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Research Article

Storage Dependent Cellular Changes in Blood Smears Prepared from EDTA Added Venous Blood Samples

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Abstract

Ethylene diamine tetra acetate (EDTA) added blood is commonly used to perform various analysis like the complete blood count in a hematology laboratory. Prolonged storage in EDTA can cause morphological changes in the cellular components of the blood which has some potential to tamper with the diagnosis and interpretation of results especially during peripheral blood smear reporting and differential counting. This study aims to assess the morphology related variations in WBC and RBC in peripheral smears prepared from EDTA added blood stored at room temperature at different time periods. Peripheral smears were prepared from 20 healthy volunteers after 0 hrs, 2 hrs, 5 hours, 10 hours and 24 hours of storage in EDTA added blood collection containers. Parameters affecting cellular morphology such as crenation, acanthocytes, bite cells, fragmentation and central pallor (for RBCs) and nuclear degeneration, number of lobes in nucleus and vacuolation in cytoplasm (for WBCs) were quantified microscopically in 10 fields/smear after Leishman staining. Students t-test was performed to determine if there was significant morphological variation at different time points and p value less than 0.05 was considered significant. There was significant increase in the number of RBCs with crenation, acanthocytes, bite cells in smears prepared after 2 hours while WBCs showed significant nuclear degeneration and cytoplasmic changes in smears prepared after 1 hour of storage. Maximum cellular distortion was seen in smears prepared after 24 hours of storage at room temperature. Morphological interpretations of RBCS should preferentially be done within 2 hours while for differential count should preferentially be done within 1 hour to minimize errors and misdiagnosis, when blood samples are stored at room temperature.

Keywords: Complete Blood Cell Count; Ethylene Diamine Tetra Acetate (EDTA); Crenation; Bite Cells; Acanthocytes; Nuclear Degeneration; Cytoplasmic Vacuolation

Introduction

Blood is the main sample analyzed and complete blood count Complete blood cell count (CBC) is the most common performed laboratory test for a wide variety of disease conditions in a hematology laboratory. Ethylene diamine tetra acetate (EDTA) is used as the anticoagulant for storing blood for CBC [1]. EDTA chelates calcium ions thereby inhibiting the cascade pathways that leads to haemostasis. However, blood cells stored in EDTA can undergo degenerative changes over time which can lead to misdiagnosis and serious consequences. Variations in the morphology of RBCs and WBCs and changes in relative and absolute numbers of neutrophils, eosinophils, lymphocytes platelets and other blood components can also cause potentially erroneous interpretations in the laboratory [2].

Assessment of the morphology of the blood cells such as RBCs and WBCs provide important diagnostic insights into the underlying cause and prognosis of various diseases. But deviation from normal cellular morphology of RBCs and WBCs due to prolonged storage in EDTA at room temperature can interfere with the accurate diagnosis and precise treatment of diseases [3].

Aim of the Study

This study aims to assess the morphology related variations in RBCs and WBCs in peripheral smear prepared from EDTA added blood stored at room temperature at different time period at room temperature.

Materials and Methods

Blood from 20 healthy volunteers between 18 to 21 years of age group were collected in EDTA containers (1 mg/ml of blood) as test samples by venipuncture. Smears were prepared on clean glass slides at 0 hrs, 2 hrs, 5 hours and 10 hours and 24 hours also and then stained with Leishman stain for microscopic examination [4].

Microscopic examination

The stained smears were screened in low power to focus on the tongue region where there was complete spreading of RBCs without any overlapping [5]. Ten sequential high power fields were quantified for various parameters from each samples. The various parameters of RBCs and WBCs quantified to assess morphological changes were as follows:

- Parameters studied for evaluating morphological changes in RBCs - Number of RBCs with crenation, Number of acanthocytes, bite cells, fragmentation and RBCs without any central pallor.
- Parameters studied for evaluating morphological changes in WBCs - Number of WBCs with nuclear degeneration, vacuolation in cytoplasm and number of lobes in nucleus of the WBCs.

Statistical analysis

Mean and standard deviation was calculated for each parameter. Students t test was performed to determine if there is significant morphological variation at different time points in EDTA samples. A p value below 0.05 was considered significant.

Results and Observation

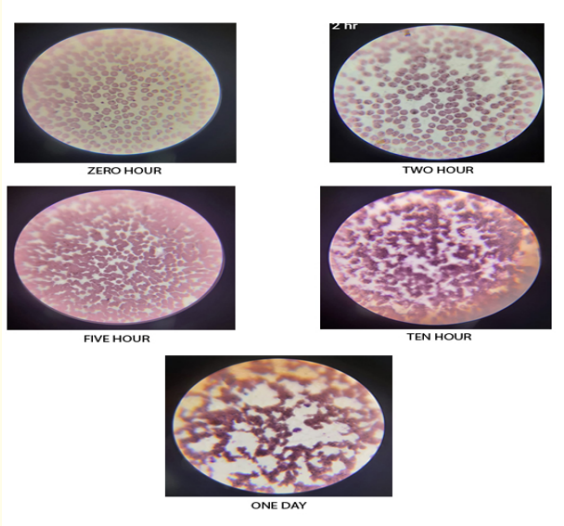
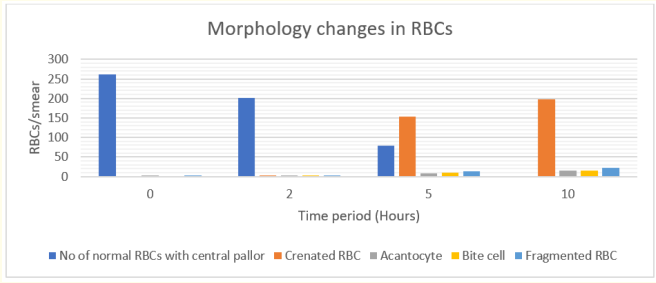


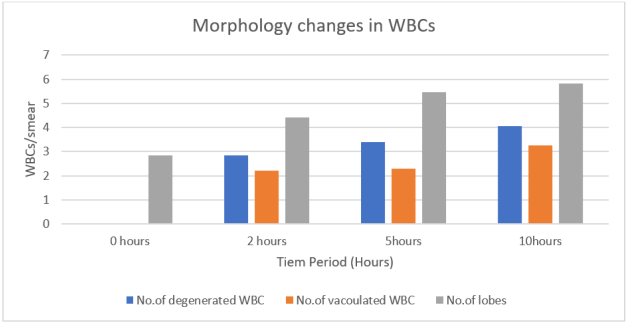
Figure 1: Representative fields of peripheral blood smears at different time points.



Graph 1: Morphology changes in RBCs.

RBCs in smears showed increase in morphological changes with increase in storage time. Smears prepared immediately after blood collection at 0 hour showed maximum number of normal RBCs with central pallor and absence of crenated RBCs, acanthocytes, bite cells and fragmented RBCs. The number of crenated RBCs, acanthocytes and bite cells progressively increased during 2, 5 and 10 hour in the smear. There was excessive stain uptake and Rouleaux formation by RBCs at 24 hours resulting hyperchromasia and

absence of central pallor thus making it impossible for microscopic quantification.



Graph 2: Morphology changes in WBCs.

Nuclear degeneration and cytoplasmic vacuolations was not seen in peripheral smears prepared immediately after collection at 0 hour. However WBCs showed gradual surge in degenerative nuclear and cytoplasmic changes as storage time increased. Maximum number of cells with nuclear degeneration and cytoplasmic vacuolation were seen in smears prepared after 24 hour of storage making it unsuitable for quantification. Surprisingly number of lobes in the nucleus also increased moderately with increase in storage time.

Discussion

Accurate diagnosis, treatment and cure of a disease is dependent on results obtained from laboratory testing. In hematology lab, an important pre-analytic factor, influencing the diagnostic result, is the (transportation or/and storage) time lapse between the collection of blood in anticoagulants and the actual analysis/testing [6]. EDTA salt, the most preferred anticoagulant in hematology lab for automated cell counting is known to cause irreversible structural and functional damage of RBCs and WBCs [7].

During peripheral smear examination, morphological changes in RBCs such as loss of central pallor can be wrongly inferred as hyperchromic RBCs whereas crenation of RBCs can be mistaken for other RBC abnormalities like the burr cells excessively seen in uremia [8]. The osmotic fragility curve is known to produce a 'shift to right' effect in stockpiled blood, due to storage dependent membrane instability [9]. Even upon refrigeration (at 4 to 8°C) of the anti-coagulated blood samples, severe noticeable changes in morphology and osmotic fragility curve was evident upon prolonged storage (4th day onwards). The cell membrane of RBCs permit water uptake on prolonged exposure to solutions resulting in swelling which explains the reason behind the 'shift to right' in osmotic fragility curves [10]. Other RBC changes such as bite cells, acanthocytes, spherocytosis, echinocytosis and spheroechinocytosis in peripheral smears can be attributed to the alterations happening in the RBC membrane due to prolonged EDTA exposure [1].

The WBCs in the blood consists of neutrophils, lymphocytes, eosinophils, basophils and monocytes. The cell membranes of RBCs are more resistant to rupture or fragmentation than cell membranes of WBCs. In our study, multi-lobed nucleus details and cytoplasmic vacuolations were examined as indicators of morphological degeneration in the WBC population. Monocytes and lymphocytes are known to show lobulation in nucleus during prolonged storage with EDTA at room temperature [8]. Neutrophils also showed ragged cell membrane with vacuolated cytoplasm with increased storage in EDTA [11]. These type of morphological changes in neutrophils is usually associated with inflammation or sepsis while the lobulation in lymphocytes may be misinterpreted for cellular benign or malignant changes [6]. Abnormal clumping of chromatin, fragmentation, degranulation or Pelguer-Huet forms are some of the WBC abnormalities observed with increase in storage time. Samples stored at room temperature for more than 24 hours is almost unacceptable for morphological interpretation of WBCs. Furthermore refrigeration of blood samples is found to be essential for morphological interpretations if smear preparation is not done within 8 hours of collection [12].

Temperature is a major factor that determines the degeneration of cells as refrigeration is known to diminish the degeneration of WBCs and RBCs [3]. Furthermore, studies conducted in countries of temperate and moderate climates showed preservation of hematological and platelet parameters up to 2 days. Whereas, in countries with hot tropical climates, degenerative changes in morphology of RBCs, WBCs and platelets were observed as early as 2 hours in unrefrigerated samples while refrigerated samples showed evidence of cellular degeneration from 6 hours onwards [13]. However as per the guidelines of International Society for Laboratory, the cells starts to degenerate within 30 minutes of sample collection and quality of smears prepared with EDTA anti-coagualted whole blood

cannot be assured after 6 hours even if the samples were stored in the refrigerator at 4C [14]. As reported by multiple studies, wide variability in parameters like RBC count, WBC count platelet count, MCV, hematocrit and hemoglobin concentration after 24 - 72 hours of storage time at different temperatures can also be accredite to the inconsistency in the cellular deterioration pattern of bloo cells evident in smears after 2 hours of blood collection [3]. Th variability reported in different storage studies of blood conducted in different parts of the world is due to the various confoundin factors such as the concentration and type of anticoagulant, mi ing of anticoagulant, analytic methods used and individual factor like the presence of antibodies and complements in the blood [3] Pre-analytic variables are responsible for the majority of lab erro (about 70%) when compared to analytic and post-analytic facto and inappropriate storage condition can be crucial for obtainir reliable high quality results in hematology laboratory.

A study of systematic review and meta-analysis have confirme that storage of blood resulted in significant time and temperatu dependent changes in routine laboratory tests that estimates num- ber size, morphology and related indices of blood such as hem gram or CBC in hematology. However basic parameters like RB count, WBC count, platelet count, Hemoglobin, MCHC, MCH repor ed acceptable stability after 24 hour. Some measures such as WB count remained almost unaltered even up to 72 hours if refrige ated [15]. However in our study, the WBC differential and morpho ogy of the cells such as RBCs and WBCs were changing over time and cellular morphologic alteration happened after 1 hour of co lection in case of WBCs and 2 hours in case of RBCs for unrefrige ated blood samples as confirmed by a previously conducted stud [16].

Conclusion

EDTA induced morphological changes in RBCs and WBCs can ensue after prolonged storage of unrefrigerated blood sample Differential count and morphological interpretation of RBCs an WBCs should preferably be done within 2 and 1 hour respective for accurate understanding of the underlying pathology and avoi ing misdiagnosis.

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Conflict of Interest

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