

EDTA INDUCED STORAGE ARTEFACTS-A STUDY ON PERIPHERAL BLOOD SMEARS

Received : 30/07/2022
Received in revised form : 08/09/2022
Accepted : 20/09/2022

Keywords:
Peripheral smears, Anticoagulant,
Platelets, Neutrophils, Artifacts.

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DOI: 10.47009/jamp.2022.4.5.39

Source of Support: Nil,
Conflict of Interest: None declared

Int J Acad Med Pharm
2022; 4 (5); 183-187



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Abstract

Background: Examination of peripheral blood smear is most important tool in investigation of various hematological disorders. Peripheral blood is most commonly collected in ethylene diamine tetra acetic acid (EDTA) vacutainer and is associated with varied morphological changes. It is important to identify storage artefacts for correct diagnosis. The aims and objectives are to analyze the storage artefacts and avoid false interpretation of peripheral blood smears. **Materials and Methods:** 50 blood samples were obtained using EDTA and 3.5% sodium citrate as anticoagulants. Smears were made immediately as well as after 2, 4 and 6hrs of storage at room temperature. Smears obtained from same patients by finger prick method served as controls. **Result:** The present study included 50 blood samples. Smears obtained by finger prick method without any added anticoagulant served as controls. These smears showed clumping of RBC's and aggregated platelets. No other significant morphological alterations/artefacts were observed in direct smears. Smears made immediately after addition of anticoagulant did not show any artefactual changes. **Conclusion:** EDTA has been recommended as the anticoagulant of choice for peripheral smear but should be examined within 1hr of collection to avoid misinterpretation as pathological findings leading to wrong diagnosis.

INTRODUCTION

The first step in analysis of hematological disorders is collection and proper assessment of the blood smears. Blood is collected into vacutainers containing different anticoagulation in specific ratio based on test required. Most commonly used anticoagulant is EDTA as it causes complete anticoagulation with slight morphological alterations of blood cells. Mechanism of action of EDTA is by removing Ca⁺⁺ from blood by converting it from the ionized to non-ionized form i.e. a chelating agent. Heparin as anticoagulant induces WBC clumping and gives faint bluish discoloration of the background stained with romanowsky stains therefore not appropriate platelet and leukocyte counts. Therefore Trisodium citrate is used as anticoagulant for platelet and coagulation studies. EDTA induces structural, biochemical and functional damage to hematological cells. Artefactual changes caused due to EDTA by a

lysocleithin formation or fall in Adenosine Triphosphate (ATP) as the blood is kept for a prolong time. hence this study was taken up to understand storage associated morphological changes induced by anticoagulants and avoid false diagnosis of the smears.^[1,2,3,4]

Aims & Objectives

To analyze the storage artefacts and avoid false interpretation of peripheral blood smears

MATERIALS AND METHODS

This study was carried out in hematology laboratory of Dr. B.R Ambedkar Medical College and Hospital, Bangalore. Blood samples were obtained from 50 patients whose haematological parameters were within normal limits. Blood samples were collected directly into commercially prepared vacutainers containing K3 EDTA and sodium citrate as anti- coagulants. The collected samples were

mixed thoroughly containing correct concentration of anticoagulant and smears were made immediately as well as after 2, 4 and 6hrs of storage at room temperature. Smears obtained from the same patients by finger prick method served as controls. The smears were stained with Leishman stain and studied under conventional microscope for identification of artefactual changes.

Patient's Selection Criteria

The study included random selection of routine complete blood count of both OPD and IPD patients of all age groups and both the genders. Patients with no history of fever or medication and those with normal haematological parameters tested on automated hematology analyser were included in the study.

Morphological artefacts to be studied are as follows

Nuclear Features: Lobulations, degeneration, karyolysis, vacuolations, rupture.

Cytoplasmic Features: Vacuolations, granularity, blebs, hairy projections, degranulation, rupture.

Platelets: Swelling, Aggregation.

Others: Swollen WBC's, crenated RBC's, smudge cells, abnormal staining characteristics.

RESULTS

The present study included 50 blood samples. Smears obtained by finger prick method without any added anticoagulant served as controls. These

smears showed clumping of RBC's and aggregated platelets. No other significant morphological alterations/artefacts were observed in direct smears. Smears made immediately after addition of anticoagulant did not show any artefactual changes. Smears from EDTA and sodium citrate showed significant morphological artefacts on storage.

Nuclear Changes

Nuclear lobulations were observed in the beginning followed by degeneration, karyolysis or pyknosis, vacuolation and rupture which was observed after 2hrs with EDTA and as early as 0hrs with citrate blood.

Cytoplasmic Changes

These included appearance of vacuoles, cytoplasmic granules, hairy projections, blebs and rupture which was observed after 2hrs with EDTA blood but as early as 0hrs with sodium citrate.

Platelets

Swelling of platelets occurred at 2hrs with EDTA and 1hr with citrate. Platelet aggregation that is pseudo agglutination of platelets occurred at 3hrs which was not observed with citrate.

These changes are shown in the [Table 1].

Other Artefacts

Changes like smudge cells, swelling of WBC's, crenated RBC's and abnormal staining characters were also observed which began at 3-4hrs with EDTA blood and as early as 1hr with citrate blood.

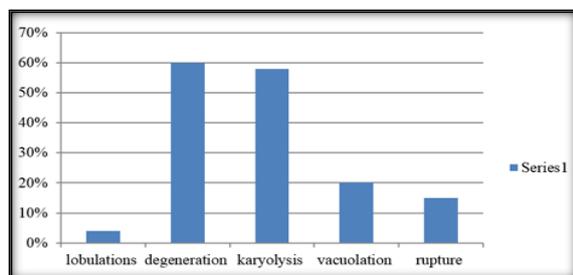
These changes are shown in [Table 2]

Table 1: EDTA induced storage artefacts

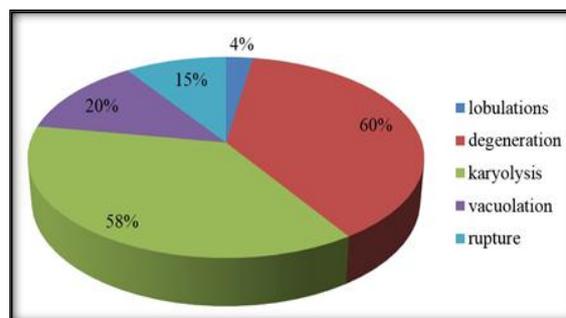
Duration	WBC cytoplasmic changes	WBC Nuclear changes	Platelet swelling	Platelet aggregation
0hrs	No change	No change	No change	No change
2hrs	Vacuoles (97%) and rupture (40%)	Degeneration (60%)	80%	8%
4hrs	Hairy projections (80%)	Lobulations (97%) and vacuolation (25%)	85%	25%
6hrs	Blebs and rupture (90%)	Rupture (95%)	90%	80%

Table 2: EDTA induced other storage artefacts

Duration	Smudge cells	Swollen WBC	Crenated RBC	Abnormal staining
0hrs	No change	No change	No change	No change
2hrs	30%	35%	45%	10%
4hrs	12.5%	40%	40%	25%
6hrs	80%	82%	90%	85%



Graph 1: Graphical representation of EDTA induced nuclear artefacts.



Graph 2: Graphical representation of EDTA induced cytoplasmic changes

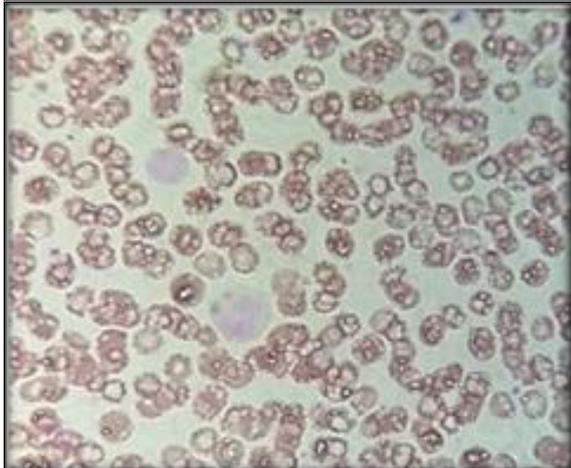
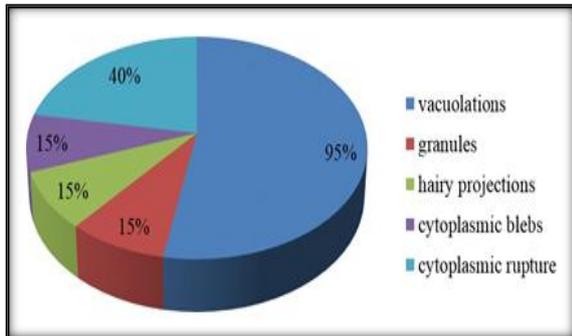
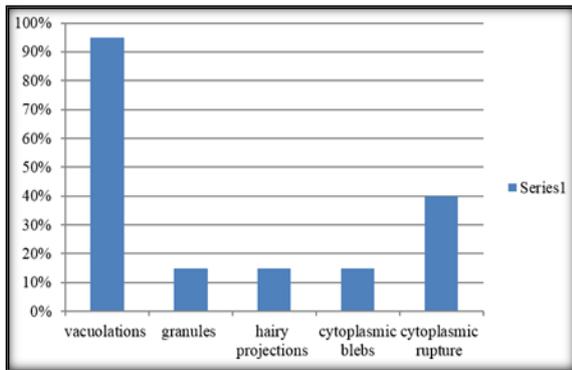


Figure 1: Abnormal staining with crenated RBCs-1

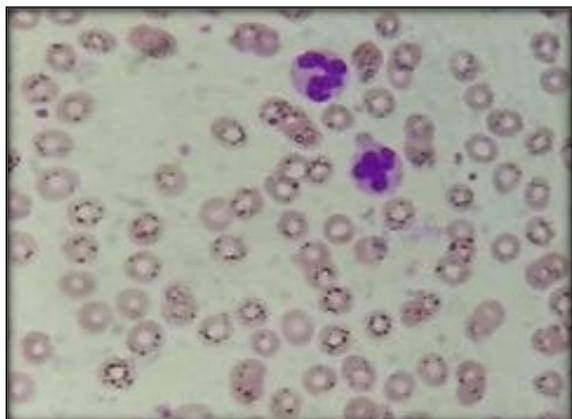


Figure 2: Cytoplasmic blebs-1

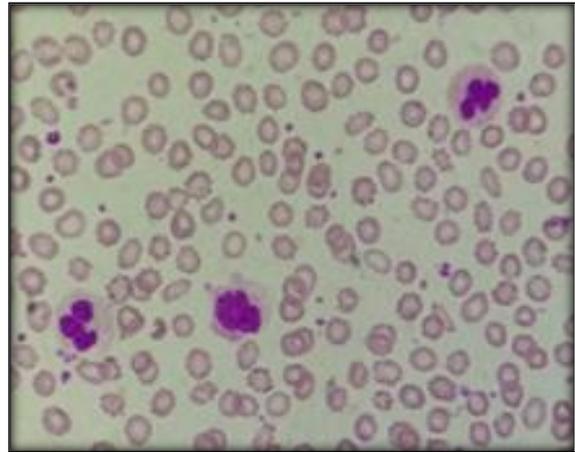


Figure 3: Cytoplasmic vacuolation and nuclear pyknosis-1

Storage Artefacts Misinterpreted on Peripheral Blood Smear

WBC Storage Artefacts

1. Neutrophil-nuclear lobulation and cytoplasmic vacuolation → Infection/sepsis
2. Lymphocyte → nuclear lobulation → Hyperchromatic nuclei
3. Monocytes → cytoplasmic vacuolation and nuclear degeneration → Infection/sepsis
4. Platelet Storage Artefacts: Platelet aggregation → Pseudothrombocytopenia

RBC Storage Artefacts

1. Crenated RBC → Burr cells
2. Loss of central pallor → Spherocyte

Therefore while examination of peripheral blood smears all these changes should be kept in mind so that they are not misinterpreted as pathological findings.

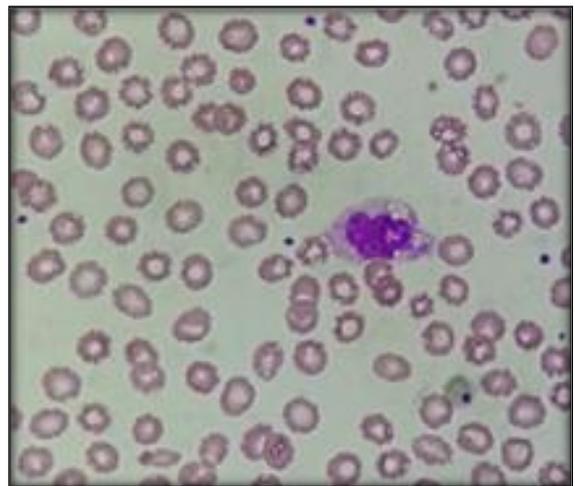


Figure 4: Cytoplasmic vacuolation-1

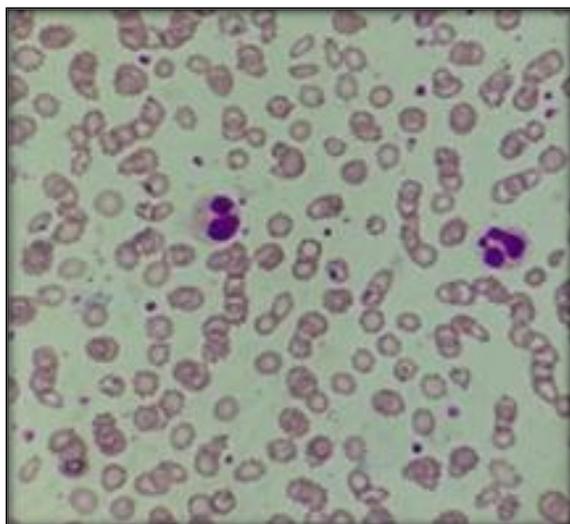


Figure 5: Nuclear pyknosis-1

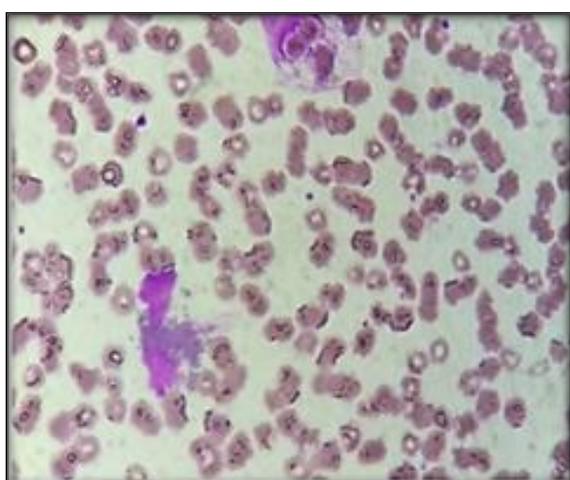


Figure 6: Nuclear vacuolation-1

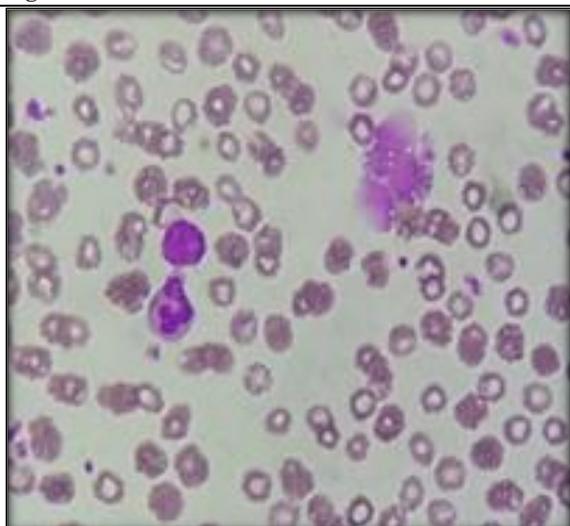


Figure 7: Smudge cells -1

DISCUSSION

Screening, diagnosis and monitoring of hematological disorders is done firstly by examination of the peripheral smear. Morphology of various blood cells gives has a significance to many diseases hence

manifest with changes in peripheral blood.^[1,5] EDTA is the ideal anticoagulant for automated blood cell counts. But it is associated with various morphological artefacts on prolonged storage leading to misinterpretation.^[6] controls as finger prick smears showed only platelet aggregation. Smears studied immediately after addition of anticoagulant did not show any artefactual changes. EDTA blood stands in the test tube causes abnormalities in leukocyte morphology to take place. Pathologist should have knowledge about the artifactual changes which leads omission false interpretation and information to the physician. Clinical history plays an important role there is deviation from normal in case of morphology of blood components like RBC's, WBC's and platelets. In the current study we came across different artifactual changes induced due to storage blood in anticoagulants.

Observed findings were as Follows

Nuclear Changes

Initially lobulations were seen as early as 2hrs followed by nuclear degeneration, karyolysis, nuclear vacuolations and nuclear rupture which started as early as 0hrs with citrate. Similar findings were noted by Koolwal, Vajpayee and Raphael et al.^[7,8,9,10]

Cytoplasmic Changes

Cytoplasmic vacuoles seen to start as early as 0hrs with sodium citrate with completely different morphology as hairy projections, blebs and rupture at the end of 6hrs. The changes are seen upto 2hrs with EDTA blood.

Platelets

Swelling of platelets seen as early as 2 hrs with EDTA and 1hr with citrate. EDTA induced pseudo – agglutination of platelets initiated at 3 hrs and marked at 6hrs no significant platelet aggregation was observed with citrate blood. The findings correlates with Koolwal and Chavda et al.^[7,8]

Other artefacts noted like smudge cells, increase in size of WBCs, crenated RBC's and abnormal staining were also seen which began as early as 1 hr with citrate but delayed upto 3-4hrs with EDTA blood. Similar findings were noted by Narasimha et al.^[10]

These above-mentioned changes can be misinterpreted as pathological abnormalities. With respect to WBC morphology vacuolisation of monocytes is noted. Neutrophils show separation of nuclear lobes and cytoplasmic vacuolation. Cytoplasmic vacuolation of neutrophils false interpreted as infection/sepsis. On other hand lymphocytes show nuclear lobulation which is misdiagnosed as malignant cells.^[5] EDTA samples sometimes may lead to pseudo thrombocytopenia. This is because of poor mixing of blood with EDTA or due to inadequate EDTA.12 0.1% of individuals

have EDTA dependent agglutinins which can induce platelet clumping.

CONCLUSION

In present study our observations were that marked changes occur in RBC, WBC and platelet morphology if the blood samples collected in EDTA anticoagulant are stored over a period of time. It may mislead the Pathologist towards false positive/false negative reports. EDTA has been recommended as the anticoagulant of choice for peripheral blood smear as it allows preservation of cellular components and does not alter the cellular morphology. Citrate should be avoided as it may result in increase in cell lysis and altered morphology.^[9,10] Therefore it is recommended that analysis of PBS should be made within one hour of collection of blood sample which is permissible with EDTA blood but not beyond that time duration.^[8]

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