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RESEARCH ARTICLE

SOME STUDIES ON RED BLOOD CELLS MORPHOLOGY OF HEALTHY AND DIABETIC PATIENTS IN TAIZ, YEMEN

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Abstract

The aim of this study was to: 1- Identify and quantify the prevalence of RBC abnormalities in healthy and diabetic subjects. 2- Provide supporting evidence about the relation between RBC storage duration at 4ºC and alterations to RBC morphology (compare with the morphology at the time of collection). 3- The obtain information about how the number of normal cells in different times of storage declines as a function of the storage period. 4- Estimate the prevalence of red cell morphological changes in diabetic patients.

One hundred and ninety-six slides of 49 healthy and 49 diabetic patients of different age groups were collected from November 2019 to March 2020.

Human venous blood samples were taken and anticoagulated with EDTA. samples were divided into 4 groups, group 1 was examined at once, and groups 2-4 were stored at 4ºC for 24, 48, and 72 hours respectively.

During the current study, abnormalities of erythrocyte morphology, prevalence, and histological effects of storage duration on the human blood cells were evaluated. 16 different types of abnormality in shapes of the red blood cells were identified in healthy subjects and 19 different shapes in diabetic subjects, with the difference in the prevalence percentage. Analysis of variance (ANOVA) exhibited statistically significant effects of storage time (24, 48, and 72 hours at 4^0C) on RBC morphology. The present result also shows that the change in erythrocyte shapes at once beginning and during time storage were statistically significant between healthy and diabetic donors. These results are in line with previous laboratory studies on other parameters.

In conclusion, our observations 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.

Keywords: RBC, Abnormality, Taiz, Yemen.

1. Introduction

Central laboratories have been established in many countries in addition to local initiative hospital laboratories. These labs cannot arrange all tests required by their patients. These labs therefore have to send their samples to some central labs for investigation. In addition, specimen collection units have been established that collect the blood samples and then send to central laboratories for processing the samples for final tests [1]. The samples suffer long delays until they reach the central lab. Transport of collected blood specimens to centralized laboratories can cause a delay in testing for several hours. Excessive delay in analysis may have caused different results in both normal and pathological specimens [2,3].

Although blood collection and administration is safer and more efficient than ever before, red cells undergo multiple metabolic and structural changes during storage that may compromise their functionality and viability following transfusion. The clinical relevance of these

changes is a hotly debated topic that continues to be a matter of intense investigation [4,5,6].

During transportation, the samples need long refrigeration to prevent the deterioration due to high environmental temperature. The condition is worst in tropical countries, where humidity and high temperature are at the peak. It is therefore very important to know how to save the stability of samples in conditions existing in tropical areas [1,7].

Refrigerated storage of anticoagulated blood has been noted to improve the stability of CBC. However, the standardization of blood specimen collection, storage, and transmission to the laboratory for hematological tests was published by the ICSH in 2002 [8]. It is well known that, within 30 minutes of collection, morphological changes of blood cells begin, and that they increase with the time and conditions of blood storage [9,10]. Some of the metabolic lesions that RBCs undergo during refrigerated storage are somewhat reversible following transfusion. ATP and 2,3-DPG (diphosphoglycerate) levels recover by 7-72 h after circulating in the recipient [11], though at a rate that may be insufficient to meet the sudden and supra-physiological metabolic demand of trauma or critically ill recipients. Accordingly, in some circumstances, transfer process of collected blood samples to a centralized laboratory may become unsuitable for blood cell morphology (BCM) examination and therefore for clinical diagnosis [12].

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 [13,14].

The morphologic, biological and molecular changes induced by the storage of erythrocytes are subjects of basic and clinical investigation because of their effects in transfused patients [15].The main result of this investigation is to demonstrate that there is a direct relation between storage time and percentage of abnormalities in the RBC. 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 [16,17,18].

Red blood cells are the most abundant type of blood cells in the human body, delivering oxygen to body tissues. The count of these vital cells is often the first step done in analyzing a patient's pathological condition. Normal 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, [19].

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. Blood is the major transportation system throughout thebody, and with this role, it affects and is affected by all parts of the body. Therefore, blood tests can be used to determine the health of major organs as well as hormones, the immune system, respiratory function, and metabolism [20,21].

Although the more often observed shape of red blood cells (RBCs) is that of a biconcave discocyte, it has long been recognized that during interactions with various drugs, such as chemotherapeutic agents, and diseased conditions, such as uremia, liver ailments, etc., the cells can often undergo transformations to stomatocytic or echinocytic shapes [22, 23, 24, 25]. Effects of such shape transformations on the functional behavior of RBCs, for example, capacity for transporting oxygen, have been studied to understand the functional changes in the RBCs that may occur with the shape transformations will likely help in better understanding the prognosis and manifestations of the concerned diseases [26].

Diabetes mellitus is a syndrome characterized by disordered metabolism and abnormally high blood sugar (hyperglycemia) resulting from either low insulin level or insulin resistance at many body cells. Hemorheological parameters in diabetes mellitus are often disturbed. These parameters include hematocrit, plasma proteins, deformability and erythrocyte abnormality [27,28,29].

Accordingly and to our knowledge, a systematic analysis of the occurrence of a broad spectrum of RBC abnormalities in healthy and diabetic patients in Yemen has not been performed so far. So, the aim of this study was to:-

1- Identify and quantify the prevalence of RBC abnormalities in healthy and diabetic subjects.
2- Provide supporting evidence about the relation between RBC storage duration at 4°C and alterations to RBC morphology (compare with the morphology at the time of collection).
3- The obtain information about how the number of normal cells in different times of storage declines as function of storage period.
4- Estimate the prevalence of red cell morphological changes in diabetic patients.

2. Material and methods

According to VivesCorrons *et al.* [30], Barbara *et al.* [31], Silva *et al.* [32], Ford [33], Bosman [34] and Duke [35], the microscopic examination of stained blood smear of red blood cells morphology remains an important tool for the pathologist to estimate the erythrocyte abnormality. So, 196 blood slides of 98

individuals comprised of 49 healthy and 49 diabetic patients of different age groups were collection from November. 2019 to March 2020. The samples from both patients and controls (healthy) were chosen randomly. During our study, all studied individuals were subjected to full history taking and Laboratory examination.

Human venous blood samples were taken and anticoagulated with EDTA. samples were divided into 4 groups, group 1 was examined at once and groups 2-4 were stored at 4ºC for 24, 48 and 72 hours respectively. 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 [36] and Shah *et al.* [37].

The data are represented as the mean and the SE. To evaluate the correlation of the variables, time of storage and morphologic alterations, we used the Pearson correlation coefficient. Group comparisons were performed by one-way ANOVA and unpaired t-test. The statistical analysis was performed using the statistical package SPSS version 9.0 (SPSS, Chicago, IL). *p*-value less than 0.05 were considered as statistically significant.

3. Results

Red cell morphology is evaluated in terms of size, shape, colour, distribution and intra cytoplasmic inclusions. During the current study, 16 different types of abnormality in shapes of the red blood cells were identified in healthy subjects and 19 types in diabetic subjects (Tables 1,2 & Fig. 1).

In current study, the rouleaux, macrocytes, hypochromsia, burr cells and acanthocytes were observed in 96, 90, 82, 69 and 65% of individuals for healthy subjects. On the other hand, the rouleaux, macrocytes, burr cells, hypochromsia and acanthocytes was represented in 96,94,94,92 and 90% of diabetic subjects respectively (Tables 1 & 2). The rouleaux, burr cells, hypochromsia and macrocytes found in large number in the analyzed. On other hand, treadrop, ovalocytes, schistocytes and elliptocytes were occasionally found.

Except the macrohypochromasia in healthy group and microhypochromasia and micropolychromasia in diabetic group, the one – way analysis of variance (ANOVA) exhibited statistical significant effects of storage time (at 4^0C) on RBC morphology (Tables 3& 4).

The erythrocyte abnormality–time storage relationship in two groups was observed, Examination of Person correlation and P – values between variables clarifies these relationship. Except macrocytes and macrohypochromasia in healthy subjects and macrohypochromasia, microhypochromasia, micropolychromasia and elliptocytes in diabetic subjects, a positive correlation was evident between the erythrocyte abnormality and time storage (at 4^0C) of the two groups investigated (Table 5 & Fig. 2).

The result also showed that the change in erythrocyte shapes at once and during storage were statistically significant between two groups (Table 6).

Table1: Occurrence of erythrocyte abnormality in 49 healthy subjects at different times of storage (at 4^0C) treated with EDTA as anticoagulant.

Morphological	Prevalence, n (%)			
abnormalities	At once	After 24 hours	After 48 hours	After 72 hours
Rouleaux	47 (96)	49 (100)	49 (100)	49 (100)
Macrocytes	44 (90)	47 (96)	47 (96)	47 (96)
Microcytes	20 (41)	19 (39)	21 (43)	20 (41)
Hypochromasia	40 (82)	45 (92)	45 (92)	46 (94)
Macrohypochromasia	1 (2)	1 (2)	2 (4)	1 (2)
Microhypochromasia	n.o.	n.o.	n.o.	n.o.
Polychromasia	11 (22)	13 (27)	15 (31)	14 (29)
Macropolychromasia	n.o.	n.o.	n.o.	n.o.
Micropolychromasia	n.o.	n.o.	n.o.	n.o.
Target cells	4 (8)	18 (37)	20 (41)	19 (39)
Spherocytes	5 (10)	25 (51)	26 (53)	27 (55)
Keratocytes	1 (2)	24 (49)	27 (55)	27 (55)
Stomatocytes	4 (8)	21 (43)	23 (47)	23 (47)
Burr cells	34 (69)	49 (100)	49 (100)	49 (100)
Acanthocytes	32 (65)	49 (100)	49 (100)	49 (100)
Treadrop	n.o.	37 (76)	38 (78)	43 (88)
Ovalocytes	n.o.	12(24)	14 (29)	14 (29)
Schistocytes	n.o.	12 (24)	13 (27)	14 (29)
Elliptocytes	n.o.	2 (4)	4 (8)	6 (12)

n.o., none observed

Table2: Occurrence of erythrocyte abnormality in 49 diabetic subjects at different times of storage (at 4^0C) treated with EDTA as anticoagulant.

Morphological	Prevalence, n (%)			
abnormalities	At once	After 24 hours	After 48 hours	After 72 hours
Rouleaux	47(96)	49(100)	49(100)	49(100)
Macrocytes	46(94)	48(98)	49(100)	49(100)
Microcytes	29(59)	28(57)	27(55)	28(57)
Hypochromasia	45(92)	49(100)	49(100)	49(100)
Macrohypochromasia	9(18)	10(20)	12(24)	12(24)
Microhypochromasia	1(2)	2(4)	2(4)	2(4)
Polychromasia	29(59)	36(73)	37(76)	39(80)
Macropolychromasia	4(8)	7(14)	8(16)	9(18)
Micropolychromasia	n.o.	2(4)	1(2)	1(2)
Target cells	8(16)	29(59)	29(59)	35(71)
Spherocytes	22(45)	33(67)	38(78)	43(88)
Keratocytes	3(6)	26(53)	28(57)	35(71)
Stomatocytes	11(22)	30(61)	34(69)	38(78)
Burr cells	46(94)	49(100)	49(100)	49(100)
Acanthocytes	44(90)	49(100)	49(100)	49(100)
Treadrop	n.o.	41(84)	47(96)	48(98)
Ovalocytes	n.o.	1(2)	14(29)	20(41)
Schistocytes	n.o.	3(6)	10(20)	20(41)
Elliptocytes	n.o.	1(2)	2(4)	2(4)

n.o.: none observed

Table3: Means ± SE, range and the percentages of erythrocyte abnormality in healthy subjects at different times of storage (at 4^0C) treated with EDTA as anticoagulant (n=49)

Morphological abnormalities	At once	After 24 hours	After 48 hours	After 72 hours	F	p-value
Rouleaux	3.23±0.112	4.82±0.108	5.86±0.106	7.02±0.108	219.83	**0.000**
	0-17	0-19	0-15	0-15		
Macrocytes	1.27±0.048	1.82±0.046	2.14±0.051	2.54±0.055	118.37	**0.000**
	0-9	0-6	0-6	0-5		
Microcytes	0.11±0.013	0.27±0.027	0.39±0.035	0.54±0.044	45.57	**0.000**
	0-3	0-3	0-3	0-4		
Hypochromasia	1.98±0.107	3.71±0.118	4.65±0.131	5.35±0.146	154.68	**0.000**
	0-16	0-16	0-14	0-14		
Macrohypochromasia	0.01±0.005	0.01±0.009	0.02±0.011	0.02±0.013	0.41	0.742
	0-3	0-4	0-4	0-5		
Microhypochromasia	n.o.	n.o.	n.o.	n.o.		
Polychromasia	0.05±0.01	0.18±0.023	0.29±0.031	0.34±0.036	31.82	**0.000**
	0-3	0-3	0-4	0-3		
Macropolychromasia	n.o.	n.o.	n.o.	n.o.		
Micropolychromasia	n.o.	n.o.	n.o.	n.o.		
Target cells	0.05±0.015	0.34±0.032	0.46±0.041	0.62±0.051	58.78	**0.000**
	0-6	0-4	0-6	0-6		
Spherocytes	0.02±0.007	0.52±0.038	0.66±0.046	0.92±0.055	127.59	**0.000**
	0-3	0-4	0-4	0-4		
Keratocytes	0.002±0.002	0.54±0.037	0.69±0.045	0.92±0.053	150.67	**0.000**
	0-2	0-3	0-3	0-4		

Stomatocytes	0.01±0.005	0.45±0.034	0.65±0.045	0.82±0.053	123.22	**0.000**
	0-3	0-3	0-5	0-5		
Burr cells	2.52±0.119	4.61±0.092	6.04±0.11	7.22±0.112	339.70	**0.000**
	0-17	0-15	0-16	0-19		
Acanthocytes	0.4±0.026	1.58±0.035	1.9±0.041	2.37±0.04	668.63	**0.000**
	0-3	0-3	0-4	0-4		
Treadrop	n.o.	0.66±0.036	0.92±0.043	1.3±0.048	325.38	**0.000**
		0-4	0-3	0-3		
Ovalocytes	n.o.	0.16±0.028	0.19±0.034	0.25±0.044	17.64	**0.000**
		0-5	0-7	0-8		
Schistocytes	n.o.	0.07±0.016	0.1±0.024	0.15±0.03	13.67	**0.000**
		0-3	0-4	0-4		
Elliptocytes	n.o.	0.01±0.005	0.02±0.01	0.04±0.014	4.27	**0.005**
		0-2	0-3	0-4		
%	29.82%	49.79%	55.44%	62.22%		
N.0.cell*	27034	22603	23638	23965		

* number of cells has been determined by light microscope. n.o.: none observed.

The significant p-values are in bold ($p < 0.05$).

Table 4: Means ± SE, range and the percentages of erythrocyte abnormality in diabetic subjects at differenttimes of storage (at 4^0C) treated with EDTAas anticoagulant (n=49)

Morphological abnormalities	At once	After 24 hours	After 48 hours	After 72 hours	F	p-value
Rouleaux	3.5±0.096	5.50±0.107	4.35±0.077	5.71±0.079	121.95	**0.000**
	0-14	0-19	0-15	0-18		
Macrocytes	1.99±0.052	2.11±0.055	1.84±0.051	2.25±0.047	8.14	**0.000**
	0-9	0-9	0-8	0-9		
Microcytes	0.24±0.019	0.51±0.03	0.51±0.037	0.84±0.053	60.29	**0.000**
	0-5	0-4	0-4	0-7		
Hypochromasia	3.11±0.087	4.59±0.091	4.09±0.07	5.26±0.072	117.00	**0.000**
	0-15	0-15	0-10	0-10		
Macrohypochromasia	0.08±0.014	0.09±0.017	0.15±0.026	0.19±0.034	5.56	**0.000**
	0-4	0-3	0-4	0-4		
Microhypochromasia	0.002±0.002	0.01±0.005	0.01±0.007	0.02±0.010	1.37	0.251
	0-2	0-3	0-3	0-3		
Polychromasia	0.24±0.02	0.54±0.031	0.81±0.039	1.24±0.044	180.76	**0.000**
	0-4	0-4	0-4	0-4		
Macropolychromasia	0.01±0.005	0.03±0.01	0.04±0.014	0.08±0.023	5.36	**0.001**
	0-3	0-3	0-3	0-4		
Micropolychromasia	n.o.	0.01±0.008	0±0.002	0.004±0.004	2.06	**0.103**
		0-5	0-1	0-2		
Target cells	0.11±0.015	0.45±0.03	0.78±0.043	1.24±0.049	235.05	**0.000**
	0-4	0-3	0-3	0-4		
Spherocytes	0.24±0.019	0.71±0.038	1.25±0.051	1.84±0.051	348.58	**0.000**
	0-3	0-5	0-5	0-6		
Keratocytes	0.01±0.005	0.33±0.029	0.65±0.04	1.18±0.049	294.87	**0.000**
	0-2	0-4	0-4	0-4		
Stomatocytes	0.13±0.017	0.49±0.034	0.87±0.045	1.38±0.047	252.16	**0.000**
	0-4	0-4	0-5	0-3		
Burr cells	3.57±0.083	5.09±0.081	4.8±0.062	5.89±0.058	158.70	**0.000**
	0-15	0-16	0-11	2_11		

Acanthocytes	0.68±0.028	1.36±0.038	1.6±0.04	2.04±0.032	290.32	**0.000**
	0-4	0-5	0-4	0-5		
Treadrop	n.o.	0.40±0.026	0.97±0.036	1.5±0.033	784.76	**0.000**
		0-3	0-3	0-4		
Ovalocytes	n.o.	0.01±0.006	0.28±0.038	0.41±0.049	67.59	**0.000**
		0-3	0-5	0-6		
Schistocytes	n.o.	0.03±0.011	0.11±0.025	0.22±0.036	29.81	**0.000**
		0-4	0-5	0-5		
Elliptocytes	n.o.	0.01±0.006	0.03±0.012	0.04±0.015	4.65	**0.003**
		0-2	0-3	0-4		
%	33.48%	48.91%	54.19%	61.53%		
N.O. cell*	39869	31896	22640	24952		

* number of cells has been determined by light microscope. n.o.: none observed.

The significant p-values are in bold ($p < 0.05$).

Table 5: Pearson correlation coefficient values that describe the relationship between occurrence of abnormalities of erythrocyte morphology in healthy and diabetic subjects and different times of storage (at 4^0C) treated with EDTA as anticoagulant (n=196)

Morphological	Healthy subjects		Diabetic subjects	
abnormalities	r	p-value	r	p-value
Rouleaux	0.22	**0.002**	-0.19	**0.008**
Macrocytes	0.11	0.140	-0.33	**0.000**
Microcytes	0.22	**0.002**	0.14	**0.047**
Hypochromasia	0.22	**0.002**	-0.17	**0.018**
Macrohypochromasia	0.03	0.712	0.04	0.553
Microhypochromasia			0.08	0.266
Polychromasia	0.18	**0.011**	0.35	**0.000**
Macropolychromasia			0.13	0.070
Micropolychromasia			-0.01	0.886
Target cells	0.27	**0.000**	0.43	**0.000**
Spherocytes	0.37	**0.000**	0.5	**0.000**
Keratocytes	0.4	**0.000**	0.53	**0.000**
Stomatocytes	0.35	**0.000**	0.46	**0.000**
Burr cells	0.32	**0.000**	-0.22	**0.002**
Acanthocytes	0.63	**0.000**	0.32	**0.000**
Treadrop	0.57	**0.000**	0.83	**0.000**
Ovalocytes	0.23	**0.001**	0.36	**0.000**
Schistocytes	0.27	**0.000**	0.34	**0.000**
Elliptocytes	0.2	**0.006**	0.11	0.141

The significant p-values are in bold ($p < 0.05$).

Table 6: Comparison between total erythrocyte abnormality in healthy and diabetic subjects over different times of storage (at 4^0C) using EDTA as anticoagulant.

Morphological abnormalities	At once	After 24 hours	After 48 hours	After 72 hours
Change (%)	-65.37	-38.31	6.61	-2.63
t96	-4.76	-3.42	0.92	-0.68
p-value	**0.000**	**0.001**	0.360	0.500

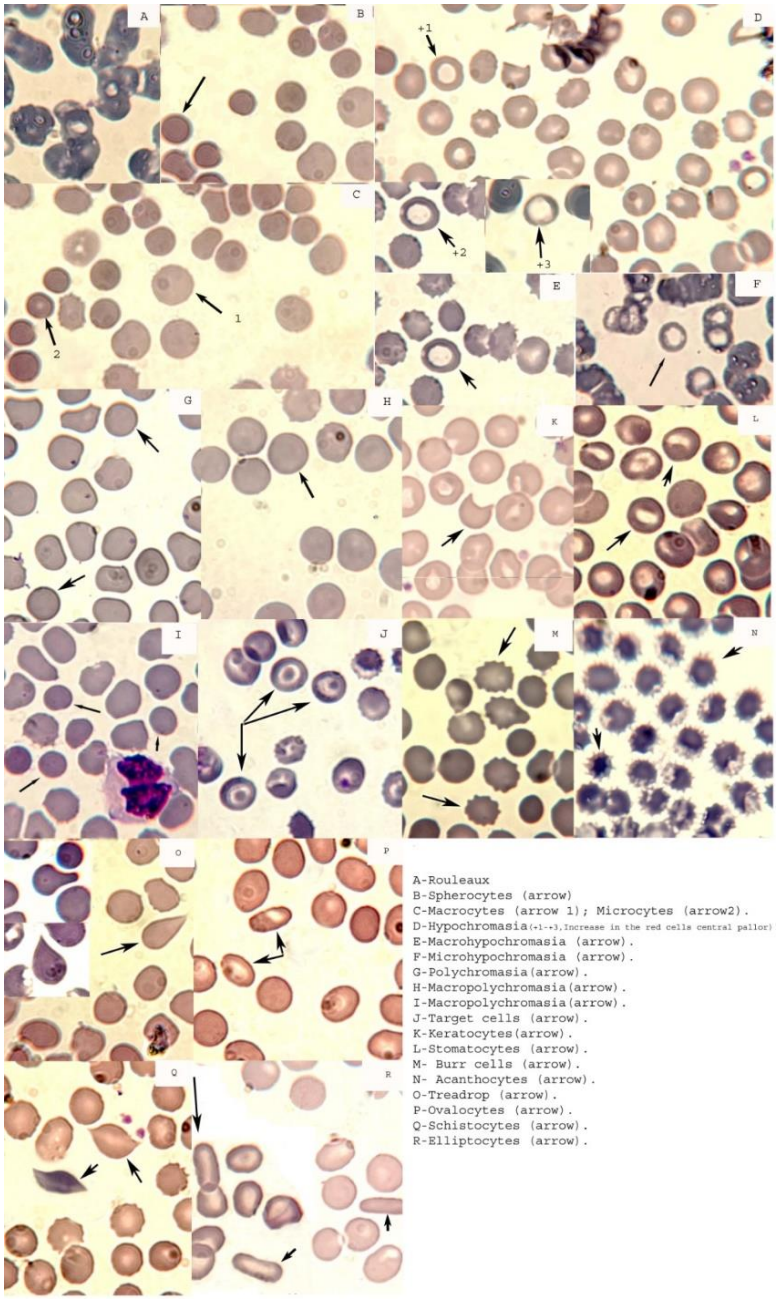

Fig. 1: Different types of abnormal red blood cells. X1000.

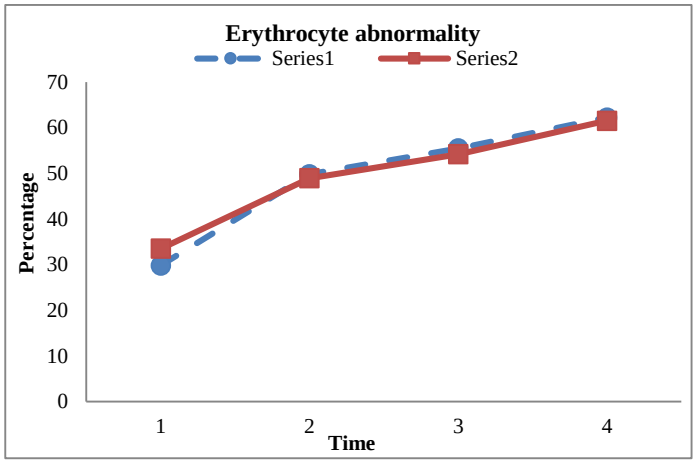

Fig. 2: Correlation between the time of storage at 4^0C and the erythrocyte changes in healthy and diabetic subjects collected in EDTA as anticoagulant.

4. Discussion

Erythrocytes are rather unique cells because they lose all organelles when mature. They only conserve a few metabolic pathways for obtaining energy and reduce the energy consumption for the key functions they need to fulfil. This makes erythrocytes highly sensitive to any disorder [38].

Red cell morphology is crucial in evaluating anemia and several blood disorders. Good quality peripheral blood smear, with special staining, coupled with the expertise of an haemato-morphologists (haematologists /haematology pathologists) remains highly valuable in patient care [39].

Erythrocyte morphology is either indicated by a clinical request or laboratory flags. And may also be indicated when significant deviations from the normal are seen in the laboratory during blood work (full blood count) irrespective of a clinical request. For instance, a significantly reduced hemoglobin level with low MCV and raised RDW may suggest iron deficiency anemia. This is an indication for red cell morphology and other ancillary investigation for abnormal morphological erythrocytes (Table 7).

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. For each one degree drop in storage temperature, there is approximately a 10% decrease in red cell metabolic rate, and at 4°C, the metabolic rate is ten times lower than at 25°C [40,41,42]. However, that red cell functionality and viability are progressively impaired during storage by three interrelated mechanisms: altered metabolism; increased oxidative stress; and membrane damage [4].

There is a complex interaction between the membrane phospholipids, transmembrane proteins and cytoplasmic components which has an impact in the morphology and deformability of the RBC during storage. This modifications decrease the RBC half life post-transfusion. RBC biomechanical changes are the result of the alterations of the lipidic bilayer, proteins and cytoskeleton membrane [43,44,45], this impacts the biomechanical by damaging the phospholipids of the membrane and the spectrine, this contributes to the formation of squistocytes and spherocytes, also contributes to the osmotic fragility. Cholesterol is another important component of the lipid layer as increased cholesterol to phospholipid ratios impair red cell viscosity and deformability, and promote alterations in red cell shape [46,47,48].

Important membrane proteins include the transmembrane protein anion exchanger 1(AE1), and the primary structural cytoskeletal proteins spectrin and ankyrin. The AE1 is a transport protein that regulates the exchange of chloride and bicarbonate across the membrane and also links the lipid bilayer to the cytoskeleton by binding to ankyrin (Fig. 3), which in turn binds to spectrin. Normal protein organisation is crucial for maintaining red cell stability, deformability and shape. AE1 is also involved in senescence signalling, as its breakdown (or clustering) generates a neo-antigen that results in the rapid clearance of the red cell from the circulation [46,47,48].

Oxidative damage is another important mechanism of RBC damage, Metabolic alterations due to cold-storage and reduced glycolysis as well as increased oxidative stress have profoundly deleterious effects on the red cell membrane [43,44,45]. Changes in the RBC during the storage produce RBC with low efficacy and effectivity to improve oxygenation in tissues, this can be related with a depletion of the 2-3 diphosphoglycerate (DPG). This 2-3 DPG depletion is bigger within the seventh day of storage [49,11].

Storage has a negative effect on RBC oxygen delivery and emerging evidence suggests that allogenic RBC infusion may actually harm some recipients. Considerable evidence suggests that transfusion increases the risk of serious complications and death in critically ill patients, especially in patients who are undergoing cardiac surgery. 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 [50].

These changes reduce red cell functionality and viability by causing membrane instability and loss, reduced deformability, alterations in red cell discoid shape and increased senescence signalling. Microvesicles (MVs) as one of storage lesions may be derived from various cell types and have key roles in several biological processes. Microvesicle formation and accumulation in the supernatant increases exponentially during storage [51,46,52,47,53, 54,55,56,48,40,57,58,59,60,61,62,14].

There has been a plethora of studies investigating the effects of prolonged blood storage on clinical outcomes. These have included healthy and patient volunteers [63,64,65,66]. It is well known that, within 30 minutes of collection, morphological changes of blood cells begin, and that they increase with the time and conditions of blood storage [9,10].

Examined the effect of blood storage on post-transfusion physiological variables, including anemia-induced cognitive dysfunction [65], extravascular haemolysis and serum iron elevation [64], attenuation of NO-mediated hyperanemia [65] and gas exchange [66] Iron-deficient erythropoiesis in blood donors and red blood cell recovery after transfusion [67]. All of these studies involved cross-over designs where autologous blood was used for transfusion following both a short (anywhere from 3–4 h to 3–7 days) and prolonged (anywhere from 23 to 42 days) storage duration.

The increased hemoglobin (HGB) and hematocrit (Hct) values will lead to hyperviscosity, which may affect RBC morphology during storage and further affect RBC deformability, which is an important factor of RBC oxygen-carrying capacity after clinical transfusion [14,68]

High blood glucose level (hyperglycemia) is a leading indicator of diabetes mellitus (DM). Diabetes mellitus is a metabolic disorder characterized by varying or

persistent hyperglycemia either due to insufficient or inefficient insulin action or improper utilization of glucose. Erythrocytes are the most abundant cells in the circulation and remain in hyperglycemic environment throughout their lifespan and thus are subjected to series of compositional changes. Long-lasting hyperglycemia affects the structure and function of erythrocytes, which in turn affects their flow properties through alteration of deformation and aggregation [69,70]. The detection of erythrocyte-related indicators can provide a valuable reference for the prevention, diagnosis, and treatment of DM and its complications.

In diabetic patients without micro- and macroangiopathy there was an increase in erythrocyte aggregation associated with an increased fibrinogen level while albumin levels were decreased. Blood viscosity was reported to be significantly higher in patients with longstanding diabetes than in matched non diabetic controls and it was suggested that hyperviscosity and reduced erythrocyte deformability might be potentially important factors in the etiology or progression of microcirculatory disease in diabetes [71,28].

In diabetic individuals, the elevated blood glucose stiffens the erythrocyte membrane, adversely altering the natural behavior of erythrocytes. One of the most important consequences of altered erythrocytes is the elevated whole blood viscosity. This elevation is explained as the increased aggregation and reduced deformability of red cells, also hematocrit was found to be elevated due to increased permeability of capillary vessel wall, which in turn increases the whole blood viscosity [72].

The rise of erythrocyte rigidity, while hampering blood-flow through already injured microvessels, could contribute to the development of retinal abnormalities irrespective of the total glycosylated hemoglobin (HbA_1) level. Consequently, the abnormalities in the retinal microcirculation could, directly or indirectly, influence red cell filterability, worsening the rheologic behaviour in the local microvessels [73]. The erythrocyte deformability is reduced, whereas its aggregation increases, both of which make whole blood more viscous compared to healthy individuals [27].

During the current study, abnormalities of erythrocyte morphology, prevalence and histological effects of storage duration on the human blood cells were evaluated. 16 different types of abnormality shapes of the red blood cells were identified in healthy subjects and 19 shapes in diabetic subjects, with the difference in the prevalence percentage. Analysis of variance (ANOVA) exhibited statistical significant effects of storage time (24, 48 and 72 hours at 4^0C) on RBC morphology. The present result also show that the change in erythrocyte shapes at once and during time storage were statistically significant between healthy and diabetic donors. These results is in line with previous laboratory studies on other parameters.

Shah *et al.*,[37] concluded that human blood cells have 12 different shapes of morphological shapes and size. Greenberg [74] reported that in the marginal blood smear of two patients with Heinz bodies hemolytic anemia morphological abnormal red blood cells were observed. Wagner *et al.*,[75] reported that red blood cells form aggregates in the form of rouleaux and this aggregation process is reversible by the activation of platelets aggregation mechanism is caused in case of wound healing aggregation lead to clot formation which is lifesaving but in case of thrombus induce strock, it cause death. During our current study rouleaux cells were observed in 94 individual. The rouleaux formation is caused by the presence of high concentration of plasma protein such as fibrinogen or immunoglobulin in the blood[37].

Loría *et al.* [76] worked on erythrocyte lifespan in iron deficiency anemia. They conclude that hypochromic cells have short lifespan and which is the risking factor for microcytic anemia. Reinhart and Chien [77] worked on echinocytes (Burr cell), stomatocyte transformation and shape control of human erythrocyte.They conclude that the erythrocyte has an energy needy shape, y-globulin was found to induce echinocytic transformation. During our current study we observed 34 echinocyte and 4 stomatocyte cells in healthy subjects and 46 echinocyte in diabetic and 11 stomatocyte subjects.

The presence of teardrop shape cell in peripheral blood has alter myelofibrosis. Farolino *et al.*[78] observed the presence of tear drop shape cell in two patients with the splenomegaly having hemolytic anemia. In our findings, the tear drop shape cells were detected in76% of the blood smear of healthy individuals and 84%of diabetic patients.

Körber *et al.*,[79] Study erythrocyte morphology in patients with β-thalassemia minor. They show that Target cells in 33 (100%) subjects, ovalocytes 96.9%, dacrocytes (treadrop)(81.8%), stomatocytes (81.8%)and elliptocytes (75.8%) were commonly found, while Schistocytes (15.2%)were occasionally found. On other hand, they observed elliptocytes, ovalocytes, dacryocytes and irregular contracted cells in large number in the analyzed slides. Their findings disagree with our findings.

Carrillo-Esper *et al.* [15] stated Red blood cells with more than seven days of storage, present significant morphologic alterations, erythrocyte abnormalities. In 2012,Kholoussi*et al.*[80] stated erythrocytes experience various changes due to changes in plasma composition that occurs in diabetes mellitus. These changes were reflected on cellular parameters directly in the form of erythrocyte aggregation and deformability. Morphological changes were revealed by SEM as

spheroidal aggregates, many flat cells in addition to spherocytes with surface &/or marginal irregularities. Echinocytes were also occasionally encountered. Gupta *et al.*, [81] recorded that with storage percentage, the discocytes decreased and that echinocytes and spherocytes increased. Blasi *et al.*,[82] stated that significant alterations to RBC morphology over storage duration occur soon after the 14th day of storage, as to become significant enough within the 21st day.

Petropoulos *et al.*[29]recorded several rheological disorders of the erythrocytes in diabetes mellitus as reduced deformability and increased aggregation and they also related these findings to the development of diabetic microangiopathy. Moreover; in 2000, Foresto *et al*, [83] noted that the rouleaux pattern of RBC aggregate morphology characterizes normal aggregates while the formation of RBC clusters characterizes disease states and seems to be increased in the case of diabetic patients compared with normal controls. Moreover, they added that aggregates of diabetic patients take a spheroidal shape compared to normal classical cylindrical shape aggregates known as rouleaux; both findings support our results.

5. Conclusion

Our observations 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, and which may be due to other disease conditions, such as uremia and liver ailments, in healthy subjects. These findings 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. However, there is a general need for clinical studies to determine how the long storage and RBC abnormality present in all blood components might affect the clinical laboratory results and transfusion recipients.

Table 7: Conditions associated with abnormal RBC morphology.

Abnormal RBC	Reasons The RBC abnormality can be seen in patients with:-	References
Rouleaux	Infections, inflammatory, connective tissue disorders and cancer.1-Hyperfibrinogenemia. 2-Multiple myeloma	[84, 36, 85,86, 87,39,37]
Macrocytes	1-The occurrence of immature red blood cells, vitamin B12 or folate deficiency. 2- Liver disease	
Microcytes	1-Iron deficiency. 2- HbH disease. 3-Thalassaemia. 4-Sideroblastic anemia.	
Hypochromasia	1-Iron deficiency anemia (less of hemoglobin) 2-β-thalassemia major	
Macrohypochromasia	Hydrops fetalis	
Microhypochromasia	1-α-Thalassaemia trait. 2- HbE trait	
Polychromasia	1-Megaloblastic Anaemia. 2-Autoimmune haemolytic anaemia. 3-Paroxysmal nocturnal haemoglobinuria.	
Target cells	1-Postsplenectomy.2-Thalassemia.3-Liverdisease (cholestasis).	
Spherocytes	1-Post splenectomy. 2-Liver disease. 3-Hemoglobinopathies 4-Heart valve prosthesis. 5-Heinz body hemolytic. 6-Anemia 7-Hypersplenism. 8-Autoimmune hemolytic	
Keratocytes	Indicates an infection of the blood vessel walls	
Stomatocytes	1-Artefact (due to slow drying in humid environment). 2-Liver disease. 3- Alcoholism, Rh-null disease. 4-Obstructive lung disease	
Burr cells	Uremic patients	
Acanthocytes	1-Severe burns.2-Sideroblastic anemia. 3-Enzymes deficiencies 4-Vitamin E deficiency. 5-Hypothyroidism. 6-Renal disease 7-Liver disease.	
Treadrop	1-vitamin B 12 deficiency. 2-iron deficiency anemia	
Ovalocytes	1-Megaloblastic anaemia. 2- South-East Asian ovalocytosis	
Schistocytes	1-Hemolytic anemia. 2-Hypersplenism. 3-Thalassemia major 4-Microangiopathic hemolytic. 5- HIV infection	
Elliptocytes	Increased in iron deficiency anemia. 1-Megaloblastic anemia. 2-Hereditary pyropoikilocytosis 3-Hereditary elliptocytes	

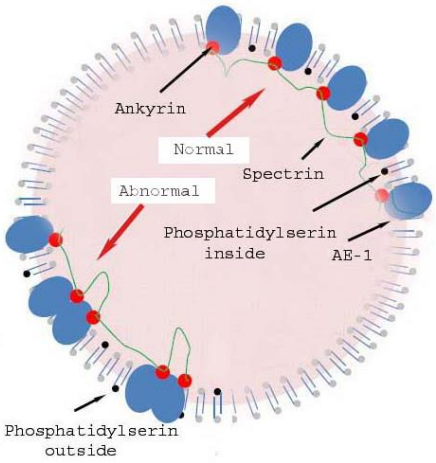

Fig. 3: Red cell changes associated with storage.
(modified from Orlov & Karkouti 2015)

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مقالة بحثية

بعض الدراسات على شكل خلايا الدم الحمراء للأصحاء و مرضى السكري في تعز،اليمن

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المُلخّص

لأجل معرفة التشوهات المختلفة في خلايا الدم الحمراء وتأثير مرض السكري والتخزين عليها تم فحص مائه وستة وتسعين شريحة، من تسعه وأربعين متبرع غير مريض بسكري وتسعه وأربعين أخرى مصابين بالسكري من نوفمبر 2019 إلى مارس 2020.

جمعت عينات الدم من الوريد في أنابيب تحتوي على EDTA كمانع للتجلط ثم قسمت إلى أربع مجموعات، المجموعة الأولى فحصت مباشره، المجموعة الثانية فحصت بعد 24 ساعة، المجموعة الثالثة فحصت بعد 48 ساعة، المجموعة الرابعة فحصت بعد 72 ساعة من التخزين عند 4درجه مئوية.

تم إحصاء 16 نوع من التشوهات الحاصلة في خلايا الدم الحمراء في مجموعة الأصحاء و19 نوع في مرضى السكري مع الاختلاف في نسبة الانتشار. وقد وجد أن زمن التخزين يؤثر على شكل خلايا الدم الحمراء. كما أثبتت نتائج الدراسة الحالية تأثير مرض السكري على شكل كرات الدم الحمراء.

نستخلص من ذلك، وجود التشوهات في شكل خلايا الدم الحمراء في كلا من مجموعة الأصحاء وكذلك في مرضى السكري، وأن مرض السكري له تأثير بسيط على شكل خلايا الدم الحمراء في الإنسان مقترنا مع ازدياد فترة التخزين.

الكلمات المفتاحية: خلايا الدم الحمراء، التشوه، تعز، اليمن.

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