Original Article

EDTA induced morphological changes in the cells of peripheral blood smear

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Abstract

Microscopic evaluation of peripheral blood smear [PBS] is a very important investigation. PBS is often prepared using blood samples collected in an anticoagulant. EDTA is an anticoagulant of choice for various laboratory analyses. However it can cause morphological changes in blood cells. These artefacts can lead to misinterpretation of PBS. The present study was undertaken to identify EDTA induced artefacts in PBS. Blood samples were collected directly in EDTA, mixed thoroughly and PBSs were made immediately and at 1hr, 2hr, 4hr and 6hr after storing the samples at room temperature. Finger prick smears prepared simultaneously from same patients served as controls. Artefacts were noticed initially at 2hr and marked changes were observed at 6hr. It is important to make PBS from EDTA anticoagulated blood samples within 1hr of blood collection to avoid morphological changes in blood cells however it is highly advisable to prepare PBS immediately after sample collection to ensure perfect interpretation of PBS.

Key Words: EDTA, Peripheral Blood Smear, Artefacts.

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INTRODUCTION

Peripheral blood smear [PBS] is a basic and highly informative haematological tool at the Clinician's disposal in screening, diagnosis and monitoring of disease progression and therapeutic response. An adept understanding of peripheral blood smear interpretation is important for a successful clinical practice. The literature reveals that as much as 70% of clinical decisions and diagnoses are supported by laboratory medicine.[1] Peripheral Blood Smear is a very important part of laboratory medicine, the diagnostic relevance of which has not been lessened by advances in haematology automation and molecular techniques. The diagnostic importance of PBS is enormous as it exposes the morphology of peripheral blood cells and thus ensures its place in the diagnosis of various primary and secondary blood related diseases. Morphologic evaluation of PBS is very important as many diseases manifest themselves with changes in peripheral blood. PBS_s are often prepared from samples of anticoagulated blood. Ethylenediamine tetracetic acid [EDTA] salt either sodium or potassium, is regularly used as an anticoagulant in blood samples meant for routine laboratory analysis.[2] EDTA is the preferred choice of anticoagulant for automated blood cell counts due to its general availability, ease of preparation, widespread use and relatively low cost.[3] It is a routine practice that blood is collected in anticoagulant for automated cell counts and PBSs are simultaneously prepared from it for morphological evaluation of blood cells. The EDTA anticoagulant action is based on inhibiting platelet aggregation and various reactions of the haemostatic cascade by chelating free calcium (Ca^{2+}) ions.[4]However EDTA causes structural, biochemical and functional damage to blood platelets and other cells and the alterations induced are considered irreversible.[5]These anticoagulant induced morphological changes or artefacts in blood cells could lead to misinterpretation of PBSs. Morphological analysis may be greatly hampered due to

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occurrence of artefacts.[6,7]These artefacts are likely to be caused by a lysolecithin formation or fall in Adenosine Triphosphate (ATP) as the blood is kept for a long time.[8] Identifying storage related morphological changes in blood cells of PBS is important so that these artefactual changes are not misinterpreted as pathologic findings.

MATERIALS AND METHODS

This study was carried out in haematology OPD of Indira Gandhi Government Medical College and Hospital, Nagpur from January 2016 to December2016. A total of 80 blood samples were collected from patients who had come to the OPD as a part of routine medical check up. The blood samples were collected into commercially prepared Tripotassium Ethlene Diamine Tetra Acetic acid [K_3 EDTA] vacutainer tubes and were thoroughly mixed and smears were prepared immediately and at 1hr, 2hr, 4hr and 6hr respectively after storing the blood samples at room temperature. The vacutainer tubes contain the correct concentration of the anticoagulant and when filled appropriately, and mixed thoroughly help in minimizing error. Finger prick PBSs were also prepared from the same patients, which served as control. The smears were stained with Leishmann stain and studied under conventional microscope for identification of storage induced artefacts.

Inclusion Criteria

Patients with no history of fever, or those who were not on chemotherapy or on any medication and whose haematological parameters, when tested on automated haematology analyser, were within normal limits, were included in this study.

Exclusion Criteria

Patients with history of fever or those who were on chemotherapy or any medication and whose haematological parameters when tested on automated haematology analyser, were not within normal limits, were excluded from this study.

RESULTS

The present study included 80 blood samples which showed normal haematological parameters on automated haematology counter. Direct blood smears made by finger prick method served as control Finger prick smear showed clumping of RBCs and platelet aggregation. Apart from this no other morphological abnormality was observed in RBCs or WBCs in the finger prick PBSs. Table 1 shows the prominent morphological artefacts in blood cells of PBSs prepared from samples stored in EDTA.

The morphological artefacts seen in WBCs were

- Cytoplasmic changes namely vacuoles seen prominentlyat 2hrs in 63.75% PBSs, hairy projections seen prominently at 4hrs in 50% of PBSs and blebs (Fig1) and rupture seen prominently at 6hrs in 62.50% of PBSs. Nuclear changes namely lobulations seen prominently at 2hrs in 57,50% f PBSs, pyknosis (Fig.3) seen prominently at 4hrs in 67.50% of PBSs, and vacuolation and rupture seen prominently at 6hrs in 65% of PBSs. Smudge cells (Fig2)initiallyseen at 4hrs in36.25% of PBSs and these changes were markedly present at 6hrs in72.50% of PBSs.
- Crenated RBCs(Fig4) initially seen at 4hrs in41.25% of PBSs and were prominently seen at 6hrs in 97.50% of PBSs.
- Swelling of platelets was observed as early as 2hrs in 20% of PBSs.

Table 1: Showing morphological artefacts in PBSs made from EDTA stored blood

Duration of storage in EDTA	WBC Cytoplasm	WBC Nucleus	RBC Crenation	Smudge cells
1hr	No significant change	No significant change	Not seen	Not seen
2hrs	Vacuoles 51 PBSs (63.75%)	Nuclear lobulations 46 PBSs (57.50%)	Not seen	Not seen
4hrs	Hairy Projections 40 PBSs (50%)	Nuclear pyknosis 54 PBSs (67.50%)	Seen 33PBSs (41.25%)	Seen 29PBSs (36.25%)
6hrs	Blebs and Rupture 50 PBSs (62.50%)	Vacuoles and rupture 52 PBSs (65%)	Marked 78PBSs (97.50%)	Marked 58PBSs (72.50%)

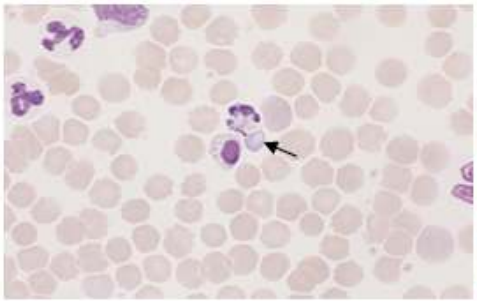

Figure 1: Cytoplasmic blebs in WBCs

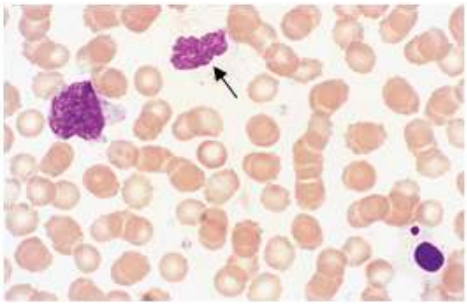

Figure 2: Smudge cell

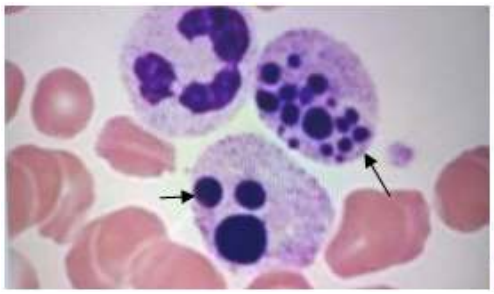

Figure 3: Nuclear pyknosis in WBCs

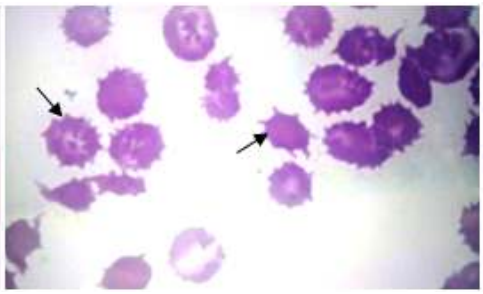

Figure 4: Crenated RBCs

DISCUSSION

Laboratory testing is a very essential part of clinical decision making process. The test results strongly influence the medical diagnosis as well as the therapy applied.[9]Appropriate evaluation of haematologic disease needs a very careful observation of a well prepared PBS. Whole blood is often treated with anticoagulants to prevent it from clotting.[10]Haematological results are often influenced by a number of pre-analytical variables. These include anticoagulants used, method of analysis and the time lapse between the initial procurement of sample and the time at which it is analysed.[11]For a perfect interpretation it is very important not to ignore this. Delayed sample analysis could result in haematological changes in measured parameter which could complicate the interpretation of the resulting data.[12]Immediate analysis of samples reduces artefactual changes in the samples which may be misinterpreted as pathological findings.[13] Morphological analysis may be greatly hampered by poorly prepared or stained blood smears due to occurrence of artefacts in cell appearance and staining that may be induced by the anticoagulant.[7] Artefacts produced either during sample collection or slide processing can often cause considerable confusion.[14] This is likely to result in wrong reporting and subsequently wrong diagnosis. A blood smear may either made by finger prick method or from venous blood added to an anticoagulant which commonly is EDTA. It is important to note that though blood for various laboratory analyses is commonly kept in EDTA, it can cause morphological changes in blood cells if the storage is prolonged. As the EDTA blood stands in the test tube changes in leukocyte morphology begin to take place.[15] Optimal morphology and staining are obtained from non anticoagulated blood, most often from a finger prick procedure.[7]In this study peripheral blood smears made by finger prick method showed prominent platelet aggregation and clumping of RBCs Apart from these no other morphological abnormalities were seen in the PBSs made by finger prick method. Similarly PBSs which were made immediately from blood collected in EDTA also did not show any morphological changes either in RBCs, WBCs or in platelets. However morphological artefacts were observed in these cells in PBSs made from samples which were allowed to stand beyond 1hr in EDTA. Following changes were prominently observed-

- NUCLEAR CHANGES IN WBCs: Nuclear lobulations were observed initially which began as early as 2hrs and were later on followed by morphological abnormalities lie nuclear degeneration, pyknosis, vacuolation and rupture. Similar findings were noted by Vajpayee *et al*.[16]
- CYTOPLASMIC CHANGES IN WBCs: At 2hrs cytoplasmic vacuoles were observed. Later on hairy projections, blebs and finally rupture were observed. WBCs showed a completely altered morphology at the end of 6hrs.
- ALTERATIONS IN PATELET MORPHOLOGY: Swelling of platelets was seen as early as 2hrs. This finding correlates with that of Zeiglar *et al*.[17]
- Smudge cells and crenated RBCs were also noted by 3-4hrs. Similar findings have also been reported by Raphael[14] and Vajpayee *et al*.[16]

All of the above morphological alterations in RBCs, WBCs and platelets correlate well with the observations made by Chavda*et al*.[18] and Narsimha *et al*.[19] However it is also important to note that the degree of change varies in different individuals according to Vajpayee *et al*.[16]

CONCLUSION

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. This may result in erroneous reporting which can lead to wrong diagnosis. We recommend that analysis of PBSs, made from samples collected in EDTA anticoagulant, should be made within 1hr of collection. In situations where the laboratory has decided to carry out the investigation of the sample that has been delayed, comments indicating the age of the specimen and its possible side effects on interpretation should be clearly mentioned on the results.

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