



# ANTICOAGULANT EDTA INDUCED STORAGE EFFECT (ARTIFACTS) ON PERIPHERAL BLOOD CELLS

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**Abstract:** **BACKGROUND :** Changes in blood cell morphology occur easily, even after short periods of time in storage. Microscopic evaluation of a peripheral blood smear is one of the most beneficial tests to identify morphological changes. But anticoagulant-induced artefacts could lead to misinterpretation of the smears. By 2 hours, changes may be discernible, and by 24 hours, they become striking. In our topic, we will discuss how long EDTA can preserve morphology and determine the best time to preserve blood samples.. **METHOD :** Thin smears were made immediately at the time of blood collection (0 hr) and after 2, 6, 12, and 24 hours of storage at room temperature. Smears stain with Leishman's stain and are observed under a microscope to check the morphology of cells. **RESULT :** The present study included 30 blood samples. Smears were prepared at 0 hours, 2 hours, 8 hours, and 24 hours using the venous blood collection method with EDTA anticoagulant. There are no other significant morphological alterations or artefacts observed in direct smears (0 h). Two-hour smears from EDTA did not show any artifactual changes. 8-hour and 24-hour smears from EDTA showed significant morphological artefacts on storage. **CONCLUSION:** By collecting blood samples in EDTA anticoagulant and storing them over time, our investigation has found that significant morphological changes occur in RBC, WBC, and platelet. This could lead to an inaccurate estimation and a wrong diagnosis

**Index Terms** - artifacts, Peripheral blood smear, morphological changes, EDTA anticoagulant

## I. INTRODUCTION

Blood cells are composed of RBCs, WBCs, and Platelets. Changes in blood cell morphology of stored samples occur within a few hours of blood collection. The changes are not solely due to the presence of an anticoagulant for they also occur in defibrinated blood. Irrespective of anticoagulant, films made from blood which has been standing for <1 hr at room temperature are not easily distinguished from films made immediately after collection of the blood. By 3 hr, changes may be discernible and by 12–18 hr these become striking.[1] Blood smears are often prepared from samples of anti coagulated blood. However morphological analysis may be greatly hampered due to occurrence of artifacts. Mainly EDTA samples are used for CBC. The main property of EDTA is ability to chelate metal ions in 1:1 metal EDTA complexes. Due to its strong complex ion with metal ions that are Cofactors for Enzymes [2]. EDTA is widely well as a sequestering agent to prevent some enzyme reactions from occurring. When blood is collected with no additives with is an appropriate container, it clots quickly as calcium ions are necessary for this process[3].

Artifacts in blood samples for hematologic testing stem mainly from either sample collection, aging of sample, or poor maintenance of the staining solutions (stain precipitate and water artifact).

Collection artifacts

These are associated with problems with sample collection. The most common problems are: Difficult venipuncture: Slow or traumatic venipuncture (poking around a lot for the vein, exiting the vein during sample withdrawal) can precipitate platelet clumping and induce small microclots within the sample or even clotting of the sample[4]

Low sample volume: Collection of a small blood volume (e.g. 0.5-1 mL) with placement into a standard 5 mL EDTA tube will cause shrinkage of red blood cells, because EDTA is hypertonic. This will cause a false decrease in the mean cell volume (MCV) and false increase in mean cell hemoglobin concentration (MCHC) of red blood cells. Crenation of red blood cells (erythrocyte formation) will also be evident on the blood smear. [5] This is a common artifact that we see in hematologic samples. Inappropriate mixing with anticoagulant: The blood should be thoroughly mixed with anticoagulant during or immediately after sample collection (by several gentle inversions). Collection directly into a vacutainer tube (allowing the vacuum to draw the appropriate amount of blood) is optimal, but difficult to achieve in small or pediatric patients with small veins, that collapse easily. Inadequate mixing will result in sample clotting, which may not be visible to the naked eye (microclots). Rough handling: Shaking of blood tubes, forcing blood through needles, vigorous expulsion into tubes, can cause shearing of red blood cells (hemolysis) and platelet clumping. Samples should be handled with kid gloves. Storage (sample age-related) artifacts[6]

Storage of blood can result in many false changes in hematology results. These changes are minimized but not eliminated by cold storage (refrigerated, shipping on ice packs). Ensure that the slides are maintained at room temperature and are not stored cold or placed in direct contact with "cool packs", as the slide can freeze or become moist, thus rupturing the cells. Age-related changes that occur are:

Red blood cells: crenation (echinocyte formation), lysis (this will decrease the RBC count and HCT, leading to a falsely high MCH and MCHC), hemoglobin crystallization. Red blood cells also swell with storage (take up water). This causes a falsely increased mean cell volume (MCV) and decreased mean cell hemoglobin concentration (MCHC). Because the HCT is dependent on the MCV, the HCT may be higher than normal.[7]

White blood cells: Swelling and smoothing of the nuclear chromatin (mimicking band neutrophil formation), pyknosis and karyorrhexis of nuclei, cell smudging, and prominence of Döhle bodies (mimicking toxic change). Pyknotic leukocytes resemble (and can be misinterpreted as) nucleated red blood cells. These changes can decrease a white cell count (lysed cells are not counted) and can affect the accuracy of a differential cell count. Sometimes, the changes are so severe, that an accurate differential cell count cannot be performed. Platelets: Clumping, degranulation (the latter makes platelets difficult to see and enumerate). Platelet clumping decreases the platelet count and increases the mean platelet volume (MPV), since a small clump of platelets is seen as a single large platelet. Large platelet clumps are excluded from the count altogether 48hour old blood8

48 hr old blood sample with extreme RBC crenation. Stain artifacts[8]

Diff-Quik®, Hemacolor®, and other commonly used quick stains for hematology and cytology can provide good staining quality if properly used and maintained:

Keep tightly capped when not in use: This prevents evaporation, minimizes contamination of solutions, and prevents water from the air getting into the fixative.

Do not "top-off" the solutions: When fluid level drops or staining quality declines, empty, clean, and dry the jars, then refill with fresh solution.

Common stain-related artifacts are water artifact and stain precipitate. Water artifact: When severe, this results in a moth-eaten appearance to the cell. It is a refractile artifact that results from the presence of water in the fixative; it usually occurs when using poorly-maintained quick stains. Refractile means that, as one focuses up and down on the cell, the artifact "flashes"; in one plane of focus, it may appear dark, while in another plane it appears bright. Such artifact, is often mistaken for some form of red blood cell inclusion, such as a parasite[9]

## II. MATERIALS AND METHODS

A total of 30 samples were collected. The blood samples were taken using sterile, disposable needles and syringes and were collected into commercially prepared EDTA tubes and direct smear in non-anticoagulated blood at 0 hour, EDTA blood thoroughly mixed and smears were prepared 2hr, 8hr and 24hr after storing them at room temperature. For controls finger prick peripheral smears were also prepared from same patients. The containers are labeled. Blood smear were stained with leishman stain and studied under conventional microscope for identification of EDTA induced changes.

## 2.1 INCLUSION CRITERIA

- 1) All the EDTA blood samples collected from healthy individuals.

## 2.3 EXCLUSION CRITERIA

- 1) All the haemolysed blood samples
- 2) Anemic and leukemic patients

## 2.4 COLLECTION OF VENOUS BLOOD

The most commonly used sites for venipuncture are the veins inside the bend of the elbow (the antecubital fossa). The blood is collected on EDTA tube. Thin smear is prepared from Direct blood at 0hr from syringe and EDTA anticoagulated blood is using at 2hr,8hr,24 hr.

## 2.5 STAINING REAGENTS

1. Leishman Stain
2. Buffer solution (pH 6.8)

### 2.6 Staining technique

- (i) Prepare a thin smear and dry the smear in air.
- (ii) Cover the smear completely with Leishman stain.
- (iii) Stain for 1 to 2 minutes.
- (iv) Dilute the stain with twice its volume of the buffer solution. The slide will almost be completely flooded. And keep it 7-10 minutes and wash with tap water.
- (v) Same procedure is repeat at 2 hour, 8 hour, 24 hour smears

## III. RESULT

The present study included 30 blood samples. 120 Smears obtained by venous blood method with EDTA anticoagulant. We observed in 0hour, 2hour, 8hour, 24hour. There is no other significant morphological alterations/artefacts were observed in direct smears(0 hour). 2 hour smears from EDTA did not show any artefactual changes. 8 hour and 24 hour Smears from EDTA showed significant morphological artefacts on storage.

**RBC Changes:** Crenated rbc(Burr cell) and loss of central pallor(Spherocytes) were observed

**Nuclear Changes:** Nuclear lobulations were observed in the beginning followed by degeneration vacuolation and rupture which was observed after 8hrs with EDTA and as early

**Cytoplasmic Changes:** These included appearance of vacuoles, cytoplasmic granules, hairy projections, blebs and rupture which was observed after 2hrs with EDTA blood but as early.

**Platelets:** Swelling of platelets occurred at after 2hrs with EDTA and 1hr with citrate. Platelet aggregation that is pseudo agglutination of platelets occurred at 8hrs.

These changes are shown in the below

TIME	NO SIGNIFICANT CHANGE	CRENATED RBC(Burr cell)	LOSS OF CENTRAL PALLOR(Spherocytes)
0 hour	30(100%)		
2hour	30(100%)		
8 hour		28(93.33%)	20(66.7%)
24 hour		30(100%)	30(100%)

TABLE NO : 1 RBC STORAGE ARTIFACTS

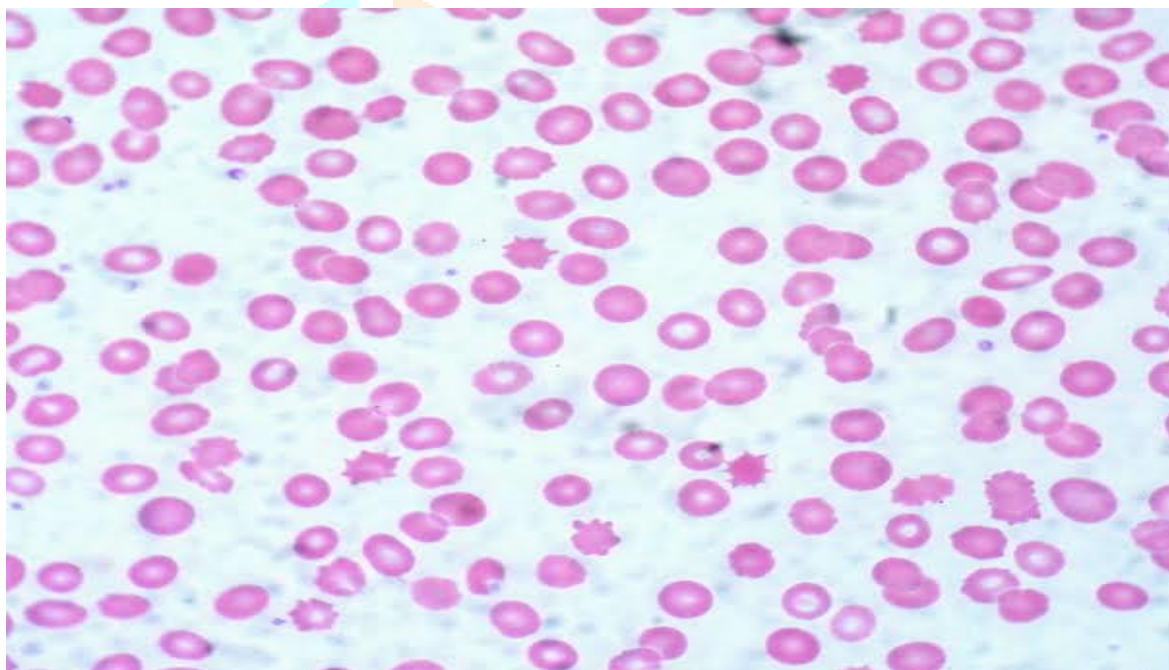
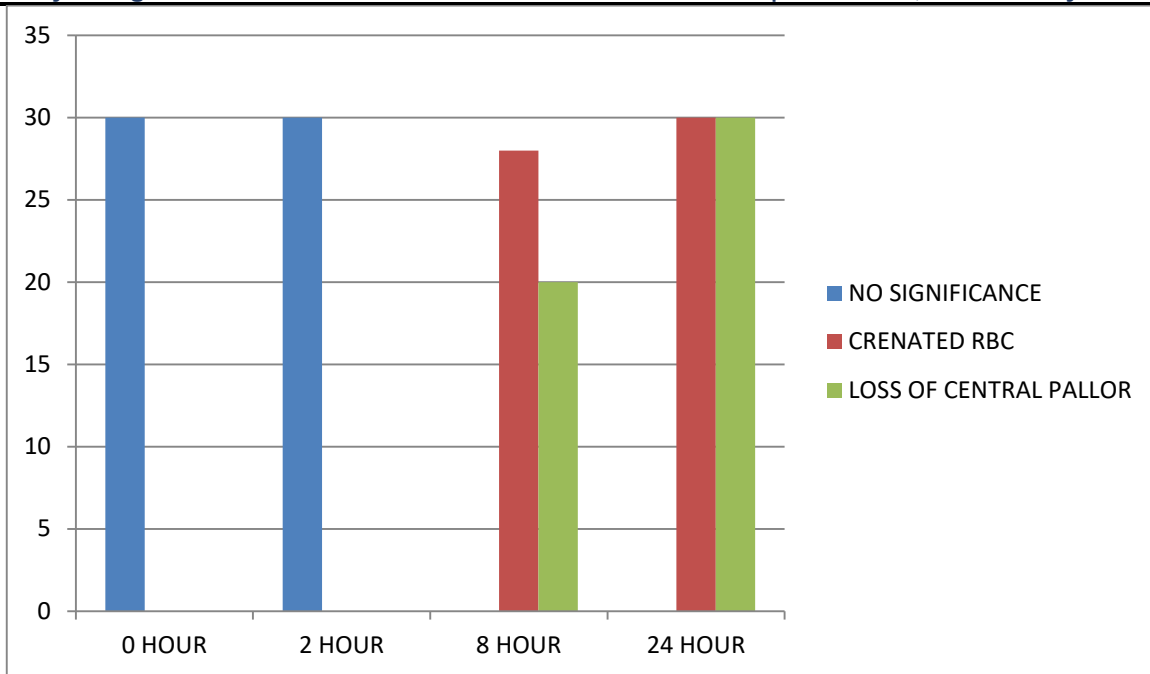


Fig.1CRENATED RBC(Burr cell) and LOSS OF CENTRAL PALLOR(Spherocytes)

**WBC STORAGE ARTIFACTS**

TIME	NO SIGNIFICANT CHANGE	WBC NUCLEAR CHANGE	WBC CYTOPLASM CHANGE
0 hour	30(100%)		
2 hour	30(100%)		
8 hour		28(93.33%) (Lobulation & vacuolation)	25(83.33%) (Hairy projection & vacuolation)
24 hour		30(100%) (Rupture Lobulation & vacuolation)	30(100%) (Blebs,rupture, Hairy projection & vacuolation)

TABLE NO : 2 WBC STORAGE ARTIFACTS RESULT

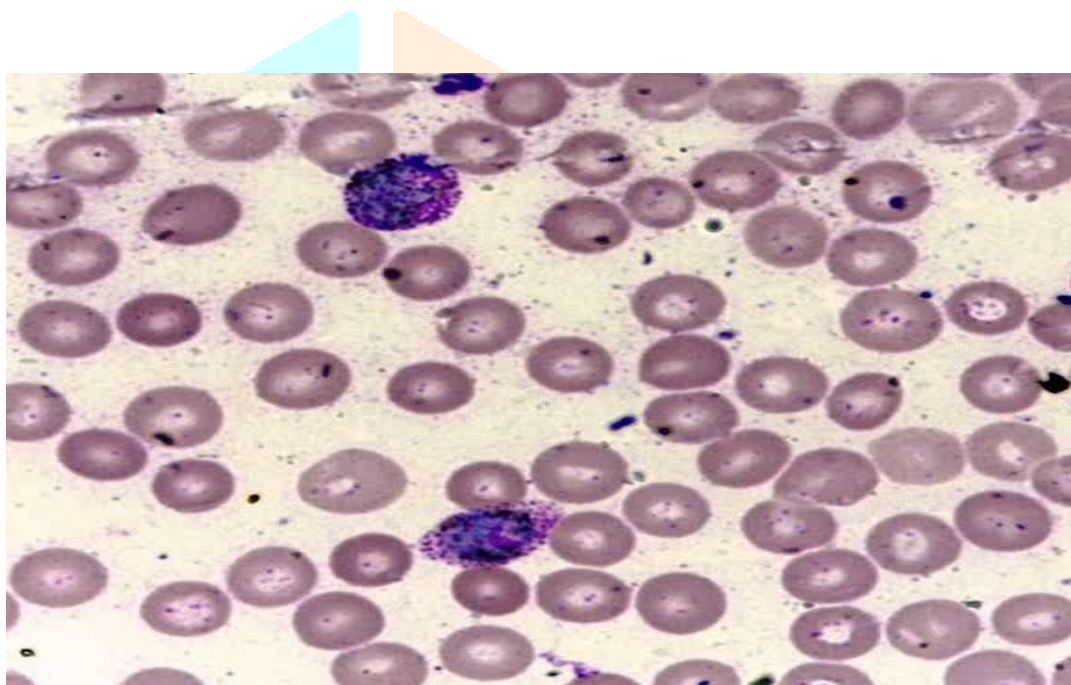
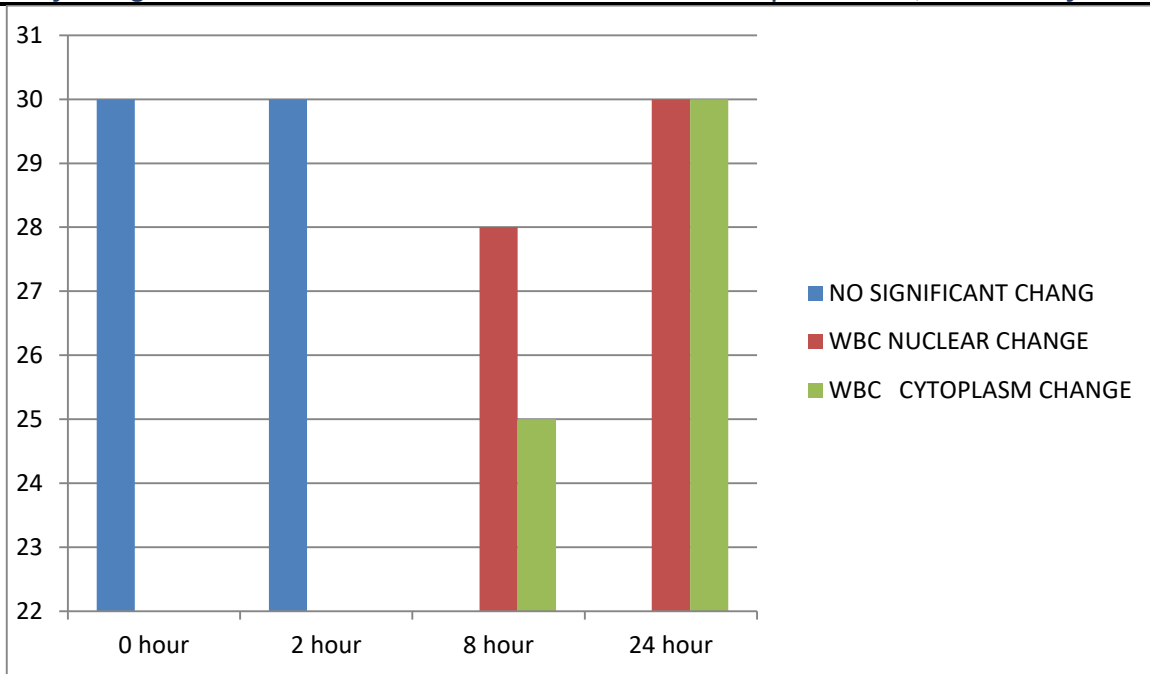


Fig : 2 WBC NUCLEAR CHANGE and WBC CYTOPLASM CHANGE

**PLATELET STORAGE ARTIFACTS**

TIME	NO SIGNIFICANT CHANGE	PLATELET SWELLING	PLATELET AGGREGATION
0 hour	30(100%)		
2hour	28(93.33%)	2(6.7%)	
8 hour	10(33.33%)	20(66.7%)	5(16.7%)
24 hour	4(13.33%)	26(86.7%)	15(50%)

TABLE NO : 3 PLATELET STORAGE ARTIFACTS RESULT

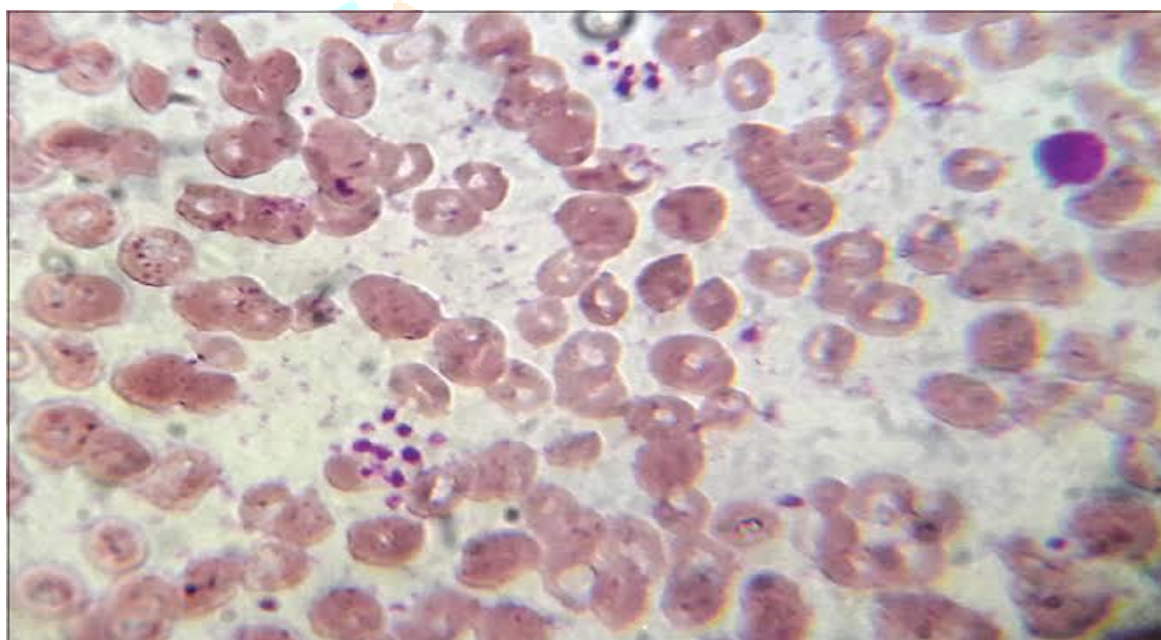
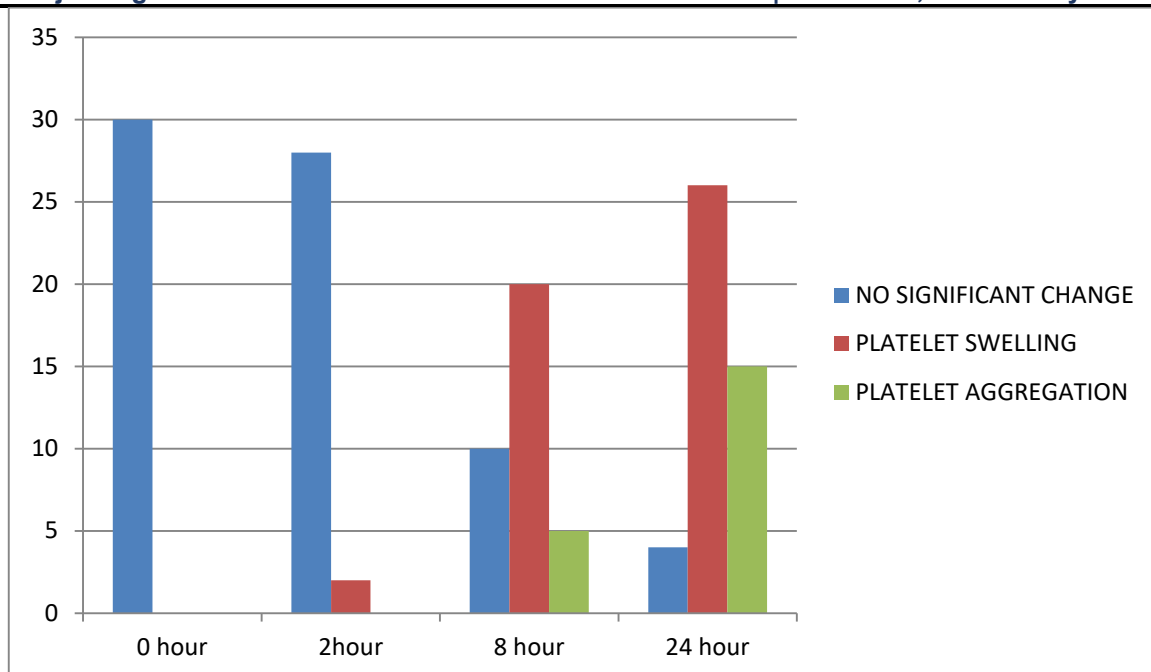


Fig. 3 PLATELET AGGREGATION

#### IV DISCUSSION

In our study 30 EDTA blood samples collected in healthy students and 120 smears stained in leishman stain. 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. EDTA is the preferred anticoagulant for automated blood cell counts. However it causes morphological alterations on prolonged storage leading to erroneous diagnosis. Smears studied immediately after addition of anticoagulant did not show any artifactual changes. As the EDTA blood stands in the test tube changes in leukocyte morphology begin to take place. The need of the 2,8,24 hours are to have knowledge about the artifactual changes which can mimic various mild to serious disorders hence giving false/biased information to the clinician. And therefore clinical history must be asked for in cases where it is not provided especially when one comes across deviation from normal in case of morphology of blood components like RBC's, WBC's and platelets. In the present study we came across various artifactual changes.

Following changes were observed:

### RBC STORAGE ARTIFACTS

In this study 0 hour and 2 hour shows no significance change in RBCs(30 smears).But in the 8 hour crenated RBC (Burr cell) seen in 28 smears and loss of central pallor(Spherocytes) seen in 20 smears. crenated RBC (Burr cell) and loss of central pallor(Spherocytes) cells seen in the all smears (30 smears) of 24 hour.

### WBC STORAGE ARTIFACTS

In this study 0 hour and 2 hour no significance change in WBCs(30 smears).

Nuclear Changes: Initially lobulations and vacuolation were observed at 8 hour in 28 smears. In 24 hour all smears shows lobulations and vacuolation.

Cytoplasmic Changes: cytoplasmic vacuoles and hairy projections initially observed at 8 hour in 28 smears. cytoplasmic vacuoles and including hairy projections, blebs and rupture observed at 24 hour in 30 smears.

### PLATELET STORAGE ARTIFACTS

In our study no significance change at 0 hr in all smears(30 smears).In 2 hour the 28 smears shows no significance change, but 2 smears show platelet swelling. In 8 hour the 10 smears observed no significance change, and 20 smears show platelet swelling and platelet aggregation seen in 15 smears. In the 24 hour no significance change observed in the 4 smears and platelet swelling in 26 smears and platelet aggregation seen in 15 smears.

A study by Archana Shrivatava et al, is “Effects of prolonged storage of blood in EDTA on blood cells morphology in peripheral blood smears”. [10]In that study the smears were examined and changes in smears were observed at different hours of duration of storage. At 1st hr there are no significant changes, though at 3rd and 6th hr more changes start appearing in blood cells. The most significant changes start next day i.e. after 24hrs. There is separation of nuclear lobes, loss of granules and vacuolation of cytoplasm in neutrophils. Where as in lymphocytes and monocytes there is irregular lobulation of nucleus to disintegration and smudging and spehering.

A study by Shaista Choudhary et al, is “ Storage artefacts in peripheral blood smear”.In that study EDTA has been recommended as the anticoagulant of choice for peripheral smear but should be examined within 1hr of collection to avoid misinterpretation as pathological findings leading to wrong diagnosis. Initially lobulations were observed in WBC as early as 2hrs followed by nuclear degeneration, karyolysis, nuclear vacuolations and nuclear rupture and cytoplasmic change. Platelets Swelling of platelets occur as early as 2 hrs with EDTA and agglutination of platelets initiated at 3 hrs and marked at 6hrs.[11]

A study by Dr.Alpesh Chavda et al, is “Storage artifacts in peripheral blood smears”. In that study EDTA has been recommended as the anticoagulant of choice for peripheral smear but should be examine within 2hrs of storage. Up till 2 hrs it allows the best preservation of cellular components and morphogy of blood cells.In case of WBC nuclear change seen after 2hours in EDTA blood. In case of RBC crenation seen after 3-4 hrs [12]

### V 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, 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. Therefore it is recommended that analysis of Peripheral blood smear should be made with in 2 hr of collection which is permissible with EDTA blood but not beyond.

### REFERENCES

- [1] Vinay Kumar; Abul K. Abbas; Nelson Fausto; Richard N. Mitchell (2007). Robbins Basic Pathology (8th ed.). Saunders.
- [2] . District Laboratory Practice in Tropical Countries, Part 2 - Page 323  
Monica Cheesbrough · 2005 · Preview
- [3] International Council for Standardization haematology. recommendations of the international council for standardization in haematology for ethylenediaminetetraacetic acid anticoagulation of blood for blood cell counting and sizing. Am J Clin Pathol. 1993;100:371-72.
- [4] International Society of Hematology · 1964 · Snippet view

- [5] . Lewis SM, Stoddart CT. Effects of anticoagulants and containers (glass and plastic) on the blood count. Lab Pract. 1971;10(10):787-922.
- [6] Aparna et al. 'anticoagulant induced artefacts in peripheral blood smears' Indian J. Hematology. Blood transfuse 24(2):43 48.
- [7] Methods for Laboratory Technicians - Issues 8-227 - Page 358. United States. War Department · 1941.
- [8] Dacie and Lewis – practical hematology (10th edition). 8
- [9] White 2nd GC, Scarborough DE, Brinkhous KM. Morphological study of early phases of platelet adhesion to foreign surfaces: effect of calcium. Ann. N.Y. Acad. Sci. 1983;416:351-62.
- [10] Shrivatava, Archana, Upasana Uniya and Reeni Malik. "Effects of prolonged storage of blood in EDTA on blood cells morphology in peripheral blood smears." *MedPulse International Journal of Pathology* (2019):
- [11] Choudhary S, Katkar RS, Nagaram D. Storage artefacts in peripheral blood smears. *IP Journal of Diagnostic Pathology and Oncology*. 2018;3(3):187-91.
- [12] Dr. Alpesh Chavda, Dr. Dimple Mehta, Dr. Nikunja Chavda, Chetal Suva "Storage artifacts in peripheral blood smears" *Indian Journal of Basic and Applied Medical Research*; March 2016: Vol.-5, Issue- 2, P. B . 8 - 12

