%	3 0%	18097 99.3	124 0.7%	77703
Case of discrepancy	None	3	none	4	
G2	548 95%	29 5%	495 85.8%	82 14.2%	1154
G1: numbers and percentage of type and subtype without using Anti-A1 reagent/ G2: numbers and percentage of type and subtype with using Anti A1 reagent					

Figure 4. *Comparative analysis of A subgroup detected by routine work and by using Anti A1 reagent*

Discussion

This study represents the ABO and Rh D blood types in all Iraqi who donated blood to the national blood transfusion center in Baghdad, Iraq. Our results indicated that blood type O was the most prevalent followed by blood type B, A type and then AB type. The results also demonstrated that the majority of donors were positive for Rh (D) antigen (90.3%) while minorities (9.7%) were negative. The results are the same in terms of distribution the blood type in the local study conducted in southern Babylon, Iraq[19], while in previous studies conducted in Iraqi population in both Blood Donors in Babylon [15] and north Baghdad, Iraq [18] the result was different in the second group which refers to that blood type A is considered the second blood type after O instead of B in our study. The same results were also obtained from the previous study in the Kurdistan region, which was characterized by ethnic diversity where they found that A blood type was the second most common blood type [27]. This indicates changes in the distribution of ABO blood type in Iraqi society. Chandra and Gupta explained that despite the constant of ABO antigens throughout life distribution of blood groups among different communities, ethnic groups and geographical boundaries varies over time [20,21].

When comparing the results with studies conducted in the neighboring countries of Iraq, in the Iranian Kurdish population, the northern Asir region in Saudi Arabia and the donor's population of Amman, Jordan type O is also the dominant blood type but the second type was A whereas in our study as mentioned above was B and still AB type the lowest blood group [11,12,13]. However, in the study conducted in Syrian Arabs and Diyarbakır, turkey where predominant of type A instead of O [16,28] . In the current study focused on the reactions between red blood cells and anti-A and anti-A1 reagents,which are important in blood typing and cross-matching for blood transfusions, as they help determine an individual's ABO blood type and subtype.

The published studies regarding the prevalence of A subtype in the Iraqi society are very limited and there is not enough data to show how it spreads. thus, this study represented a sample from the community because this center receives donors from different regions of Iraq.

In comparison, the result of the current study noted that there is a huge difference between the numbers of subtypes detected by depending only on the weakest degree or discrepancy (first group) and detected with Anti A1 reagent (second group). The prevalence of A subtypes in the first group was 0.07% (124) interpreted as A2B whereas only three donors were classified as A2, the reason for the low detection of the A2 subtype belonged to the fact that monoclonal anti-A reagent reacted equally strong with red blood cells from A1 and A2 individuals in the forward typing test [6]. So, detected A2B not A2 in this group depends on the weak degree of agglutination (Ab-Ag reaction) in the forward typing test, the reason is mainly due to the fewer number of antigens on the RBC suspension of these donors so that the reactivity in the serological test will be weaker. Klein and Anstee clarify that the gene of A1 creates antigen sites between (810000-1170000) on the adult A1 red blood cells, whereas the production of the A2 gene results only (240000-290000) antigen sites on the adult A2 red blood cells[7]. in addition to the above, the detection of A2B is more than A2 due to the efficiency of the enzymes that two genes responsible for the ABO system inherited together in the AB type. the efficiency of the enzyme responsible for the formation of B antigen seems to be more competitive than the enzyme responsible for the formation of A antigen so according to this competition of enzyme will be fewer amount of antigen sites of A on the AB type than on the A-type[8].

The increase in the percentage of the A2 subtype in the second group (reaching 5% (29) and 14.2% (82) in A and AB types respectively) is due to the specification of Anti A 1 reagent, which contains antibodies that react with only A1 antigen. A1 antigen is a subtype of A antigen that is mainly present in the A1 and A1B types and absent from the

A2 and A2B subtypes. So, if the RBCs being tested have the A1 antigen on their surface, the agglutination will be visible due to the reaction between Anti A1 reagent and the A1 antigen that causes agglutination. As mentioned in a previous study conducted by Gorakshakar and Ghosh, which explained that using Anti A1 lectin in serological testing has some degree of specificity, lectin is extracted from the seed of some type of plant that agglutinated with A1 but not agglutinated with A2 and others A subtype, therefore, it is considered to differentiate reagent between A1 and A2 phenotype [9].

From previous results, there are few cases of discrepancy, which can be defined as that result in unexpected reactions in the ABO forward and reverse typing reactions in this study the unexpected formation of Anti A1 in reverse grouping [24]. Anti-A1 presents as an alloantibody in the serum of 1% to 8% of A2 individuals and 22% to 35% of A2B individuals and may be present in the other weaker subgroups[25].

It cannot be determined if the subtype was A2 or another weaker subgroup by serological methods only, adsorption- elution tests, molecular testing and secretor studies can be used to subdivide A individuals into A2 or others A3, Ax, Aend[10].

Conclusion

In the current study, the most prevalent blood type among Iraqi donations was O then B>A>AB blood type, respectively. The percentage of discrepancy between forward and Reverse grouping in Iraqi individuals was very low, the same fact was observed about the weaker degree of agglutination that helps in the detection of subtypes. So, it is difficult to detect the ABO subtype in Iraqi donors by routine work only rather than using an Anti-A1 reagent to detect this subtype. We can't distinguish A2 from other subtypes (A3, Ax, Am, and Ael) by serological method only, it should be using molecular technique and other techniques.

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