Section: Pathology

Original Research Article

INDUCED ARTEFACTS IN PERIPHERAL BLOOD SMEARS: A PROSPECTIVE THREE-YEAR OBSERVATIONAL STUDY

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ABSTRACT

Background: Microscopic examination of peripheral blood smears (PBS) remains a cornerstone of hematological diagnostics. However, pre-analytical variables such as storage duration and anticoagulant type can introduce morphological artefacts, compromising diagnostic accuracy. **Objective:** To assess the spectrum and progression of artefactual changes in peripheral blood smears prepared from EDTA-anticoagulated blood samples stored under routine laboratory conditions. **Materials and Methods:** This prospective observational study was conducted over a period of three years (January 2022 January 2025) at a tertiary care teaching hospital. A total of 500 peripheral blood samples collected in K3EDTA vacutainers were studied. Smears were prepared at 0, 2, 4, 6, 12, and 24 hours post-collection, stained with Leishman stain, and examined by two independent pathologists for artefactual changes affecting red blood cells (RBCs), white blood cells (WBCs), and platelets. **Result:** Artefactual changes were evident as early as 2 hours post-collection. WBC vacuolation (48%) and platelet swelling (39%) were common early findings. By 6 hours, RBC crenation (70%) and platelet aggregation (68%) predominated. At 24 hours, smudging, karyolysis, and cytoplasmic degeneration were widespread. Many artefacts closely mimicked pathological conditions, including pseudothrombocytopenia and toxic granulation. **Conclusion:** Peripheral blood smears should be prepared within 12 hours of blood collection to avoid significant artefactual changes. Recognizing these changes is crucial to prevent diagnostic misinterpretation.

INTRODUCTION

Peripheral blood smear evaluation is an essential diagnostic tool for various hematological and systemic diseases, offering unmatched insights into cellular morphology. Despite increasing automation in hematology laboratories, manual smear analysis remains irreplaceable in many clinical settings.

Ethylene diamine tetra-acetic acid (EDTA) is the preferred anticoagulant for hematologic studies due to its effective chelation of calcium, thereby preventing coagulation. However, EDTA-induced morphological artefacts emerge with increasing storage time, leading to cellular distortions that may mimic genuine pathological features.

This study aims to systematically evaluate and document the timeline and types of artefactual changes observed in peripheral blood smears stored in EDTA over a 24-hour period.[1]

MATERIALS AND METHODS

Study Design and Setting: A prospective observational study was carried out in the Department of Pathology at a tertiary care teaching hospital between January 2022 and January 2025.

Sample Collection: A total of 500 venous blood samples from patients undergoing routine hematologic testing were included. Blood was collected in standard 2 mL K3EDTA vacutainers and thoroughly mixed.

Smear Preparation: For each sample, peripheral blood smears were prepared at six time intervals post-collection: 0 (immediately), 2, 4, 6, 12, and 24 hours.

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All smears were stained using Leishman stain under uniform conditions.

Microscopic Evaluation: Two experienced hematopathologists independently evaluated each smear using a light microscope under oil immersion (1000x magnification). The following parameters were analyzed:

RBCs: Crenation, central pallor loss, acanthocytes, bite cells, schistocytes.

WBCs: Pyknosis, karyolysis, nuclear lobulation, vacuolation.

Platelets: Swelling, aggregation, satellitism.

Statistical Analysis: Data were compiled using Microsoft Excel and analyzed with SPSS v26.0. Chi-square and Student's t-test were applied. A p value < 0.05 was considered statistically significant.

RESULTS

Of the 500 analyzed samples:

2 Hours Post-Collection:

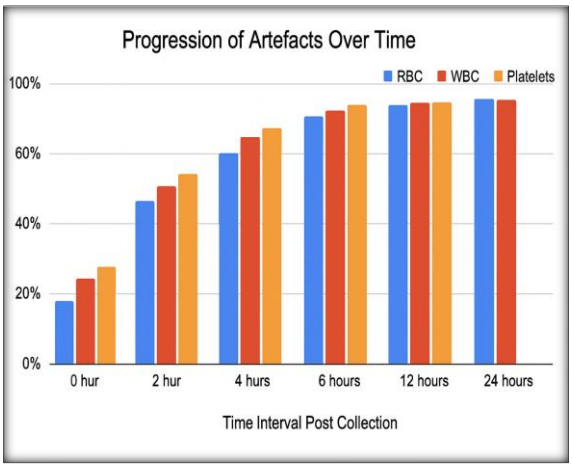
- WBC Vacuolation: Seen in 48% of smears.
- Platelet Swelling: Noted in 39% of samples.
- RBC Morphology: Mostly preserved, with minimal crenation.

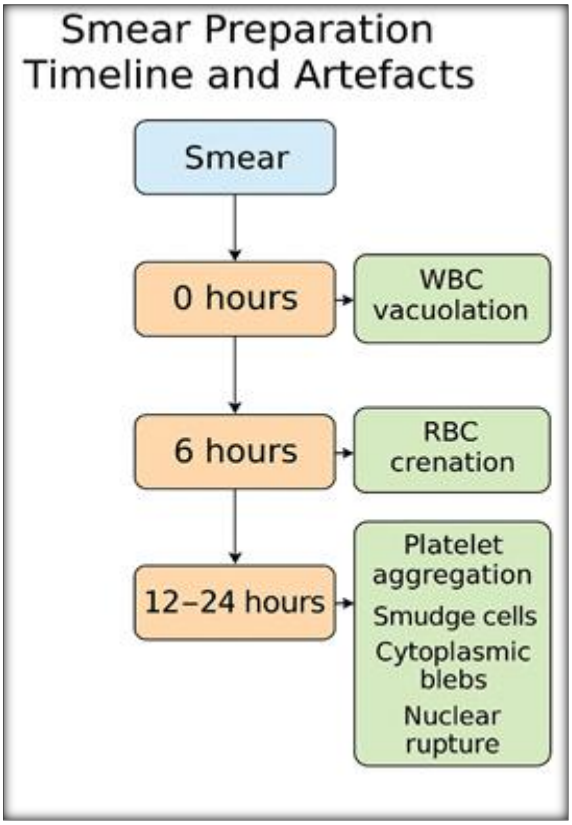
4-6 Hours Post-Collection:

- RBC Changes: Crenation observed in 70%, loss of central pallor in 30%.
- WBC Alterations: 55% with nuclear lobulation; 38% showed cytoplasmic blebbing.
- Platelet Artefacts: 68% demonstrated aggregation; satellitism in 12%.

12-24 Hours Post-Collection:

- RBC Morphology: Marked crenation, fragmentation observed in arround 90% of samples
- WBC Changes: Pyknosis, karyolysis in arround 80% of samples.
- Platelet Clumping: Observed in 88% of smears.
- Smudge Cells: Present in 90% of cases.





Common Misinterpretations

- Toxic granulation Bacterial sepsis
- RBC spherocytosis Hereditary spherocytosis
- Platelet clumping Pseudothrombocytopenia
- WBC cytoplasmic vacuoles Acute infection or neoplasia

DISCUSSION

This study highlights the impact of storage time on blood smear morphology. EDTA, though a stable anticoagulant, induces time-dependent degenerative changes that can significantly alter interpretation if smears are delayed.

Earlier studies by Dalal & Brigden (2002), Chavda A (2016) and Choudhary et al. (2022),[2,3,4,5] corroborate our findings, noting that artefactual changes begin within 24 hours and worsen thereafter. RBC crenation, a hallmark of storage-related dehydration, peaked by 6 hours. Platelet satellitism, a well-known EDTA-associated artefact, increased markedly after 4 hours, often mimicking thrombocytopenia.

This study reinforces the importance of preparing peripheral smears promptly and training personnel to recognize artefacts to avoid diagnostic pitfalls.

CONCLUSION

EDTA-induced artefactual changes can significantly distort peripheral smear interpretation, beginning as early as 2 hours post-collection and becoming pronounced by 6 hours. Diagnostic fidelity is best preserved when smears are made within 2 hours of

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blood collection. Awareness and recognition of artefactual patterns are vital to prevent misdiagnosis.

REFERENCES

1. Angadi RS, Mamatha R, Kumar BS, Murthy NC. Anti-Coagulant EDTA Induced Storage Artifacts on Peripheral Blood Smear. Int J Curr Pharm Rev Res. 2025;17(3):364370.
2. Dalal BI, Brigden ML. Artifacts that may be present on a blood film. Clin Lab Med. 2002;22(1):81100. doi:10.1016/S0272-2712(03)00068-4
3. Choudhary S, Katkar RS, Punneshetty DS, Begum F, Shilpa SJ. EDTA Induced Storage ArtefactsA Study on Peripheral Blood Smears. Int J Acad Med Pharm. 2022;4(5):183187. doi:10.47009/jamp.2022.4.5.39
4. Choudhary S, Katkar RS, Nagaram D. Storage artefacts in peripheral blood smears. J Diagn Pathol Oncol. 2018;3(3):187191. doi:10.18231/2581-3706.2018.0039
5. Chavda A, Mehta D, Chavda N, Suva C. Storage artifacts in peripheral blood smears. Ind J Basic Appl Med Res. 2016;5(2):B812.

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