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The relation between age, gender and the percentage of abnormalities in the RBC Morphology

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العلاقة بين العمر والجنس ونسبة التشوه في شكل كريات الدم الحمراء

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الملخص

العمر والجنس يمثلان عاملان حيويان يمكنه تعديل الفسيولوجيا المرضية وشدة الأمراض التي تصيب الأنسان والتي تشمل تشوهات كريات الدم الحمراء. الأبحاث الحديثة تشير الى ان كريات الدم الحمراء المأخوذة من المتبرعين الرجال تتحلل أسرع من تلك المأخوذة من المتبرعات النساء. ان الغرض الرئيسي من هذه الدراسة هو لتحديد العلاقة بين العمر والجنس ونسبة التشوه في كريات الدم الحمراء وذلك لمعرفة التباين بين الرجال والنساء في تشوه كريات الدم الحمراء. قد تم فحص ثمانية وتسعون شريحة مأخوذة من 21 رجل و28 أنثى يشكلون 49 متبرع من أعمار مختلفة. بشكل عام أظهر تحليل مربع كاى وجود تأثير ذو أهمية إحصائية للعمر على شكل كريات الدم الحمراء (χ^2=166.89, P=0.000). وقد تم تحديد 10 أنواع من التشوهات الشكلية لكريات الدم الحمراء في الرجال و11 نوع في النساء. وقد استنتج من الدراسة الحالية أن هناك تأثير للعمر والجنس على التشوه في شكل كريات الدم الحمراء، وان هناك اختلاف ولو كان صغيرا بين الرجال والنساء في أشكال التشوه في كريات الدم الحمراء بشكل عام.

الكلمات المفتاحية: العمر، الجنس، شكل كريات الدم الحمراء.

The relation between age, gender and the percentage of abnormalities in the RBC Morphology

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Abstract

Age and gender represents biologic variables that can modulate the pathophysiology and severity of human diseases, including RBC disorders. Recent reports have indicated that stored RBCs from male donors hemolyze more than those from female donors at the end of storage or in response to applied mechanical stress. The main purpose of this study was to determine the relation between age, gender and percentage of abnormalities in the RBC, to known variation RBC abnormality in women and men. Ninety eight blood slides of 49 individuals comprised of 21 male and 28 female of different age classes were collection and subjected to full history taking and Laboratory examination. In general, the Chi square (χ^2) test exhibited statistical significant effects of age on RBC morphology (χ^2 = 166.89, *P*=0.000). During the current study, 10 different types of abnormality in shapes of the red blood cells were identified in male samples and 11 types in female samples. In conclusion, The age and gender effects on the human blood cells were evaluated. There was a significant albeit very small difference in RBC abnormality between women and men in the entire cohort.

Keywords: Age, Gender, RBC morphology.

Introduction:

Blood is a connective tissue made up of cells (RBCs, WBCs and Platelets) suspended in a fluid medium known as plasma. The plasma is made up of minerals, salt, vitamins, coagulation factors cell and organic elements. Full blood count (FBC) consist of cellular content, white blood cells (leucocytes) and its different types platelets (thrombocytes) and the red blood cell (erythrocyte), and plasma with related parameters (packed cell volume, hemoglobin concentration).

The erythrocyte is a unique cell, with a cytoplasm consisting of 95% hemoglobin, the protein responsible for oxygen transfer from the lungs to the rest of the body [1]. Red blood cells are the most abundant type of blood cells in the human body. The count of these vital cells is often the first step done in analyzing a patient's pathological condition. Normal red blood cells (RBC's) are biconcave in shape with a central pale area, and any deviation in size, shape, volume, structure or color represents an abnormal cell. Such abnormalities are detected by viewing the blood-smear images through a microscope [2]. Examination of blood cells can reveal blood cell abnormalities, which may be characteristic of different diseases, or variation in number of cell types, which could reveal a response to infection. [3,4].

Blood samples used in haemorheological studies may need to be stored for a period of time prior to being measured. This period may sometimes include shipping to a remote laboratory and thus the storage might be prolonged for 24 hours or longer. Red blood cells (RBCs) undergo biochemical and morphologic alterations during storage that are known as the storage lesions causing decreased RBC quality and are correlated with transfusion reactions in certain groups especially in infants and critically ill patients [5,6].

There is enough scientific evidence that storage time from the transfused RBC during transoperatory or during their stay in the intensive care unit is associated with an increased risk of death, more complications and more hospital stay [7-9].

Little is known about age-related changes in red blood cell (RBC) abnormality. Various hematological parameters have also been shown to change and develop with age, although other factors such as genetics, sex,

altitude, and life style may affect this process. Most of these factors vary depending on the population and geographical area studied [10-14]. Sex represents a biologic variable that can modulate the pathophysiology and severity of human disease, including cardiovascular disease, renal failure, and RBC disorders. recent reports have indicated that stored RBCs from male donors hemolyze more than those from female donors at the end of storage or in response to applied mechanical stress [15,16]. Therefore, the main purpose of this study was to determine the relation between age and gender and abnormalities in the RBC, as well as to known variation RBC abnormality in women and men.

Material and methods:

Ninety eight blood slides of 49 individuals comprised of 21 male and 28 female of different age groups were collection from January 2023 to June 2023. The samples from both genders were chosen randomly, and subjected to full history taking and Laboratory examination.

Human venous blood samples were taken and anticoagulated with EDTA and the samples was examined at once. For each individual, two blood smears were prepared and stained with Giemsa stain and then were utilized to determine the morphology and percentage of erythrocyte abnormalities. Detection of abnormal red blood cells was according to Rozenberg [17] and Shah *et al.* [18].

To evaluate the correlation of the variables and morphologic alterations, the Chi square (χ^2) test was used to compare proportions between sex and ages. *P*-value of ≤ 0.05 was considered to be significant. The statistical analysis was performed using software PAST package release 3.25 [19].

Results:

Taking both genders together, the abnormal RBC showed 12 different type of abnormality in shapes of the red blood cells were identified in Table (1) and Figure (1). Only, the Macrohypochromasia was observed in 12.5% of individuals of 34_39 age class. However, the 28_33 age class has a higher percentage 52.6% of prevalence of RBC morphological diseases relative to age classes. The Rouleaux, Macrocytes, Hypochromasia, Burr and Acanthocytes found in high percentage (100%, 100%, 76.9%, 69.2% and 69.2% respectively) in 28_33 age class. On the other hand, the

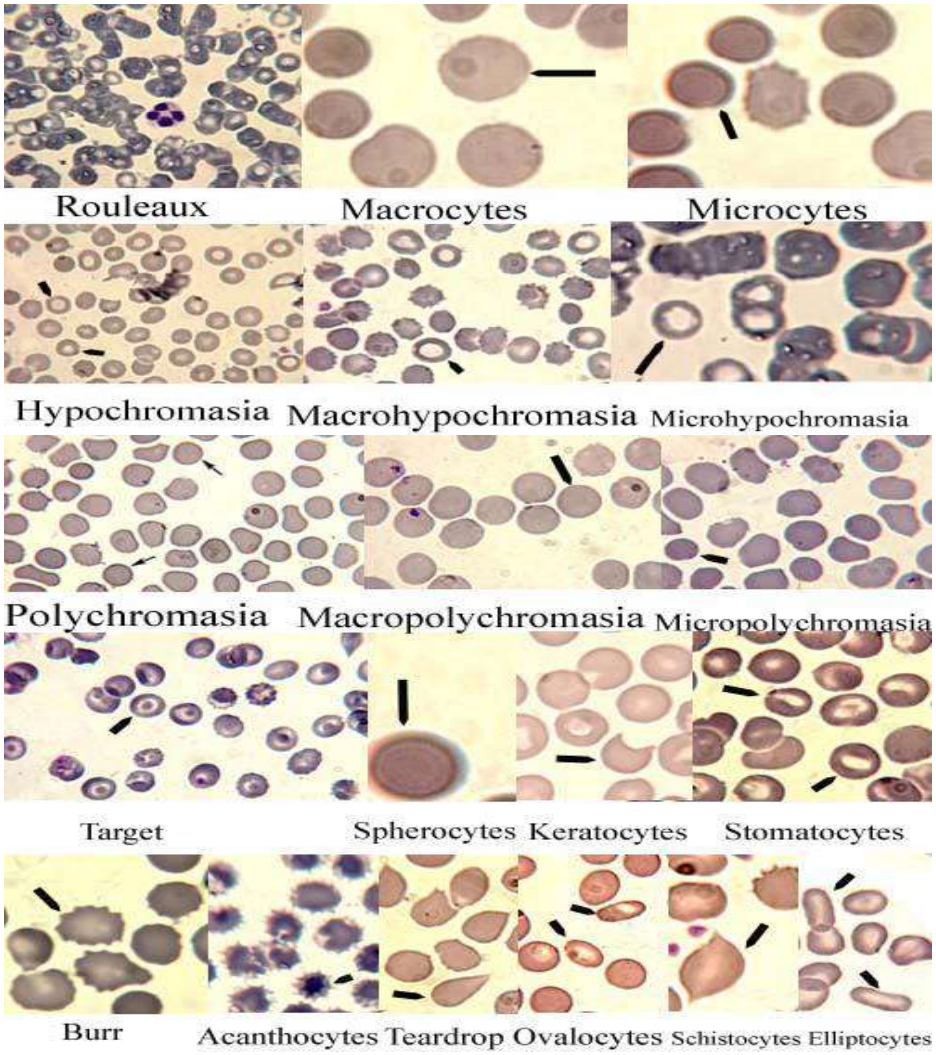
Keratocytes was observed in 33.3% of individuals of 16_21age class only. In general, the Chi square (χ^2) test exhibited statistical significant effects of age on RBC morphology ($\chi^2 = 166.89$, $P = 0.000$).

Table (1): The Prevalence of RBC morphological diseases relative to age groups in 49 subjects.

Age groups / RBC abnormality	Prevalence, n (%)							
	16_21 N=3	22_27 N=9	28_33 N=13	34_39 N=8	40_45 N=10	46_51 N=3	≥52 N=3	Total
Rouleaux	3(100)	8(88.9)	13(100)	8(100)	9(90)	3(100)	3(100)	7(100)
Macrocytes	3(100)	7(77.8)	13(100)	7(87.5)	9(90)	3(100)	2(66.7)	7(100)
Macrohypochromasia	n.o.	n.o.	n.o.	1(12.5)	n.o.	n.o.	n.o.	1(14.3)
Macropolychromasia	n.o.	n.o.	n.o.	n.o.	n.o.	n.o.	n.o.	n.o.
Microcytes	2(66.7)	3(33.3)	7(53.8)	3(37.5)	3(30)	0	2(66.7)	6(85.7)
Microhypochromasia	n.o.	n.o.	n.o.	n.o.	n.o.	n.o.	n.o.	n.o.
Micropolychromasia	n.o.	n.o.	n.o.	n.o.	n.o.	n.o.	n.o.	n.o.
Hypochromasia	3(100)	7(77.8)	10(76.9)	8(100)	7(70)	3(100)	2(66.7)	7(100)
Polychromasia	1(33.3)	2(22.2)	5(38.5)	n.o.	1(10)	1(33.3)	1(33.3)	6(85.7)
Target	n.o.	n.o.	1(7.7)	1(12.5)	n.o.	1(33.3)	n.o.	3(42.9)
Spherocytes	n.o.	1(11.1)	2(15.4)	n.o.	1(10)	1(33.3)	n.o.	4(57.1)
Keratocytes	1(33.3)	n.o.	n.o.	n.o.	n.o.	n.o.	n.o.	1(14.3)
Stomatocytes	1(33.3)	n.o.	2(15.4)	n.o.	1(10)	n.o.	n.o.	3(42.9)
Burr	3(100)	6(66.7)	9(69.2)	5(62.5)	6(60)	2(66.7)	3(100)	7(100)
Acanthocytes	3(100)	6(66.7)	9(69.2)	3(37.5)	6(60)	2(66.7)	3(100)	7(100)
Teardrop	n.o.	n.o.	n.o.	n.o.	n.o.	n.o.	n.o.	n.o.
Ovalocytes	n.o.	n.o.	n.o.	n.o.	n.o.	n.o.	n.o.	n.o.
Schistocytes	n.o.	n.o.	n.o.	n.o.	n.o.	n.o.	n.o.	n.o.
Elliptocytes	n.o.	n.o.	n.o.	n.o.	n.o.	n.o.	n.o.	n.o.
Total	9(47.4)	8(42.1)	10(52.6)	8(42.1)	9(47.4)	8(42.1)	7(36.8)	

n.o., none observed.

Fig. (1): Different types of abnormal red blood cells in men and women (X1000).



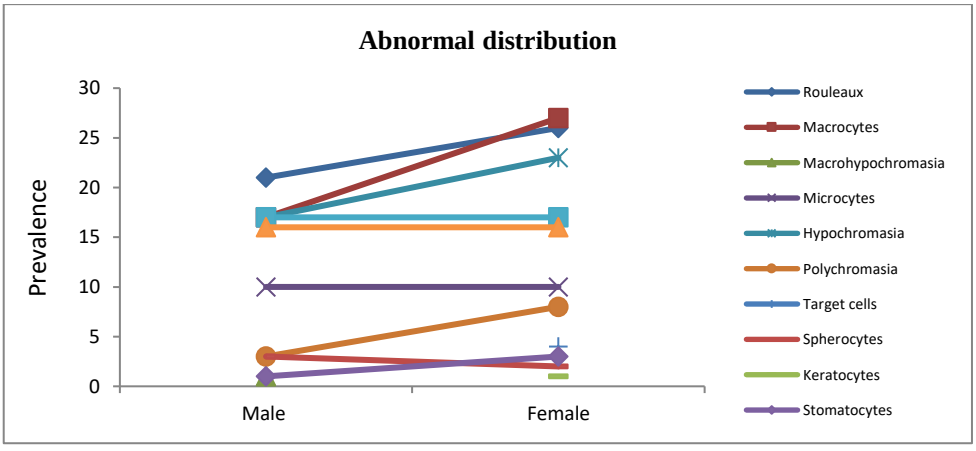
During the current study, 10 different types of abnormality in shapes of the red blood cells were identified in male samples and 11 types in female samples. Comparing RBC abnormality between genders, the women displayed higher percentage of macrocytes (96.4%) than men (81%) (Table (2) & Fig. (2)). However, both genders displayed significantly variation in the prevalence of RBC abnormality in males and females. The analysis of RBC abnormality revealed that the target cells and keratocytes associated with female samples vs males in macrohypochromasia.

Table (2): Occurrence of abnormalities prevalence of erythrocyte morphology in 49 samples according to sex.

Morphological abnormalities	Prevalence, n (%)	
	Male (N=21)	Female (N=28)
Rouleaux	21(100)	26(92.9)
Macrocytes	17(81)	27(96.4)
Macrohypochromasia	1(4.8)	n.o.
Macropolychromasia	n.o.	n.o.
Microcytes	10(47.6)	10(35.7)
Microhypochromasia	n.o.	n.o.
Micropolychromasia	n.o.	n.o.
Hypochromasia	17(81)	23(82.1)
Polychromasia	3(14.3)	8(28.6)
Target cells	n.o.	4(14.3)
Spherocytes	3(14.3)	2(7.1)
Keratocytes	n.o.	1(3.6)
Stomatocytes	1(4.8)	3(10.7)
Burr cells	17(81)	17(60.7)
Acanthocytes	16(76.2)	16(57.1)
Treadrop	n.o.	n.o.
Ovalocytes	n.o.	n.o.
Schistocytes	n.o.	n.o.
Elliptocytes	n.o.	n.o.
χ^2 (*P- value*)	**χ^2= 146.09 (*P= 0.000*)**	**χ^2= 164 (*P= 0.000*)**

n.o., none observed. The significant p-values are in bold ($p \leq 0.05$).

Fig. (2): Correlation between the sex and the erythrocyte changes in samples collected in EDTA as anticoagulant



Initial statistical analysis revealed that none index was constantly distributed. None of the age classes demonstrated a significant gender difference in RBC abnormality, although women in 40_45 years class showed a tendency to have a somewhat higher rouleaux, macrocytes and hypochromasia than men of the same age. The men have higher microcytes in 28_33 years class (Table (3)). On the other hand, the men have higher RBC abnormality shapes in 28_33 and 34_39 years classes than women of the same age.

Table (3): illustrates the age- gender- dependent occurrence of abnormalities prevalence of erythrocyte morphology in 49 samples according to sex and age.

Morphological abnormalities	Prevalence, n (%)											
	Male (N=21)						Female (N=28)					
	16_21 N=1	22_27 N=2	28_33 N=7	34_39 N=4	40_45 N=4	≥52 N=3	16_21 N=2	22_27 N=7	28_33 N=6	34_39 N=4	40_45 N=6	46_51 N=3
Rouleaux	1(100)	2(100)	7(100)	4(100)	4(100)	3(100)	2(100)	6(85.71)	n.o.	n.o.	5(83.33)	3(100)
Macrocytes	1(100)	1(50)	7(100)	3(75)	3(75)	2(66.67)	2(100)	6(85.71)	n.o.	n.o.	6(100)	3(100)
Macrohypochromasia	n.o.	n.o.	n.o.	1(25)	n.o.	n.o.	n.o.	n.o.	n.o.	n.o.	n.o.	n.o.
Macropolychromasia	n.o.	n.o.	n.o.	n.o.	n.o.	n.o.	n.o.	n.o.	n.o.	n.o.	n.o.	n.o.
Microcytes	n.o.	n.o.	5(71.43)	2(50)	1(25)	2(66.67)	2(100)	3(42.86)	2(33.33)	1(25)	2(33.33)	n.o.
Microhypochromasia	n.o.	n.o.	n.o.	n.o.	n.o.	n.o.	n.o.	n.o.	n.o.	n.o.	n.o.	n.o.
Micropolychromasia	n.o.	n.o.	n.o.	n.o.	n.o.	n.o.	n.o.	n.o.	n.o.	n.o.	n.o.	n.o.
Hypochromasia	1(100)	2(100)	6(85.71)	4(100)	2(50)	2(66.67)	2(100)	5(71.43)	4(66.67)	4(100)	5(83.33)	3(100)
Polychromasia	n.o.	n.o.	2(28.57)	n.o.	n.o.	1(33.33)	1(50)	2(28.57)	3(50)	n.o.	1(16.67)	1(33.33)
Target	n.o.	n.o.	n.o.	n.o.	n.o.	n.o.	n.o.	n.o.	2(33.33)	1(25)	n.o.	1(33.33)
Spherocytes	n.o.	n.o.	2(28.57)	n.o.	1(25)	n.o.	n.o.	n.o.	n.o.	n.o.	n.o.	1(33.33)
Keratocytes	n.o.	n.o.	n.o.	n.o.	n.o.	n.o.	1(50)	n.o.	n.o.	n.o.	n.o.	n.o.
Stomatocytes	n.o.	n.o.	1(14.29)	n.o.	n.o.	n.o.	1(50)	n.o.	1(16.67)	n.o.	1(16.67)	n.o.
Burr	1(100)	2(100)	5(71.43)	3(75)	3(75)	3(100)	n.o.	4(57.14)	4(66.67)	2(50)	3(50)	2(66.67)
Acanthocytes	1(100)	2(100)	5(71.43)	2(50)	3(75)	3(100)	1(50)	4(57.14)	4(66.67)	1(25)	3(50)	2(66.67)
Teardrop	n.o.	n.o.	n.o.	n.o.	n.o.	n.o.	n.o.	n.o.	n.o.	n.o.	n.o.	n.o.
Ovalocytes	n.o.	n.o.	n.o.	n.o.	n.o.	n.o.	n.o.	n.o.	n.o.	n.o.	n.o.	n.o.
Schistocytes	n.o.	n.o.	n.o.	n.o.	n.o.	n.o.	n.o.	n.o.	n.o.	n.o.	n.o.	n.o.
Elliptocytes	n.o.	n.o.	n.o.	n.o.	n.o.	n.o.	n.o.	n.o.	n.o.	n.o.	n.o.	n.o.

n.o., none observed

Discussion

Erythrocyte morphology researches may be useful to help in understanding of RBC storage lesion, predict the cell fate after the collection and accurately evaluate the risk of mistake results in clinical laboratory analyses routine on both normal and pathological specimens of the long-stored RBC units and transfusing these units into circulation. Additionally, erythrocyte-related indicators can provide more clinical information and can be used to monitor the progression of diabetes and its complications [20].

The function of red cell storage is to maintain the functionality and viability of red cells throughout the approved storage period. Cold-storage of red cells at 4±2°C helps maintain red cell functionality and viability by reducing the red cell metabolic rate. Storage has a negative effect on RBC oxygen delivery. The research indicated that the RBC storage lesion is responsible for impaired tissue oxygen use, pro-inflammatory and immunomodulatory effects, increased infections, multiple organ system failure and ultimately increased morbidity and mortality [21-24].

Study of Alareeqi *et al*., [20] indicate that morphological abnormalities of erythrocytes are common in healthy and diabetic subjects, and the slight effects of diabetic mellitus on the changes observed in erythrocyte compare to healthy subjects over 72 hours of storage. Effects of age-dependent membrane transport changes on the homeostasis of senescent human red blood cells was studied by Lew *et al*. (2007) [25]. van Lochem *et al.* (2004) [26] suggested that the studied of differentiation lineages, reflecting age-related changes or disease-induced bone marrow abnormalities.

Dawson *et al.* [27] found that there is some differences between men and women in blood count. Furthermore, Kemona *et al.* [28] recorded higher count of blood platelets and thrombopoietic activity of plasma in women than men. Farhangi *et al.* [29] suggested obese women had higher WBC, platelet count, and inflammatory biomarkers compared to non-obese women. In contrast with the data reported by Hoffmann *et al.* [30], Alis *et al.* [13] showed that there is a significant positive association between age with sex and red blood cell distribution width (RDW) and that this association is stronger in women than in men. Huang *et al.* (2011) [31] suggested that close correlation was found between the surface charge on an aging RBC and its structure and functions, from the cell morphology, the membrane deformability to the intracellular Hb structure and oxidation ability.

Kanias *et al.* [32] emphasized on recognition of sex as a biologic variable that needs to be taken into consideration in cellular therapeutics and in studies of human hemolytic diseases, such as sickle cell disease (SCD) and malaria.

Sex differences in predisposition to hemolysis have been largely ascribed to premenopausal women, in whom factors such as menstruation, lower hematocrit (Hct) and iron levels, and the protective action of female sex hormones (primarily estradiol and progester one) may modulate rheological properties to enhance membrane deformability [17,33-35]. In current study, red cell morphology is evaluated in terms of size, shape, color, distribution and intra cytoplasmic inclusions. The age and gender effects on the human blood cells were evaluated. There was a significant albeit very small difference in RBC abnormality between women and men in the entire cohort.

Conclusion:

These findings may help identify heritable variants with effects on RBC abnormality, which may be highlight the important implications in clinical and research practice which dealing with RBC abnormality and its association with several conditions, as age and gender should be considered an important confounding factor.

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