

Original Article**Storage Induced Alterations in Erythrocyte Morphology and Platelet Count****Oshan Shrestha*, Reetu Baral, Bhaktaraj Majhi**

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Article Received: 14th April, 2024; Accepted: 5th June, 2024; Published: 30th June, 2024**DOI: <https://doi.org/10.3126/jonmc.v13i1.68104>****Abstract****Background**

Examination of blood smears and various hematologic parameters is the first step in assessment of hematologic function and diagnosis of possible underlying diseases. The aim of this study was to identify artefacts in stored red blood cells and classify the occurrence of such alterations according to the age of the stored sample. This study also aimed to note the changes in platelet count dispatched by automated hematology coulter in such stored blood samples.

Material and Methods

A prospective cross sectional study was conducted from the EDTA anticoagulated blood samples of 100 patients received in the department of Pathology after receiving ethical approval from the institutional review committee. All the samples were analyzed in 4 occasions i.e. within 2 hours, at 24 hours, at 48 hours and at 96 hours. Analysis was carried out by Sysmex 5 part analyzer for platelet counts and the Leishman stained blood films were examined to note changes in the erythrocyte morphology.


Results

There was alterations in platelet counts in 22% cases between 2-hour samples and other samples. The red blood cell morphology was not different among 2 hours samples and 24 hours sample. However, the red blood cell morphology was altered in 48 hours samples and 96 hours samples.

Conclusion

Storage of EDTA anticoagulated blood even upon refrigeration can cause changes in platelet counts as well as morphology of red blood cells. Changes in red blood cells are not significant till 24 hours while the platelet counts may alter within 24 hours.

Keywords: *Artifacts, Blood cells, Platelets*

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Introduction

Complete blood counts (CBC) by automated hematology cell counter as well as the morphological assessment of individual blood cells and platelets in a peripheral blood smear is vital for diagnosis of underlying disorders and effective patient management. Duration between sampling and analysis of the blood sample is the storage time. Most commonly used anticoagulant for these tests is Ethylene diamine tetra acetic acid (EDTA)–[1].

Alterations in CBC parameters and morphology can occur by a prolonged storage time in EDTA [2]. EDTA can cause structural, biochemical and functional damage to blood cells that are likely to be caused by a Lysolecithin formation or fall in Adenosine Triphosphate (ATP) as the blood is kept for a long time [3]. These changes thus could lead to erroneous results that would complicate diagnostic as well as management part of the patient.

The present study was thus undertaken with an objective to identify storage related changes in platelet counts as well as changes in morphological parameters of the red blood cells at different intervals.

Material and Methods

This was a prospective cross-sectional study that was conducted in the Department of Pathology of Nobel medical college and teaching hospital from 7th March, 2024 to 7th June, 2024 for duration of three months. Ethical clearance from the Institutional review committee of Nobel medical college and teaching hospital was obtained before the study. Peripheral blood samples referred routinely for CBC examinations with at least 2 ml blood in EDTA vial were included in the study. These were studied at different time intervals of 0-2 hours, 24 hours, 48 hours and 96 hours. These samples were refrigerated at 4 degree Celsius in between. Blood samples with abnormal counts and/or morphology at 0-2 hours were excluded from the study. The sample size was estimated to be 96 by using the formula, $n = Z^2 P (1-P) / e^2$, where Z is the confidence level at 95% (1.96); e is margin of error taken as 10% and P is expected prevalence of samples received in the department for peripheral blood film examination during the study period. One hundred peripheral blood samples were analysed and convenience sampling was performed. The parameters studied at all the time intervals were platelet counts, haemoglobin levels and total leucocyte count. The smear was stained by Leishman stain and studied for morphology of platelets, red

blood cells and leucocytes.

The morphological parameters noted in RBCs were crenation artefact with echinocytic or acanthocytic appearance, spherocytosis, tear drop or target cell appearance and polychromasia. Likewise, WBCs were assessed for cytoplasmic fragmentation, apoptotic changes and cytoplasmic degranulation. Clumping and presence of large/giant platelets were also noted.

Results

22/100 (22%) samples showed reduction in platelet count below the normal lower limit of 1,50,000/ cu.mm. beginning from 24 hours sample. Giant platelets and platelet clumps were also observed from 24 hours sample onwards. These changes were progressively more marked in 48 hours and 96 hours sample.

Red blood cell morphological changes weren't appreciable in both 0-2 hours sample and 24 hours sample. Morphological changes in the form of echinocytes and acanthocytes were most frequent and were observed in 48 hours sample and more so in 96 hours sample. Spherocytes and tear drop cells were also more in 48 hours sample and 96 hours sample. Target cell change was not noted while the polychromasia of red blood cells were noticeably absent from 24 hours sample onwards.

No appreciable differences in haemoglobin levels were noted in any of the stored samples.

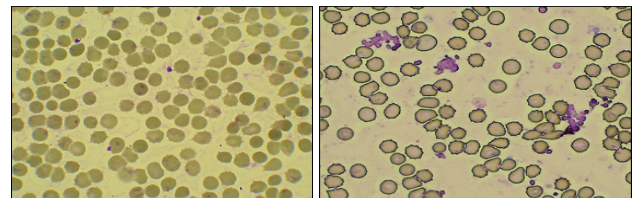


Figure 1: Leishman stained peripheral blood smear of a sample stored for 48 hours (a) Crenated RBCs (400x) (b) Multiple platelet clumps (400x)

Cytoplasmic fragmentations and vacuolations started appearing in WBCs of samples beginning from 24 hours. Other changes noted were apoptosis of neutrophils observed in 25/100 (25%) cases starting from 24 hours stored sample.

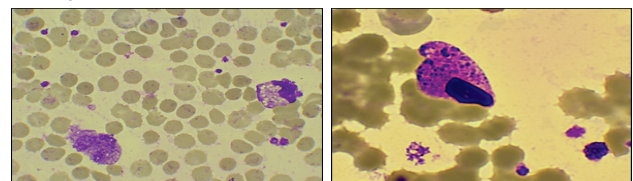


Figure 2: Leishman stained peripheral blood smear of a sample stored for 98 hours (a) Cytoplasmic vacuolations and degeneration of neutrophils (400x) (b) Apoptotic neutrophil (1000x)



Table 1: Storage induced Platelet alterations in EDTA blood:

Duration	Platelet count reduction below normal (%)	Giant platelets (%)	Platelet clumps (%)
0-2 hours	0	0	0
24 hours	22	18	20
48 hours	28	25	20
96 hours	30	28	22

Table 2: Storage induced RBC alterations in EDTA blood:

Duration	Crenated cells (%)	Spherocytes (%)	Tear drops (%)	Loss of polychromasia (%)
0-2 hours	0	0	0	0
24 hours	2	0	0	70
48 hours	44	12	12	94
96 hours	84	14	27	100

Table 3: Storage induced WBC alterations in EDTA blood:

Duration	Cytoplasmic fragmentations (%)	Cytoplasmic vacuolations (%)	Apoptotic forms (%)
0-2 hours	0	0	0
24 hours	64	26	25
48 hours	68	28	32
96 hours	76	28	38

Discussion

Being an indispensable tool for screening, diagnosis and monitoring of any haematological disease, accurate reporting of peripheral blood sample is must. Many a times, request to repeat test on samples with abnormal platelet counts or any other parameters are sent and a fresh sample may not be available to the laboratory due to various reasons like unavailability of the patient, patient's denial or difficulty in re-sampling. These conditions lead to re-evaluation of the stored sample already stored in the lab. Samples from peripheral collection centers or blood donation camps may also have a delay in transport to the central lab. However, it is to be noted that interpreting these stored samples after few days may produce erroneous results.

According to guidelines of International Society for Laboratory Hematology morphological changes in blood cells begin within 30 minutes of a collection of the sample. It also emphasizes that the quality of smears cannot be guaranteed after 6 hours of sample drawing even if refrigerated at 4 degrees [4]. Analytical stability of hematological parameters after varying conditions of sample storage has been researched by

previous studies. Daves et al in 2015 found no significant differences in platelet counts or red cell parameters in samples stored at 4 degrees upto 6 hours while they observed differences in platelet count in samples stored 24 hours and beyond at 4 degrees [5]. Zini et al in 2014 showed that films made from blood stored not more than one hour do not show any morphological changes while by 12-18 hours, morphological changes, especially in WBC, are noted at room temperature thus emphasizing the need of refrigeration of samples at 4 degrees [6].

In our study we noticed considerable morphological changes like crenation in the RBCs; degeneration of WBCs in the form of apoptosis, cytoplasmic fragmentation/ vacuolization, and platelet clumps with large/giant forms. The changes in platelets and WBCs started within 24 hours while the RBC changes began after 48 hours. They then progressively increased with time at 96 hours even on refrigeration. These findings are in agreement with findings by Koolwal et al also didn't find any significant difference in platelet count or morphology or RBC morphology in samples of 0-2 hours while the morphological changes were noted in samples stored thereafter [7]. Similar findings were reported by Buoro et al [8]. Narasimha et al also didn't find any artefacts like crenation of RBCs, alterations in platelet or WBC morphology in samples stored at EDTA blood for 3-4 hours duration [3]. There is some evidence that morphologic changes in RBCs upon storage correlates with the ATP depletion of the RBC [9]. Nakao et al also showed that re-establishing the ATP levels with adenosine made the RBC to obtain their normal biconcave structure after being crenocytes [10]. Baca et al however contrastingly did not find significant changes in RBC, haemoglobin or platelet parameters in EDTA stored blood for up to 72 hours even on room temperature [11].

Gulati et al stated that parameters like RBC, WBC, Hemoglobin and platelets remain stable at room temperature even after 48 hours [12]. A study by Basu et al in 2019 found platelet counts altered significantly in the 6 hour un-refrigerated samples as compared to the refrigerated one. In the 24 hours (37°C) samples along with platelet counts, the total leucocyte count (TLC) had also been significantly changed. In the 24 hour un-refrigerated samples, all the blood indices were altered along with TLC with respect to the refrigerated one [13].

A potential limitation is that this study was done with random samples from a varied group of patients and individual factors like presence of



antibodies and activated complement affecting the results was not considered.

Conclusion

Storage of blood in EDTA vials do not cause alterations in the morphology of platelets or RBCs up to 2 hours. RBC morphology, but not platelets, is also stable up to 24 hours at 4 degrees. However, results obtained after interpretation of peripheral smears or platelet counts by hematology analysers beyond 24 hours are unreliable and lead to a wrong diagnosis and management.

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Conflict of interest: None

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