Anticoagulant EDTA Induced Storage effect (Artifacts) on Peripheral Blood Cells

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Abstract
Aims & Objectives: *The EDTA induced RBC & WBC morphological storage artifacts. Identify the EDTA induced platelets related artifacts. Exclude the EDTA induced storage artifacts. Identify and exclude the misinterpretation of peripheral blood smears examination.*
Material & Methods: *Blood was collected in a sterile EDTA containing tube and processed following our established laboratory protocol. A complete blood counting including HB%, PCV, Red cell indices, platelet count and total white cell count and differential was done by Automated blood cell counter and peripheral blood smear examination then a sterile EDTA containing blood sample tube stored at room temperature. The all cell count indices including RBC, WBC count with differential along with morphological storage artifacts and platelet count with storages artifacts, was further confirmed by manual oil immersion smear study method. Peripheral smears study was done with field A and B stain and leishman stain.*
Conclusion: *EDTA cause the various storage artefacts encountered on peripheral blood smear examination when smear prepared from prolong stored sterile EDTA containing blood sample tube at room temperature. EDTA cause RBC, WBC morphological artifacts and platelets related artifacts. These artifacts lead to various misinterpretation of peripheral blood smear examination so exclude them.*
Keyword: *Burr RBC, Nuclear lobe, Platelets aggregation.*

Material & Methods
Study area and design- This present study was conducted at the Advanced institute of medical sciences and research Bhopal and associated referral hospital Bhopal mp. The study was designed as a observational retrograde with prospective hospital based study over a period of time from 2016.
Ethical Consideration- Blood was collected in a sterile EDTA containing tube and processed following our established laboratory protocol

then generate the report of each patient. Take informed consent was obtained from all study participant for use of your blood sample for medical research after doing physician request investigating and generate the report.
Patient's Selection Criteria- The study target random selection of routine complete blood count patient. We include both OPD and IPD patients with all age groups, male and female both gender for study. Sample size is 1000 patients.

Laboratory investigations Blood was collected in a sterile EDTA containing tube and processed following our established laboratory protocol .A complete blood counting including HB%, PCV, Red cell indices, platelet count and total white cell count and differential was done by Automated blood cell counter and peripheral blood smear examination then a sterile EDTA containing blood sample tube stored at room temperature. The all cell count indices including RBC, WBC count with differential along with morphological storage artifacts and platelet count with storages artifacts, was further confirmed by manual oil immersion smear study method. Peripheral smears study was done with field A and B stain and leishman stain. Smear prepared from prolong stored sterile EDTA containing blood sample tube at room temperature.

Complete Blood Count (CBC) and Peripheral Smear
Materials
1) Purple vacutainer tube or capillary collector (EDTA) ethylenediaminete-traacetate
2) Slides and blue capillary tube
3) Needle or lancet
4) Vacutainer holder
5) Alcohol swab
6) Cotton balls
7) Absorbent materials
8) Slide case

Procedure
1. Specimen is collected into EDTA (purple) vacutainer. (5 or 7ml volume)

Preparation of peripheral blood smear from prolong stored (> 4hr) sterile EDTA containing blood sample tube at room temperature.

Step 1. A small drop of venous blood is placed on a glass microscope slide, using a glass capillary pipette.

Step 2. A spreader slide is positioned at 45^0 angle and slowly drawn toward the drop of blood.

Step 3. The spreader slide is brought in contact with the drop of blood and is being drawn away.

Step 4. The spreader slide is further pulled out, leaving a thin layer of blood behind.

Step 5. The blood smear is nearly complete.

Step 6. End result will be a glass slide with a well-formed blood film. After drying for about 10 minutes, the slide is fixed in methanol & stained with field A and B stain.

A well-made peripheral smear is thick at the frosted end and becomes progressively thinner toward the opposite end. The "zone of morphology" (area of optimal thickness for light microscopic examination) should be at least 2 cm in length. The smear should occupy the central area of the slide and be margin-free at the edges.

Hematological examination

Hematological examination including HB%, PCV, Red cell indices, platelet count and total white cell count with differential count should be done on peripheral smears stained with field A and B stains.

Observation & Discussion
EDTA induced RBC storage artifacts after 5 hour.

RBC storage artifacts	Misinterpretation on peripheral blood smears examination	Total Cases (n=1000)	
		No.	%
Loss of central pallor	Spherocyte	633	63.3%
Creanated RBC	Burr cells	267	26.7 %
Raised MCV	Macrocytic cells	100	10.0%

EDTA induced WBC storage artifacts after 11 hour

WBC cells	WBC storage artifacts	Misinterpretation on peripheral blood smears examination	% (n=1000)
Neutrophils	Nuclear lobes may be separated .Cytoplasmic margin appear ragged with cytoplasmic vacuolation.	Infection /Sepsis	62.04 %
Lymphocyte	Nuclear lobulation (Budding) Give rise to nuclei with two or more lobes.	Hyper chromatic nuclei	35.84 %
Monocytes	Large progressive cytoplasmic vacuolation and nuclear under goes disintegration.	Chronic infection	2.12%

EDTA induced platelets storage artifacts

platelets storage artifacts	% (n=1000)
Platelets aggregation (Pseudo thrombocytopenia)	36.02%
Platelets satellitism	10.2%

Remaining does not show platelets storage artifacts. Data analysis in following hematological parameters with the difference under the Extended Mantel-Haenszel test for trend of chi –Squares test. Chi-sq. test X2 Value =0.873 [DF = 1] 2-sided P = 0.350 For trend in a given direction: P = 0.175

Conclusion

Prolonged storage of peripheral blood cell in EDTA produce various storage[16] artefacts .RBC is most commonly shows loss of central pallor which can be misinterpretation of normal erythrocytes as sherocytes[4] and crenated RBC which can be misinterpretation of abnormal erythrocytes as burr cells[4] which are related with uraemia .high concentration of EDTA cause RBC cell to shrink because of hypertonicity[3] of the plasma with increased ionic concentration[2,3] and may create artefacts that make RBC morphology difficult to interpretation .EDTA cause damage to membrane of erythrocytes and leucocytes [1] .With respective to the WBC morphology[2,3,6], characteristic of EDTA anticoagulated blood on storage at room temperature a mild vacuolization of monocytes was found after 09 hrs progressive to moderated after 12hrs.Neutrophils shows Nuclear lobes may be separation with cytoplasmic margin appear ragged[11] with cytoplasmic vacuolation. The cytoplasmic vacuolation of Neutrophils mimicking that seen in infection /sepsis. Neutrophils and monocytes appeared to be the cells most sensitive[10,11] to storage in EDTA.

Lymphocytes shows nuclear lobulation which can lead to the misinterpretation of benign cells as malignant cells from EDTA indused[11] storage artifacts .This type storage artifacts are started after 11 hrs. Only minimum change in the WBC morphology[10,11,12] characteristics have been reported on storage at four degree temperature for as long as 12 hrs.

EDTA reduces platelet activation by protecting the platelets during contact with the glass tube that may initiate platelets activation[5]. Activation cause platelets to clump in the presence of ca ++ and platelets adhere to the glass surface at a rapid rate chelation of calcium using EDTA results in decreased platelets adhesion[5]. Paeudothrombo-cytopenia due to many poor mixing of blood with EDTA and very small fibrin clots in the EDTA anticoagulation specimen[6,7]. Additionally improper collection of the blood sample may due to platelets aggregation[8,9] and occasional may be due to insufficient EDTA and poor quality EDTA. Patients condition in which presence od EDTA cause the platelets to clump. Few pt who have antibodies that can bind to platelets when EDTA is added to the blood[6], the antibodies are activated and cause platelets clumping[8,9]. This is most particularly at room .Platelets satellitism' when platelets form the haloing surrounding white blood cells in which neutrophils is most commonly involve. This invitro phenomena due to presence of antibodies in most of case because if we change the anticoagulant this phenomena does not occure[7].

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