Original article:

Storage artifacts in peripheral blood smears

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Abstract

Introduction: microscopic evaluation of a peripheral blood smear is very important. Blood smears are often prepared from samples of anticoagulated blood. However, morphological analysis may be greatly hampered due to occurrence of artifacts.

Aims and objectives: To identify the storage artifects and thus avoid misinterpretation of peripheral blood smears.

Materials and methods: 200 blood samples were collected directly into commercially prepared vaccutes which contains correct concentration of K_3 EDTA as anticoagulant. Smears were made immediately as well as after 2 ,6 ,12 and 24 hours of storage at room temperature. Smears obtained from the same patients by finger prick method served as controls.

Conclusion: EDTA has been recommended as the anticoagulant of choice for peripheral smear but should be examine within 2 hrs of storage. Up till 2 hrs it allows the best preservation of cellular components and morphogy of blood cells.

Introduction

Changes in blood cell morphology occur easily even in short time storage. Irrespective of anticoagulant films made from blood that has been standing for not 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.[1] Many disease process menifest themselves with changes in peripheral blood . Therefore the microscopic evaluation of a peripheral blood smear is very important. Blood smears are often prepared from samples of anticoagulated blood. However , morphological analysis may be greatly hamperd due to occurence of artifacts.[2], [3] Ethylenediamine tetraacetic acid for hemocytometry and sodium citrate for coagulation tests. The main property of EDTA is the ability to chelate metal ions in 1:1 metal-edta complexes. Due to its strong complexion with metal ions that are cofactors for enzymes, EDTA is widely used as a sequestering agent to prevent some enzymes reactions from occuring. When blood is collected with no additives within an appropriate container, it clots quickly. As calcium ions are necessary for this process, the specific association between the carboxylic groups of and calcium is a reliable solution to prevent clotting, stabilizing whole blood in a fluid state.

Aims and objectives

To identify the storage artefects and thus avoid misinterpretation of peripheral blood smears.

Materials and methods

Blood samples were obtained from Hematology laboratory at our institute. 200 blood samples were collected directly into commercially prepared vaccutes which contains correct concentration of K_3 EDTA as anticoagulant. Samples collected were mixed thoroughly and smears were made

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immediately as well as after 2 ,6, 12 and 24 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 microscope for identification of storage artifacts.

Obseravtion and result

The present study included 200 blood samples. Smear obtained by finger prick method without any added anticoagulant served as controls. These smears show clumping of RBCs and aggregates of platelets. Smears from EDTA blood showed significant morphological artefects on storage. These included

In case of WBC

 Nuclear changes: Nuclear lobulation> nuclear degeration >karyolysis/pyknotic nucleus > nuclear vacuolation > nuclear ruptute 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.

In case of RBCs crenation seen after 3-4 hrs.

Findings of our study is shown in chart 1 and chart 2.which state that significant storage artifacte seen after 6 hrs and at the end of 24 hrs marked storage artifacte seen. Various storage artefects are shown in fig1 -5.
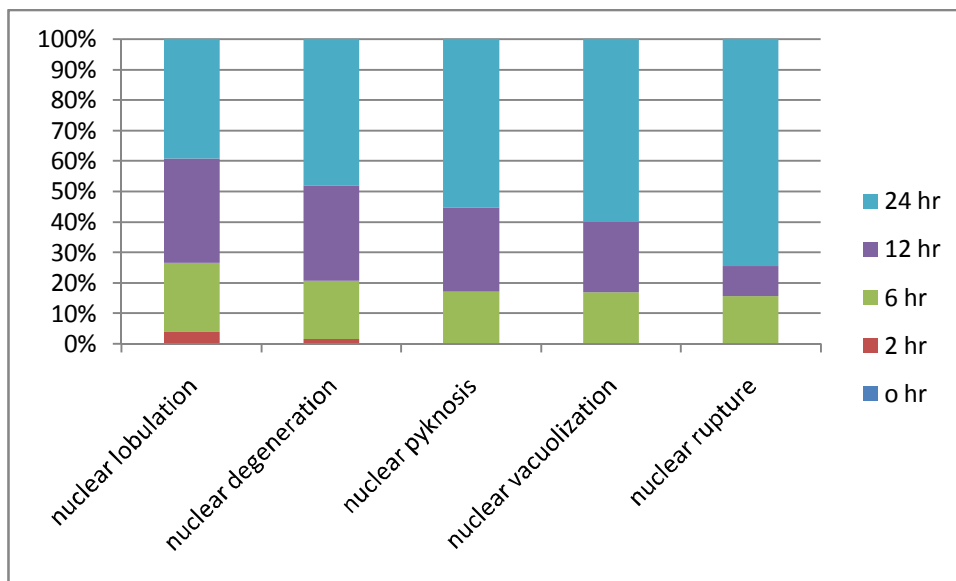

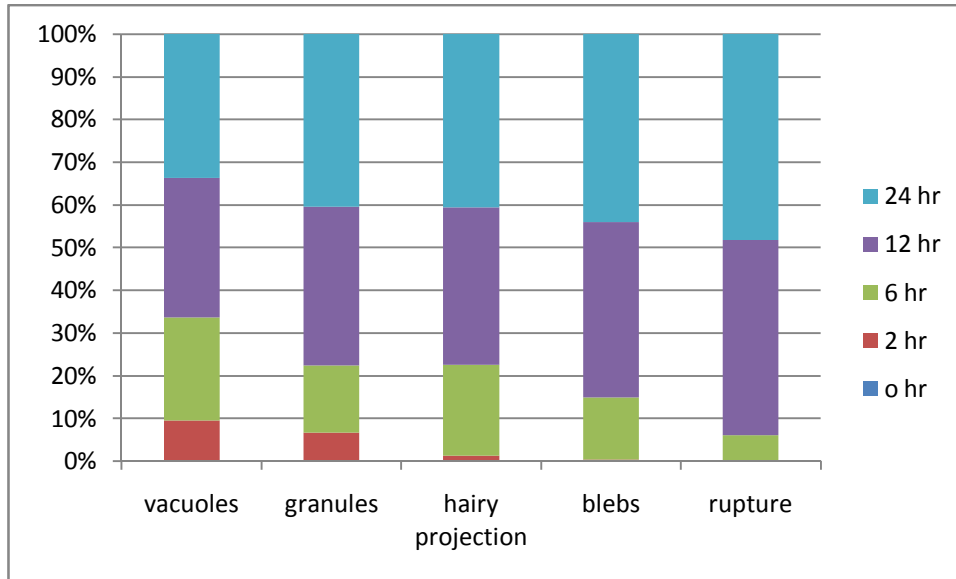Chart 1 wbc nuclear changes on storage.
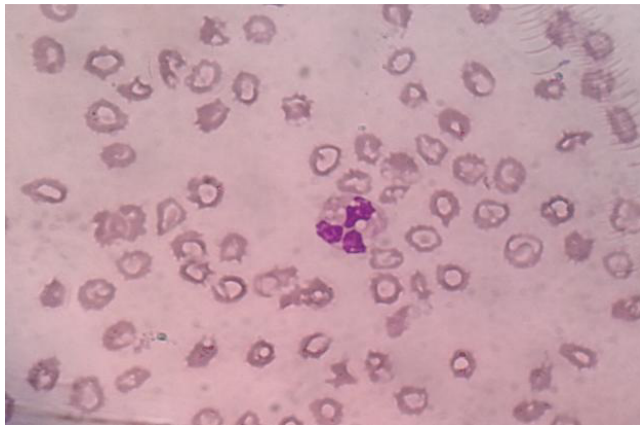
Chart 2 wbc cytoplasmic changes on storage.

Figure 1: Leishman stain 100 x nuclear and cytoplasmic vacuolations rbc crenation.

Figure 2: Leishman stain 100 x Nuclear pyknosis, cytoplasmic protrusion, smudging and apoptosis.

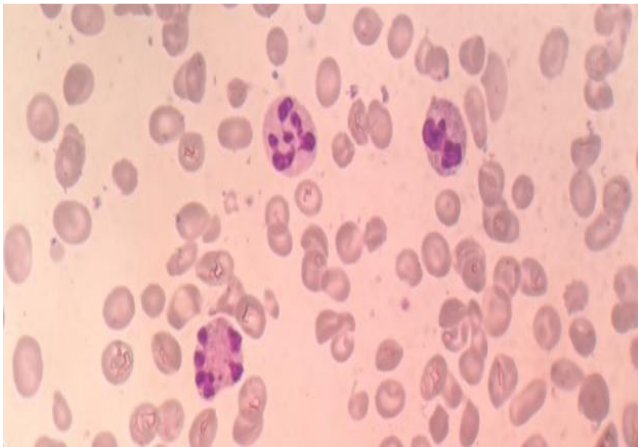Figure 3: Leishman stain 100 x cytoplasmic bleb formation.

Figure 4: Leishman stain 100 x nuclear degeneration and cytoplasmic granulation.

Figure 5: Leishman stain 100 x cytoplasmic vacuole formation.

Discussion

In non anticoagulated blood obtained by finger prick method, prominent platelet aggregations were observed. The smears made immediately after addition of anticoagulant did not show any morphological alterations in the blood cells. 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 ruptute 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.

In case of RBCs crenation seen after 3-4 hrs.

Our findings are correlate with previous study.[4],[5].

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 it shows storage artifacte . 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.

References

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