



Distribution and Allelic Frequencies of ABO and Rh Blood Groups and Their Association with Hematological Parameters in Tobra, Libya: Cross-Sectional Study

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توزيع وتكرارات الأليلات لفصائل الدم (ABO و Rh) وعلاقتها بالمؤشرات الدموية
في توبرة، ليبيا: دراسة مقطعية

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Abstract:

ABO and Rh blood group systems serve as vital biomarkers in the fields of blood transfusion, clinical diagnostics, and population genetics. This study evaluated the phenotypic distribution and allele frequencies of the ABO/Rh systems and investigated their association with complete blood count (CBC) parameters among a group of healthy individuals in Tobra, Libya. A cross-sectional study design was conducted to analyze data from 101 healthy subjects (50 males and 51 females; aged 15–55 years), while controlling for confounding factors by excluding smokers, pregnant or lactating women, substance abuse, and individuals with chronic metabolic or cardiovascular diseases. Serological testing for ABO/Rh blood groups was performed alongside complete blood count (CBC) analyses, including hemoglobin (Hb), red blood cell (RBC) count, hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), total white blood cell (WBC) count, and platelet (PLT) count. Allele frequencies were estimated using Bernstein's method, assuming Hardy-Weinberg equilibrium. Differences between groups were assessed using the Student's t-test and one-way analysis of variance (ANOVA). The phenotypic distribution of ABO blood groups followed this order: A (37.62%) > O (30.69%) > B (24.75%) > AB (6.93%). Rhesus (Rh) positive and negative phenotypes accounted for 80.20% and 19.80% of participants, respectively. Allele frequencies were calculated as follows: $r(O) = 0.5674$, $p(A) = 0.2576$, and $q(B) = 0.1749$ for the ABO system; and $p(D) = 0.5550$ and $q(d) = 0.4450$ for the Rh system. Statistically significant differences were observed among ABO blood groups regarding hemoglobin (Hb) levels ($p = 0.0113$), mean corpuscular volume (MCV) ($p = 0.0312$), and mean corpuscular hemoglobin (MCH) ($p = 0.0394$), with group O exhibiting higher values for these parameters compared to groups A and AB. In contrast, the Rh factor showed no statistically significant association with complete blood count parameters ($p > 0.05$). Significant gender-based differences ($p < 0.001$) were observed in hemoglobin levels, red blood cell counts, and hematocrit, with males exhibiting higher baseline values. This study provides updated genetic data on the distribution of ABO/Rh blood groups in Tobra, confirming the predominance of the A allele. The strong association between ABO blood group phenotypes and red blood cell indices highlights the physiological impact of blood group antigens on

blood characteristics, offering a vital reference for transfusion medicine and local epidemiological studies.

Keywords: ABO/Rh systems; Allele frequencies; Complete blood count (CBC); Hardy-Weinberg equilibrium; Population genetics; Tocra; Libya.

الملخص:

تعد أنظمة فصائل الدم (ABO/Rh) مؤشرات حيوية جوهريّة في مجالات نقل الدم، والتشخيص السريري، وعلم الوراثة السكانية. وقد قيّمت هذه الدراسة التوزيع المظهري وتكرارات الأليلات لأنظمة (ABO/Rh)، وحققت في ارتباطها بمؤشرات تعداد الدم الكامل (CBC) لدى مجموعة من الأصحاء في مدينة توكرة بليبيا. استُخدم تصميم دراسة مقطعية لتحليل بيانات 101 شخص سليم (50 ذكراً و51 أنثى؛ تتراوح أعمارهم بين 15 و55 عاماً)، مع استبعاد العوامل المربكة للنتائج من خلال استثناء المدخنين، والنساء الحوامل أو المرضعات، ومتعاطي المواد المخدرة، والأفراد المصابين بأمراض مزمنة في التمثيل الغذائي أو القلب والأوعية الدموية. أجري فحص مصليّ لتحديد فصيلة الدم ABO/Rh بالتزامن مع فحوصات الدم الكاملة، بما في ذلك الهيموجلوبين (Hb)، وعدد كريات الدم الحمراء (RBC)، ومكداس الدم (HCT)، ومتوسط حجم الكرية (MCV)، ومتوسط هيموجلوبين الكرية (MCH)، ومتوسط تركيز هيموجلوبين الكرية (MCHC)، وإجمالي عدد كريات الدم البيضاء (WBC)، والصفائح الدموية (PLT). وقُدرت التوزيعات الأليلية باستخدام طريقة برنشتاين بافتراضات توازن هاردي-واينبرغ. وقُيِّمت الفروق بين المجموعات باستخدام اختبار t-student's وتحليل التباين أحادي الاتجاه (ANOVA). جاء التوزيع المظهري لفصائل الدم (ABO) وفق الترتيب: A بنسبة (37.62%) < O بنسبة (30.69%) < B بنسبة (24.75%) < AB بنسبة (6.93%). وشكّلت الفئات المظهرية الموجبة والسالبة للعامل الريزي (Rh) نسبة 80.20% و19.80% من المشاركين على التوالي. وقد حُسبت تكرارات الأليلات لتكون: $p(D) = 0.5550$ و $q(d) = 0.4450$ لنظام Rh. كما رُصدت فروق ذات دلالة إحصائية بين فصائل الدم (ABO) فيما يتعلق بمستويات الهيموجلوبين (Hb) ($p = 0.0113$)، ومتوسط حجم الكرية الحمراء (MCV) ($p = 0.0312$)، ومتوسط كمية الهيموجلوبين في الكرية (MCH) ($p = 0.0394$)، حيث أظهرت المجموعة O قيمة أعلى لهذه المؤشرات مقارنة بالمجموعتين A وAB. في المقابل، لم يُظهر عامل Rh أي ارتباط ذو دلالة إحصائية مع معايير تعداد الدم الكامل ($p < 0.05$). وسجلت فروق ملحوظة بين الجنسين ($p > 0.001$) في مستوى الهيموجلوبين، وعدد كريات الدم الحمراء، ومكداس الدم، حيث أظهر الذكور مستويات أساسية مرتفعة. توفر هذه الدراسة أساساً جينية محدثة لتوزيع فصائل الدم ABO/Rh في توكرة، مؤكدة غلبة الأليل A. ويُبرز الارتباط الوثيق بين أنماط فصائل الدم ABO ومؤشرات كريات الدم الحمراء التأثير الفسيولوجي لمستضدات فصائل الدم على خصائص الدم، مما يوفر مرجعاً أساسياً لطب نقل الدم والدراسات الوبائية المحلية.

الكلمات المفتاحية: أنظمة ABO/Rh؛ ترددات الأليلات؛ تعداد الدم الكامل (CBC)؛ توازن هاردي-واينبرغ؛ علم الوراثة السكانية؛ توكرة؛ ليبيا.

Introduction:

ABO and Rh antigen systems are fundamental immunological markers in blood transfusion protocols, clinical diagnostics, and population genetics (Dean, 2005). Since the pioneering discovery of ABO antigens by Karl Landsteiner in 1900 and the subsequent characterization of the Rh factor in 1940, blood group determination has proven crucial for ensuring transfusion compatibility, mitigating the risk of hemolytic disease of the fetus and newborn (HDFN), and tracing human evolutionary lineages (Mitra et al., 2014).

Global population distributions reveal significant geographic and ethnic diversity regarding the alleles of the ABO system (I^A, I^B, and i) and the Rhesus (Rh) factor (D and d). These evolutionary patterns primarily attributed to mechanisms such as natural selection, genetic drift, the founder effect, and historical population migrations (Cavalli-Sforza et al., 1994). Furthermore, determining phenotypic prevalence rates and calculating allele frequencies using Bernstein's equation under Hardy-Weinberg equilibrium assumptions provides essential genetic reference data; such information is crucial for optimizing the logistics of regional blood centers and developing emergency blood transfusion protocols (Garratty et al., 2002).

Growing evidence indicates that blood group antigens (ABO system) beyond their traditional immunological roles can influence various physiological parameters, including key Complete Blood Count (CBC) indices such as hemoglobin (Hb) levels, red blood cell (RBC) count, hematocrit (HCT), and red blood cell indices (MCV, MCH, and MCHC) (Wong et al., 2018). The biological pathways governing these interactions thought to stem from pleiotropic genetic effects, cell-surface antigen presentation processes, and potential differences in erythropoietin signaling across different blood group phenotypes (Chowdhury et al., 2015).

Although numerous epidemiological reports have documented the distribution of ABO/Rh blood groups across various geographical regions in Libya, detailed genetic assessments of allele frequencies

and their potential associations with hematological parameters remain limited for the city of Tobra. Given the city's unique demographic composition, conducting a local immunogenic characterization is crucial. Accordingly, this study aimed to: (1) determine the phenotypic distribution and calculate ABO and Rh gene frequencies in a sample of healthy adults in Tobra; (2) investigating the genetic equilibrium (according to the Hardy-Weinberg principle) within this sample; and (3) systematically evaluate variations in key Complete Blood Count (CBC) parameters and their relationship with blood group patterns and gender.

Materials and Methods:

- 1. Study Design and Exclusion Criteria:** A cross-sectional observational study was conducted in Tobra, Libya, involving a sample of 101 healthy adults (50 males and 51 females) aged 15 to 55 years; informed consent was obtained from each participant prior to blood collection. Strict selection criteria were applied; exclusion criteria included active smoking, pregnancy, breastfeeding, drug abuse, and documented chronic medical conditions (such as renal insufficiency, cardiovascular disorders, hypertension, liver cirrhosis, and diabetes mellitus). In addition, we exclude Individuals with a history of blood transfusion within the preceding six months, or those receiving blood component replacement therapies (such as oral iron or folate supplements).
- 2. Specimen Collection and Diagnostic Procedures:** A peripheral blood sample of approximately 5 mL was collected from each participant via venipuncture using sterile equipment. The samples immediately transferred into tubes containing ethylenediaminetetraacetic acid (EDTA) to prevent coagulation and then subjected to the following analyses:
 - **Blood grouping (ABO/Rh):** Blood group phenotypes were determined using commercial monoclonal antisera (anti-A, anti-B, and anti-D). Serological testing combined the standard method of glass slide agglutination with the tube test method for cross-verification, with visible hemagglutination results recorded upon examination.
 - **Hematological Profiling:** A comprehensive blood cell analysis was performed using an automated method. The parameters evaluated included: hemoglobin (Hb), red blood cell count (RBC), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), total white blood cell count (WBC), and platelet count (PLT).
- 3. Allele Frequency Estimation and Statistical Modeling:** ABO system allele frequencies—denoted as p (for allele A), q (for allele B), and r (for allele O)—were calculated using standard Bernstein formulas, assuming Hardy-Weinberg equilibrium:

$$r = \sqrt{\text{Freq of phenotype O}}$$

$$p = 1 - \sqrt{\text{Freq of phenotypes O + B}}$$

$$q = 1 - \sqrt{\text{Freq of phenotypes O + A}}$$

Regarding the Rh blood group system, allelic frequencies derived based on the proportion of the recessive phenotype:

$$q(d) = \sqrt{\text{Frequency of Rh(d) phenotype}}$$

$$p(D) = 1 - q(d)$$

Biostatistical analyses and data processing were performed using IBM SPSS software (version 26.0). Continuous quantitative variables following a normal distribution were expressed as mean $\pm$ standard deviation (SD). Comparisons between groups—based on gender and Rhesus (Rh) factor—were conducted using the independent two-tailed Student's t-test. Differences among the four ABO blood group phenotypes were analyzed using one-way analysis of variance (ANOVA). Statistical significance was set at $p < 0.05$.

Results:

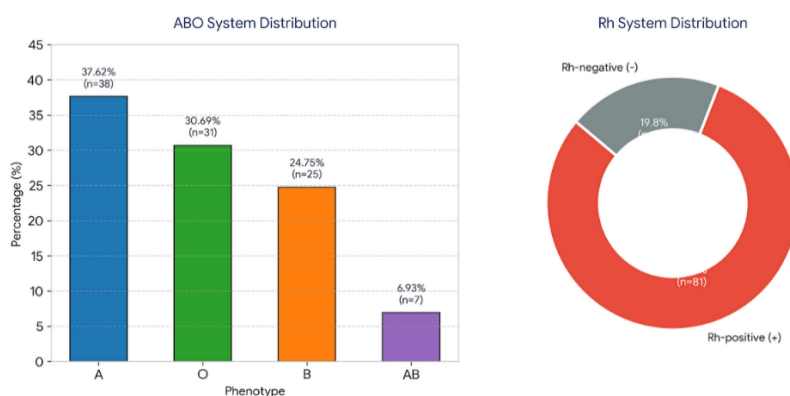
1. Phenotypic distribution and allele frequencies for the ABO/Rh systems:

Phenotypic characterization of the study sample ($n = 101$) revealed that blood group A was the most common at 37.62% ($n = 38$), followed by group O at 30.69% ($n = 31$), group B at 24.75% ($n = 25$), and finally group AB at 6.93% ($n = 7$). Regarding the Rh antigen system, 80.20% ($n = 81$) of participants were Rh-positive, while 19.80% ($n = 20$) were Rh-negative.

Using Bernstein's formula within the Hardy-Weinberg equilibrium framework, the calculated gene frequencies for the ABO system were: r (O) = 0.5674, p (A) = 0.2576, and q (B) = 0.1749. For the Rh system, the calculated allele frequencies were: p (D) = 0.5550 and q (d) = 0.4450.

Table (1): Phenotypic Distribution and Allele Frequencies of ABO and Rh Systems (N = 101)

Genetic System	Phenotype	Frequency (n)	Percentage (%)	Allele Frequency
ABO System	A	38	37.62%	p (A) = 0.2576
	O	31	30.69%	r (O) = 0.5674
	B	25	24.75%	q (B) = 0.1749
	AB	7	6.93%	Total = 1.0000
Rh System	Rh-positive (+)	81	80.20%	p (D) = 0.5550
	Rh-negative (-)	20	19.80%	q (d) = 0.4450

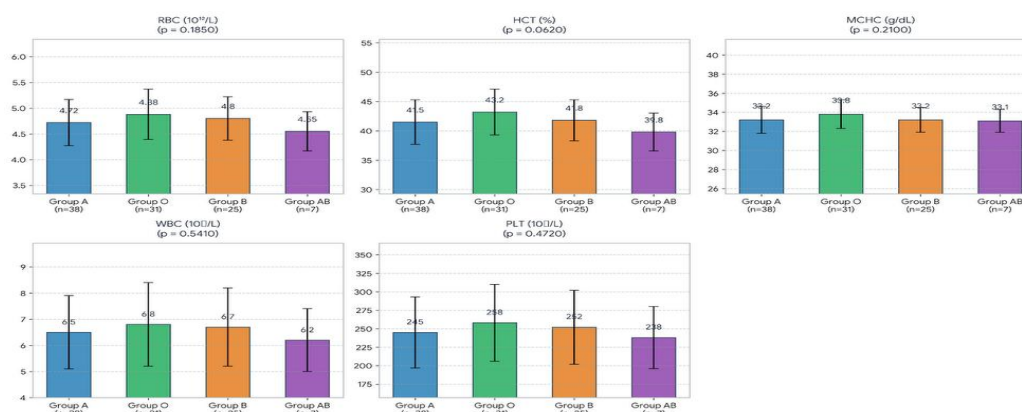
**Figure (1):** Shows Phenotypic Distribution and Allele Frequencies of ABO/Rh Systems

2. Association between blood groups (ABO system) and hematological parameters:

One-way analysis of variance (ANOVA) (Table 2) revealed statistically significant differences among ABO blood group phenotypes regarding hemoglobin (Hb) concentration ($p = 0.0113$), mean corpuscular volume (MCV) ($p = 0.0312$), and mean corpuscular hemoglobin (MCH) ($p = 0.0394$). Participants with blood group O exhibited higher mean levels of hemoglobin (14.6 ± 1.3 g/dL) and MCV (88.5 ± 5.4 fL) compared to those with blood groups A and AB. Conversely, no statistically significant associations were observed between ABO blood group phenotypes and red blood cell (RBC) count, hematocrit (HCT), mean corpuscular hemoglobin concentration (MCHC), white blood cell (WBC) count, or platelet count ($p > 0.05$).

Table (2): Comparison of Hematological Parameters across ABO Blood Groups (Mean $\pm$ SD)

Parameter	Group A (n=38)	Group O (n=31)	Group B (n=25)	Group AB (n=7)	p-value (ANOVA)
Hb (g/dL)	13.8 ± 1.2	14.6 ± 1.3	13.9 ± 1.1	13.2 ± 1.0	0.0113*
RBC (10 ¹² /L)	4.72 ± 0.45	4.88 ± 0.49	4.80 ± 0.42	4.55 ± 0.38	0.1850
HCT (%)	41.5 ± 3.8	43.2 ± 3.9	41.8 ± 3.5	39.8 ± 3.2	0.0620
MCV (fL)	87.9 ± 5.1	88.5 ± 5.4	87.1 ± 4.8	87.5 ± 4.2	0.0312*
MCH (pg)	29.2 ± 2.1	29.9 ± 2.2	28.9 ± 1.9	29.0 ± 1.8	0.0394*
MCHC (g/dL)	33.2 ± 1.4	33.8 ± 1.5	33.2 ± 1.3	33.1 ± 1.2	0.2100
WBC (10 ⁹ /L)	6.5 ± 1.4	6.8 ± 1.6	6.7 ± 1.5	6.2 ± 1.2	0.5410

**Figure (2):** Shows Comparison of Hematological Parameters across ABO Blood Groups

3. Differences related to gender and Rhesus (Rh) factor:

Statistical analysis (Table 3) showed no statistically significant differences between Rh-positive (Rh+) and Rh-negative (Rh-) groups regarding any of the evaluated hematological parameters ($p > 0.05$). In contrast, the Student's t-test revealed significant gender-related differences ($p < 0.001$) in hemoglobin (Hb), red blood cell (RBC), and hematocrit (HCT) levels, with males exhibiting higher baseline values compared to females.

Table (3): Gender Differences in Core Hematological Parameters

Parameter	Males (n=50)	Females (n=51)	p-value (t-test)
Hb (g/dL)	15.1 ± 1.1	12.8 ± 1.0	< 0.001*
RBC (10 ¹² /L)	5.08 ± 0.38	4.45 ± 0.35	< 0.001*
HCT (%)	45.2 ± 3.1	38.6 ± 2.8	< 0.001*

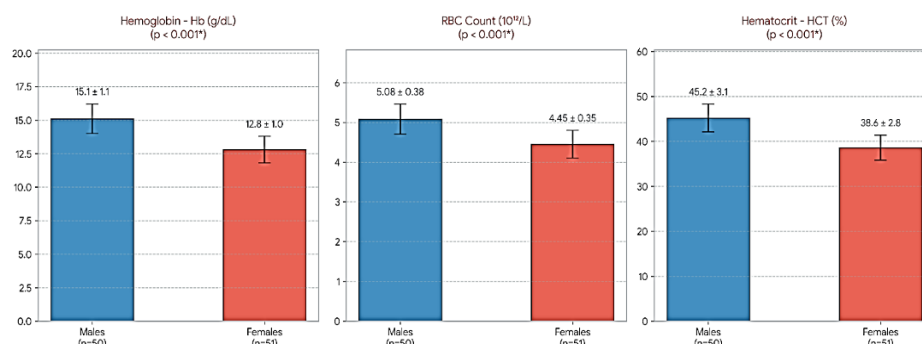


Figure (3): Shows Gender Differences in Core Hematological Parameters

Discussion:

In the present study, the prevalence of ABO blood group phenotypes followed the order: A > O > B > AB. These findings generally align with the epidemiological patterns observed in Libya and North Africa, where blood groups A and O are notably prevalent. Specifically, Al-Ghazali and Al-Mahjoub (2018) documented high frequencies of phenotypes O and A in Benghazi, reflecting the distinctive genetic composition of the Mediterranean region. Similarly, Al-Mukhtar and Al-Tarhuni (2020) observed a lower frequency of the q allele (associated with blood group B) compared to the p (associated with group A) and r (associated with group O) alleles among blood donors in western Libya.

Regarding the Rhesus (Rh) antigen system, it was found that 80.20% of individuals were (Rh+), while 19.80% were (Rh-). This observed proportion of (Rh-) individuals aligns closely with reference estimates for North African populations (ranging from 12% to 20%), figures that remain significantly higher than the rates recorded in East Asian populations (less than 1%) (Saeed & Salem, 2021). Furthermore, the calculated gene frequencies for the ABO system ($r = 0.5674$, $p = 0.2576$, $q = 0.1749$) and allele frequencies for the Rh system ($p \{D\} = 0.5550$, $q \{d\} = 0.4450$) are consistent with the Hardy-Weinberg equilibrium principle, reflecting a state of genetic stability characteristic of North African population samples (Bain, 2017).

ANOVA analysis revealed statistically significant differences among ABO blood group types regarding hemoglobin (Hb) ($p = 0.0113$), mean corpuscular volume (MCV) ($p = 0.0312$), and mean corpuscular hemoglobin (MCH) ($p = 0.0394$), with individuals of blood group O exhibiting higher mean values. These pleiotropic dynamics likely stem from interactions at the genetic locus on chromosome 9q34, where the ABO gene regulates red blood cell differentiation kinetics and the structure of cell membrane glycoproteins (Ashafa et al., 2021). Our observations align with the findings of Saeed and Salem (2021), who reported similar elevations in red blood cell indices among individuals of blood group O in eastern Libya. In contrast, Abdullah et al. (2014) did not find any significant associations between ABO blood groups and complete blood count parameters, suggesting that regional environmental influences and polygenic background variations may alter these phenotypes across distinct populations. A clear sex-based difference ($p < 0.001$) was observed in hemoglobin (Hb), red blood cell (RBC) count, and hematocrit (HCT) levels, with males exhibiting significantly higher values than females. This finding aligns fully with established physiological principles and regional reference ranges (El-Sawi et al., 2019; Bain, 2017). Physiologically, the higher RBC parameters in males attributed to androgen-stimulated erythropoietin production and the absence of menstrual blood loss. In contrast, Rhesus (Rh) antigen expression showed no statistically significant effect on any complete blood count (CBC) parameters ($p > 0.05$), reinforcing the prevailing understanding that the Rh (D) antigen functions primarily as an immunogenic structural membrane protein without directly altering quantitative RBC indices (Al-Bustan et al., 2002).

Conclusion & Recommendations:

1. Conclusion:

- **Phenotypic profiling revealed the following order of frequency:** A > O > B > AB, with the Rhesus-positive (Rh+) trait being more prevalent than the Rhesus-negative (Rh-) trait. Allele frequency values, calculated using the Hardy-Weinberg equation, confirm the existence of genetic equilibrium, reflecting demographic characteristics typical of the Mediterranean and North African regions.
- Statistically significant phenotypic differences were also confirmed among ABO blood groups regarding hemoglobin levels, mean corpuscular volume (MCV), and mean corpuscular hemoglobin (MCH). Notably, individuals with the (O) phenotype exhibited higher values for these red blood cell indices compared to other blood groups.
- The study revealed a notable sex-based difference in key red blood cell parameters (hemoglobin, red blood cell count, and hematocrit), with higher levels observed in males compared to females. In contrast, the Rhesus (Rh) factor showed no statistically significant effect on the quantitative parameters of the complete blood count.

2. Recommendations:

- **Extensive multicenter epidemiological studies:** Multicenter epidemiological studies with large sample sizes across diverse geographic regions are essential for validating regional immunological baselines.
- **Centralized national blood transfusion registry:** Establishing a national database of ABO/Rh phenotype frequencies recommended streamlining blood inventory distribution, optimizing transfusion logistics, and facilitating access to rare blood types.
- **Molecular genetics:** Further functional genomic studies are recommended to elucidate the pleiotropic pathways on chromosome 9q34 that regulate red blood cell lineage dynamics and membrane expression.
- **Sex-specific clinical reference ranges:** Clinical diagnostic laboratories should apply sex-specific reference ranges for complete blood count parameters to reflect physiological sex-based differences in clinical assessments.

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