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STORAGE ARTEFACTS IN PERIPHERAL BLOOD FILM OF ANTICOAGULATED BLOOD

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ABSTRACT **Introduction:**Examination of blood smears and hematologic parameters is often the first step in assessment of hematologic function and diagnosis of related diseases. EDTA is the preferred anticoagulant for blood counts as it produces complete anticoagulation with minimal morphological alterations of cell. Aims and objectives: The current study was undertaken to identify the anticoagulant induced artefacts and thus avoid misinterpretation of peripheral blood smears. Materials and methods Blood samples were obtained from Hematology laboratory at District Hospital, Samba. 100 blood samples were collected directly into commercially prepared vaccutiners which contain correct concentration of K3 EDTA as anticoagulant. Smears were made immediately as well as after 2 ,4, 6 hours of storage. Morphological artefacts were studied. Results and observations: Smears made immediately after addition of anticoagulant did not show any artefactual changes. Smears from EDTA stored blood show significant morphological artefacts on storage. Conclusion EDTA has been recommended as the anticoagulant of choice for peripheral smear as it allows the bes preservation of cellular components and morphology of blood cells up to 2 hours of storage. After 2hrs it shows storage artifacts .

KEYWORDS

tripotassium, trisodium, EDTA

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INTRODUCTION:

Examination of blood smears and hematologic parameters is often the first step in assessment o hematologic function and diagnosis of related diseases. Blood is collected by venipuncture into collection tubes containing anticoagulant.1 The most commonly used anticoagulants are tripotassium or trisodium salts o ethylene diamine tetra acetic acid, trisodium citrate and heparin. EDTA is the preferred anticoagulant for blood counts as it produces complete anticoagulation with minimal morphological alterations of cell. EDTA acts by removing Ca++ from blood by converting it from the ionized to non ionized form i.e. a chelating agent. Heparin causes leucocyte clumping and gives a faint blue coloration in the background stained with romanowsky stains. It is not suited for platelet and leucocyte counts.2 Trisodium citrate is the preferred anticoagulant for platelet and coagulation studies.3 However EDTA causes structural, biochemical and functional damage to blood cells. These artefacts are likely to be causedaj by a lysolecithin formation or fall in Adenosine Triphosphate (ATP) as the blood is kept for a long time.4 So the current study was undertaken to identify storage related morphological changes in blood cells so that these artefactual changes are not misinterpreted as pathologic findings. Changes in blood cell morphology occur easily even in short time storage. Irrespective of anticoagulantfilms made from blood that has been standing for no more than 1 hour at room temperature are not easily distinguished from films made immediately after collection of blood. By 3 hours changes may be discernible and by 12-18 hours these become striking.5Anticoagulant induced artefacts can lead to misinterpretation of the smears. The current study was undertaken to identify the anticoagulant induced artefacts and thus avoid misinterpretation of peripheral blood smears.

Materials and methods

Blood samples were obtained from Hematology laboratory at District Hospital, Samba. 100 blood samples were collected directly into commercially prepared vaccutiners which contain correct concentration of K3 EDTA as anticoagulant. Samples collected were mixed thoroughly and smears were made immediately as well as after 2 ,4, 6 hours 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 examined by conventional light microscope for identification of storage artifacts. Following morphological artefacts were studied:

Nuclear Features: Lobulations, degeneration, karyolysis, vacuolations, rupture. Cytoplasmic Features: Vacuolations, granularity, blebs, hairy projections, degranulation, rupture.

Platelets: Swelling, Aggregation. Others: Swollen WBCs. Crenated RBCs, smudge cells, abnormal staining characteristics.

Results and Observation:

The present study included 100 blood samples. Smears obtained by finger prick method without any added anticoagulant served as controls. These smear showed clumping of RBCs and aggregated platelets. No other significant morphological artefacts were observed in direct smears. Smears made immediately after addition of anticoagulant did not show any artefactual changes. Smears from EDTA stored blood show significant morphological artefacts on storage.

Nuclear Changes: Nuclear lobulations were observed in the beginning followed by degeneration, karyolysis or pyknosis, vacuolation and rupture after 2hrs with EDTA Cytoplasmic Changes: These included appearance of vacuoles, cytoplasmic granules, hairy projections, blebs and rupture which was observed after 2hrs with EDTA
blood

Platelets: Swelling of platelets occurred at 2hrs with EDTA and Platelet aggregation that is pseudo agglutination of platelets occurred at 3hrs

Table 1: EDTA induced storage artefacts

Duration	WBC cytoplasmic changes	WBC nuclear changes	Platelet swelling	Platelet aggregation
0 hrs	No change	No change	No change	No change
2 hrs	Vacuoles(95%) and rupture(40%)	Degeneration(60 %)	75%	10%
4 hrs	Hairy projections(75 %)	Lobulations(95%) and vacuolations(25 %)	80%	30%

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6 hrs	Blebs and rupture(88%)	Rupture(90%)	90%	80%

Other Artefacts: Changes like smudge cells, swelling of WBC's, crenated RBC's and abnormal staining characterswere also observed which began at 3-4hrs with EDTA blood

Table 2: EDTA induced other storage artefacts:

Duration	Smudge cells	Swelling of WBCs	Crenated RBCs	Abnormal staining
0 hrs	No change	No change	No change	No change
2 hrs	12.5%	35%	45%	10%
4 hrs	30%	40%	50%	30%
6 hrs	80%	85%	90%	85%

Discussion:

Peripheral blood smear is an important and informative tool for screening, diagnosis and monitoring of disease. Morphological evaluation of peripheral blood smear provides an important clue as many diseases manifest with changes in peripheral blood.1,6 EDTA is the preferred anticoagulant for automated blood cell counts However it causes morphological alterations on prolonged storage leading to erroneous diagnosis.7 The finger prick smears which served as controls showed only platelet aggregation. Smears studied immediately after addition of anticoagulant did not show any artefactual changes. Conversely, blood films made from EDTA blood beyond 2 hours show artefects. The significant changes observed with anticoagulated blood in order of sequence were: In case of WBC

Nuclear changes: Nuclear lobulation> nuclear degeration >karyolysis/pyknotic nucleus > nuclear vacuolation > nuclear rupture which began after 2 hrs with EDTA blood.

Cytoplasmic changes: cytoplasmic vacuoles> cytoplasmic granules> hairy projections>cytoplasmic blebs>cytoplasmic rupture which began as early as 2 hr with EDTA blood. Our findings correlate with those of 5,8,9 ,10. In case of RBCs crenation seen after 3-4 hrs. Our findings correlate with previous study.11,12.

Conclusion

EDTA has been recommended as the anticoagulant of choice for peripheral smear as it allows the best preservation of cellular components and morphogy of blood cells up to 2 hours of storage. After 2hrs I shows storage artifacts . Therefore smear should be made immediately or within 2 hrs of collection in case of anticoagulated blood. A delay upto 2 hour is permissible with EDTA but not beyond. The observations of our study show 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, which may result in misinterpretation leading to wrong diagnosis. 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.11 Citrate should be avoided as it may result in increase in cell lysis and altered morphology.5,9 Therefore it is recommended that analysis of PBS should be made with in 1hr of collection which is permissible with EDTA blood but not beyond.8,10

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