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ORIGINAL ARTICLE

Anticoagulant induced artefacts in peripheral blood smears

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Abstract Microscopic evaluation of a peripheral blood smear is one of the most beneficial test. But anticoagulant induced artefacts could lead to misinterpretation of the smears. The present study was undertaken to identify the anticoagulant induced artefacts and avoid misinterpretation of peripheral blood smears. The blood samples were collected using Ethylene Diaminetetraacetic acid (EDTA) and Sodium citrate, mixed thoroughly and smears were made immediately as well as 1hr apart for 6 hrs, stained and examined under oil immersion microscope. Direct smears were used as controls. Significant morphological artefacts were observed in our study. Artefacts were marked at the end of 2 hrs with EDTA but seen almost immediately with citrate blood. At 6 hrs, artefacts were marked but more severe with citrates than EDTA. Thus the practice of making blood smears before addition of anticoagulant is recommended and a delay up to 1hr is permissible with EDTA blood but not beyond.

Keywords EDTA · Sodium Citrate · Artefacts · Blood smears

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Introduction

Many disease processes manifest themselves with changes in peripheral blood cells. Therefore the microscopic evaluation of a peripheral blood smear is one of the most practical and efficient skills a pathologist can develop. It can provide evidence of ongoing disease process in a patient and thus aid in clinical diagnosis. There may be abnormalities in erythrocytes or leucocytes such as those in inflammation, infection and in leukemia. It can also reveal deficiencies of platelet numbers. Such evaluations will supplement the clinical examination and provide additional information prior to receiving further laboratory results [1]. But anticoagulant induced artefacts could lead to misinterpretation of the smears. So, the current study was undertaken to identify the anticoagulant induced artefacts and thus avoid misinterpretation of peripheral blood smears.

Materials and methods

The material for the present study was obtained from the Hematology laboratory at our institution. Blood samples were obtained from fifty patients whose hematological parameters were within normal limits. The blood samples were collected randomly using Tripotassium Ethylene Diaminetetraacetic acid (K_3-EDTA) and 3.5% Sodium Citrate as anticoagulants. Blood was collected directly into commercially prepared vacutainer tubes, which contain the correct concentration of anti-coagulant when filled appropriately thereby minimizing error [2]. The samples thus collected were mixed thoroughly and smears were made immediately as well as 1 hr apart for 6 hrs. Direct smears obtained from the same patients by finger prick method served as controls.

The smears were stained with Leishman stain and examined under oil immersion light microscopy at a final magnification of X 1000. The smears made immediately as well as those smears made 1hr apart for 6hrs were studied for identification of anticoagulant induced artefacts.

Significant morphological artefacts to be studied are as follows:

Nuclear features: lobulations, degeneration, karyolysis, vacuolations and rupture.

Cytoplasmic features: vacuolations, granularity, blebs, hairy projections, degranulation and rupture.

Platelets: swelling and aggregation

Others: Swollen WBC's, crenated RBC's, smudge cells, abnormal staining characteristics.

Exclusion Criteria: All patients with infections, septicemia, toxemia and on chemotherapy were excluded as their blood smears would show morphological alterations simulating the artefacts.

Results

The present study included 50 normal blood samples.

Direct smears made from finger prick method without any added anticoagulant served as controls. These smears showed clumping of the RBC's and aggregated platelets. No other significant morphological alterations/ artefacts were observed in direct smears.

Smears made from EDTA as well as citrate blood showed significant morphological artefacts. These included:

Nuclear changes: nuclear lobulations → nuclear degeneration → karyolysis / pyknotic nucleus → nuclear vacuolations → nuclear rupture. (Table 1) (Fig. 1)

Cytoplasmic changes: cytoplasmic vacuoles → cytoplasmic granules → hairy projections → cytoplasmic blebs → cytoplasmic rupture. (Table 2) (Fig. 2 & Fig. 3)

Platelets: platelet swelling → platelet aggregation. (Table 3) (Fig. 4 & Fig. 5)

Other features: swollen WBC's → crenated RBC's → smudge cells → abnormal staining characters. (Table 4) (Fig. 6)

Discussion

Blood smear analysis is a well known technique in medical laboratories. Careful evaluation of a well prepared blood smears is an important part of the evaluation of haematologic disease. Blood smears are often prepared from samples of anticoagulated blood. However, morphological analysis may be greatly hampered by poorly prepared or stained blood smears due to occurrence of artefacts in cell

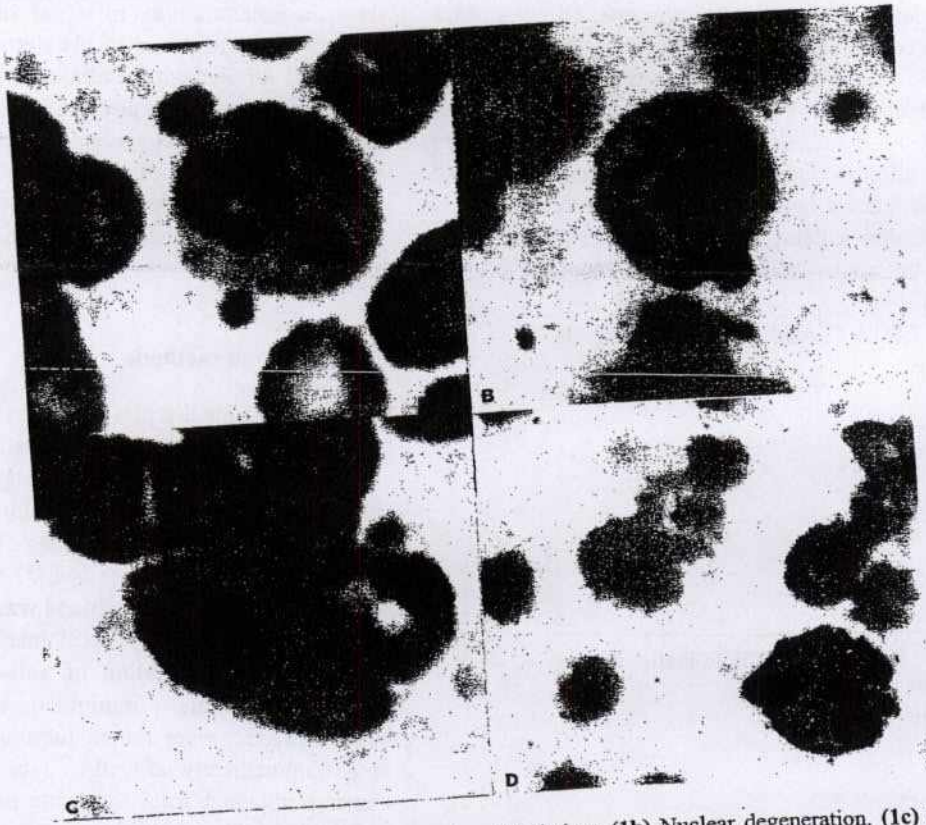

Fig. 1 Photomicrograph showing nuclear artefacts **(1a)** Nuclear lobulations **(1b)** Nuclear degeneration. **(1c)** Pyknotic nucleus **(1d** Nuclear rupture (Leishman, X 1000)

Table 1 Anticoagulant induced nuclear artefacts

Time	Nuclear lobulations		Nuclear degeneration		Karyolysis/ pyknosis		Nuclear vacuolations		Nuclear rupture	
	EDTA	SOD.CIT	EDTA	SOD.CIT	EDTA	SOD. CIT	EDTA	SOD.CIT	EDTA	SOD. CIT
0 HR		47 (94%)		23 (46%)		22 (44%)		13 (26%)		
1 HR	48 (96%)	03 (06%)	05 (10%)	27 (54%)	04 (08%)	23 (46%)	02 (04%)	33 (66%)		34 (68%)
2 HR	02 (04%)		34 (68%)	M	31 (62%)	05 (10%)	13 (26%)	02 (04%)	08 (16%)	16 (32%)
3 HR	M	M	07 (14%)	M	08 (16%)	M	23 (46%)	02 (04%)	30 (60%)	M
4 HR	M	M	04 (08%)	M	07 (14%)	M	12 (24%)	M	12 (24%)	M
5 HR	M	M	M	M	M	M	M	M	M	M
6 HR	M	M	M	M	M	M	M	M	M	M

Abbreviations: HR-hour; EDTA-ethylene diaminetetraacetic acid; Sod.Cit-sodium citrate; M-marked changes

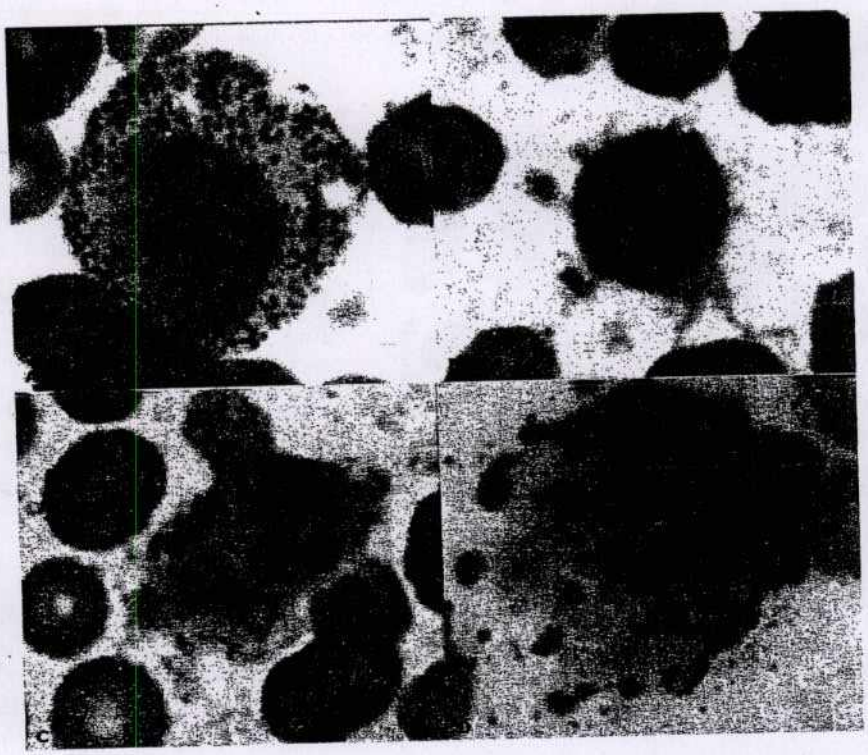

Fig. 2 Photomicrograph showing cytoplasmic artefacts **(2a)** Cytoplasmic granules **(2b)** Hairy projections **(2c)** Cytoplasmic blebs **(2d)** Cytoplasmic rupture (Leishman, X 1000)

appearance and staining that may be induced by the anticoagulant [2].

Artefacts produced during either sample collection or slide processing can often cause considerable confusion [3]. As the EDTA blood stands in the test tube, changes in leucocyte morphology begin to take place [4]. Defects owing to artefacts can range from crenation of the erythrocyte membrane to changes in the density of staining [3]. The degree of change varies among cells and in different individuals [5]. A blood film may be made from non-anticoagulated blood, obtained either from a vein or a capillary, or from EDTA anti-coagulated blood. Optimal morphology and staining are obtained from non-anticoagulated blood, most often from a finger prick procedure [2].

In non-anti-coagulated blood obtained by finger prick method, prominent platelet aggregations as well as marked rouleaux formation were observed.

The smears made immediately after addition of anticoagulant did not show any morphological alterations in the blood cells or the platelets.

Conversely, blood films made from EDTA blood beyond 1 hr did show artefacts.

The significant changes observed with anti-coagulated blood in the order of sequence were:

Nuclear changes: Initially there were nuclear lobulations followed by nuclear degeneration, karyolysis or pyknosis, nuclear vacuolations and nuclear rupture which began

Table 2 Anticoagulant induced cytoplasmic artefacts

Time	Cytoplasmic vacuoles		Cytoplasmic granules		Hairy projections		Cytoplasmic blebs		Cytoplasmic rupture/ degranulation	
	EDTA	SOD.CIT	EDTA	SOD.CIT	EDTA	SOD. CIT	EDTA	SOD.CIT	EDTA	SOD. CIT
0 HR		43 (86%)		37 (74%)		38 (76%)		40 (80%)		
1 HR	50 (100%)	07 (14%)	43 (86%)	13 (26%)	43 (86%)	12 (24%)	43 (86%)	10 (20%)		39 (78%)
2 HR	M	M	07 (14%)	M	07 (14%)	M	07 (14%)	M	22 (44%)	11 (22%)
3 HR	M	M	M	M	M	M	M	M	28 (56%)	M
4 HR	M	M	M	M	M	M	M	M	M	M
5 HR	M	M	M	M	M	M	M	M	M	M
6 HR	M	M	M	M	M	M	M	M	M	M

Abbreviations: HR-hour; EDTA-ethylene diaminetetraacetic acid; Sod.Cit-sodium citrate; M-marked changes

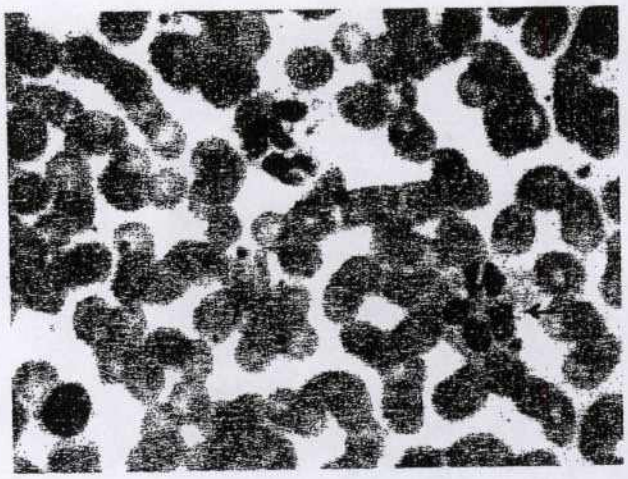

Fig. 3 Photomicrograph showing nuclear vacuolations (Thin arrow) and cytoplasmic vacuolations (Thick arrow). (Leishman, X 1000)

as early as '0' hr with citrate but delayed up to '2 hrs' with EDTA blood. Similar findings are also described by Vajpayee et al [5].

Cytoplasmic features included appearance of cytoplasmic vacuoles, cytoplasmic granules, hairy projections,

Table 3 Anticoagulant induced artefacts in platelets

Time	Platelet swelling		Platelet aggregation	
	EDTA	SOD.CIT	EDTA	SOD. CIT
0 HR		10 (20%)	-	-
1 HR	05 (10%)	37 (74%)	-	-
2 HR	45 (90%)	03 (06%)	-	-
3 HR	M	M	38 (76%)	-
4 HR	M	M	10 (20%)	-
5 HR	M	M	02 (04%)	-
6 HR	M	M	M	-

Abbreviations: HR-hour; EDTA-ethylene diaminetetraacetic acid; Sod.Cit-sodium citrate; M-marked changes

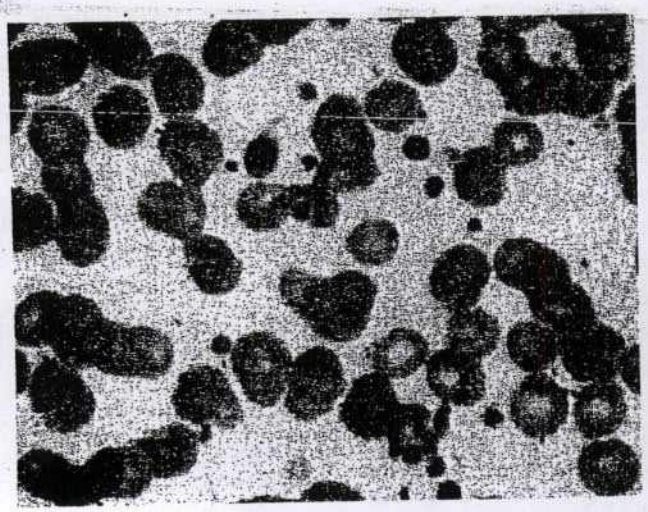

Fig. 4 Photomicrograph showing platelet swelling. (Leishman, X 1000)

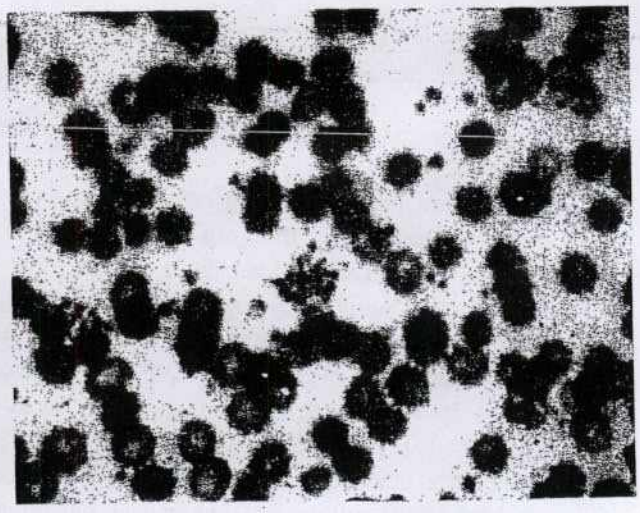

Fig. 5 Photomicrograph showing aggregated platelets with spiny projections. (Leishman, X 1000)

Table 4 Anticoagulant induced other artefacts

Time	Smudge cells		Swollen WBC'S		Crenated RBC'S		Abnormal staining of WBC'S	
	EDTA	SOD.CIT	EDTA	SOD.CIT	EDTA	SOD. CIT	EDTA	SOD.CIT
0 HR				11 (22%)		10 (20%)		06 (12%)
1 HR	05 (10%)	27 (54%)		29 (58%)		14 (28%)		07 (14%)
2 HR	26 (52%)	21 (42%)	19 (38%)	10 (20%)	23 (46%)	18 (36%)	04 (08%)	30 (60%)
3 HR	16 (32%)	02 (04%)	31 (62%)		13 (26%)	08 (16%)	40 (80%)	07 (14%)
4 HR	03 (06%)	M	M	M	14 (28%)		06 (12%)	
5 HR	M	M	M	M	M	M	M	M
6 HR	M	M	M	M	M	M	M	M

Abbreviations: HR-hour; EDTA-ethylene diaminetetraacetic acid; Sod.Cit-sodium citrate; M-marked changes

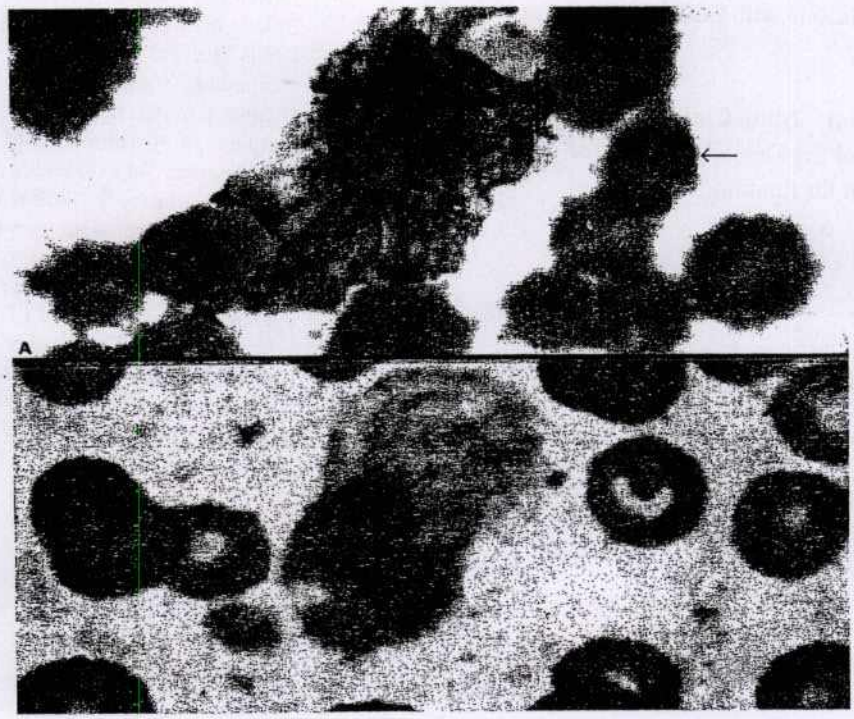

Fig. 6 Photomicrograph showing other artefacts. **(6a)** Smudge cell with crenated RBC's (Thin arrow). **(6b)** Swollen WBC. (Leishman, X 1000)

cytoplasmic blebs and finally cytoplasmic rupture. These changes began as early as '0' hr with sodium citrate with completely altered morphology at the end of 6 hrs, but delayed up to '2 hrs' with EDTA blood. The cytoplasmic vacuoles are clear and discrete with uneven distribution of cytoplasmic granules along with irregular cell membrane which was consistent with the findings of Vajpayee [5], Eastham [6], Lewis [7] and Gosett [8].

Smudge cells, increase in size of the WBC's, crenated RBC's and abnormal staining characters as described by Raphael [3] and Vajpayee [4] were also observed in our study which began as early as '1 hr' with citrate but delayed up to '3–4 hrs' with EDTA blood.

Platelets: Swelling of platelets occurred as early as '2 hrs' with EDTA and '1 hr' with citrate. Spiny projections were also observed. Zeigler observed that if films are made at 3 hrs after blood drawing, the fraction of large platelets are increased.[9]

Finally, EDTA induced pseudo-agglutination of platelets were initiated at '3 hrs' and marked at '6 hrs'. No significant platelet aggregation was observed with citrate blood.

This finding of our study was comparable to that of Shimasaki et al [10], Lippi et al.[11] At '6hrs' all these changes are marked, but more severe with citrate than EDTA [7].

Hence, preparation of satisfactory blood smears requires careful attention to preparation of blood smear with proper

use of appropriate anticoagulant and staining techniques with sound knowledge of morphologic appearances of normal and pathologic cell types.

Conclusions

EDTA has been recommended as the anticoagulant of choice for peripheral blood smear as it allows the best preservation of cellular components and morphology of blood cells. Citrate should be avoided as it may result in increase cell lysis and altered morphology. It is advisable to make smears immediately with the anticoagulated blood. A delay up to 1hr is permissible with EDTA blood but not beyond.

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