Histological and Ultrastructural Changes of White Blood Cells in *Vitro* Storage of Human Blood at Different Time Intervals

Original Article

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ABSTRACT

Introduction: Determination of the changes of blood samples is an important subject. The time passing from changing of normal morphology of WBCs to the un-identification period is useful in the determination of the age of in *vitro* storage blood which in turn helps in determination of post-mortem intervals.

Materials and Methods: This study was done to demonstrate the light microscopic and ultrastructural changes occurring in white blood cells (WBC in *vitro* samples at different time intervals. Light microscopic examination of blood films using Hematoxylin and Eosin (Hx & E), semithin sections stained with toluidine blue, and electron microscopic examinations were done at intervals of (0, 6, 12, 24, 48, and 60 hours) for in *vitro* blood samples left at room temperature.

Results: Concerning the morphological changes in different WBCs, it was observed that neutrophils had normal structure at zero- and 6-hours post-storage. Degeneration was observed at 12-hours and progressed at 24-, 48-, and 60-hours. Degenerative changes include pyknosis, and irregularity in the nuclear shape, vacuolation, pale-stained, and decreased amount of cytoplasm. At 60 hours most of cells are completely degenerated and cannot be discriminated from other WBCs. Ultrastructurally, it was noticed that the degenerative changes were observed in neutrophils after 12-hours post storage. They progressed gradually at 24-and 48-hours till they became completely unidentifiable at 60hours. Eosinophils showed degenerative changes at 12-, 24-, and 48-hours then they became completely degenerated and un-recognized after 60-hours. In addition, monocytes began to show degenerative morphological changes at 12-, 24-, 48-, and at 60-hours most of them completely degenerated. It was observed that lymphocytes were the most resistant cells as they were the only cells that could be detected at 60-hours by semithin sections.

Conclusion: It was concluded that the morphological changes of WBCs of in *vitro* blood samples could be an indicator of the age of the blood.

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Key Words: Cellular changes, post storage intervals, ultrastructure, WBCs.

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INTRODUCTION

Blood is a fluid that normally consists of 54% plasma, 45% erythrocytes, and 1% leukocytes and platelets. White blood cells (WBCs) consist of neutrophils (60-70%), lymphocytes (20-25%), monocytes (5%), eosinophils (2-4%) and basophils (0.5%)[1]. Blood is a specialised connective tissue (CT), and it has the advantage of being unaffected by factors such as gender, diet, stress, age, and diurnal cycles[2].

Previous studies used different methods for the determination of the blood age, such as changes in enzyme activities, the surface absorption spectrum of bloodstains, physiological techniques, and morphological changes of different blood cells[3]. Blood pH ranges from 7.35 to 7.45, and acid-base buffers such as bicarbonate ion and carbonic acid regulate blood pH through the respiratory system and the kidneys[4].

When blood is stored at the blood banks, Physical properties and biochemistry of blood cells are changed because of storage conditions. Under normal conditions in the body's circulation, these changes do not occur as pH, optimum temperature, nutrient concentration, and waste products removal are maintained[5].

Numerous cells in blood show varying degrees of post-storage changes and these occurring cellular changes could help in forensic field as an indicator to the post-mortem intervals[3].

This study aimed at displaying in *vitro* histological and ultrastructural changes of WBCs that occur within a period from (0-60 hours), as indicator for the age of the stored blood sample.

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MATERIALS AND METHODS

Sample Collection

Ten millilitres (10 ml) of blood were withdrawn from the cubital vein of 20 healthy human volunteers after their consent using a wide bore plastic syringe. Samples were put immediately in glass tubes containing 0.2 ml of 10% ethylene diamine tetra-acetic acid (EDTA) solution as an anticoagulant[6]. Exclusion criteria included known blood diseases or general diseases (hypertension, diabetes, or infection) and history of previous blood transfusion. The experiment took place in March with temperature varying from 26 at the day to 12 at night.

Histological Examination

Samples were left at room temperature. At times of (0, 6, 12, 24, 48, and 60 hours), a drop of blood from each sample was spread on a glass slide and left to dry then stained with Hematoxylin and Eosin (H&E) for light microscope (LM) examination. The peripheral blood smears were examined under a light microscope in an area of 1 cm x 2 cm which was presumed to contain approximately 100 cells according to Bancroft and Gamble (2008)[7]. The microscopic changes in WBCs (neutrophils; lymphocytes, monocytes, and eosinophils) had been recorded in the Histology Department, Faculty of Medicine, Assiut University.

Electron Microscope (EM) Examination

1.0 ml of peripheral blood was centrifuged, and the leukocyte buffy coat was fixed in a mixture of 5% glutaraldehyde and 4% paraformaldehyde in sodium cacodylate buffer 0.1 M, pH 7.2, post-fixed in 1% osmium tetroxide, contrasted in 1% solution of uranyl acetate, dehydrated in ethanol, and embedded in epoxy resin. Semithin sections (0.5-1 µm) were stained with toluidine blue[7]. Ultrathin sections (500-800Ao), for the selected areas in semithin sections, were contrasted with uranyl acetate and lead citrate[8], examined with the transmission electron microscope (TEM) JEOL (JEM-100 CXII, Tokyo, Japan) and photographed at 80 kV in Assiut University-Electron Microscope Unit.

Evaluation of pH Level

1ml of the blood sample was taken at different time intervals (0, 6, 12, 24, 48 and 60 hours), put into a clean tube and centrifuged at 2500 round per minute (rpm) for 15 minutes. pH meter (Analyticon Biotechnologies AGD-35104 Lichtenfeis, made in Germany) was used to measure plasma pH level. It was calibrated each time of use with pH 4.0, 7.0, and 10.0 calibrators.

Ethical Considerations

This research was approved by The Research Ethics Committee of the Faculty of Medicine-Assiut University (Ref code: 17300062). Informed consent was taken from all individuals who participated in the study. Confidentiality of the data was guaranteed.

Statistical Analysis

Statistical analysis was performed using the IBM Statistical Package for Social Sciences (SPSS) program version 20.0 software. The results are expressed as the mean ± SD. One way ANOVA test was used to determine whether there were any statistically significant differences between the means of pH levels at different time intervals. Values of $P < 0.05$ were considered statistically significant.

RESULTS

Histological results

Light microscopical examination of neutrophils stained with Hx & E showed that at zero and 6 hours, neutrophils had a normal structure with multilobed nuclei and acidophilic cytoplasm (Figures 1,2). After 12 & 24 hours the nuclei of neutrophils showed different forms of degeneration (pyknosis, and distorted shapes). cytoplasm also became pale-stained, scanty with irregular cell outline (Figures 3,5). At 48 hours the cells appeared with pyknotic or bilobed nuclei and scanty pale cytoplasm (Figure 6). While at 60 hours they revealed dense unlobed nucleus and pale scanty vacuolated cytoplasm (Figure 7). In addition to that, semithin sections stained with toluidine blue revealed that at zero, 6, 12, and 24 hours, neutrophils appeared with multilobed nuclei attached to chromatin threads, with no obvious changes being detected (Figures 8-11). Whereas, at 48 hours pyknosis of the nuclei became apparent, band-shaped and eccentric (Figure 12). Moreover, at 60 hours, all WBCs were degenerated and could not be discriminated from each other's (Figure 12).

Eosinophils stained with Hx & E at zero & 6 hours, showed normal structure with bilobed nuclei, and cytoplasm studded with acidophilic granules (Figures 1,2). At 12 hours, they showed nuclear condensation (Figure 4) and after 24 hours, the nuclei degenerated but the acidophilic granules were still present (Figure 5). In addition to that, at 48 hours, the nuclei degenerated and fragmented (Figure 6). While, at 60 hours, acidophilic granules extruded outside the cell (Figure 7). Semithin sections of eosinophils at zero, 6 & 12 hours, appeared normal with bilobed nuclei (Figures 8-10). while, at 24 hours, the nuclei became pyknotic (Figure 11) and after 48 hours, the nuclei degenerated, and the eosinophils had irregular outlines (Figure 12). The cells were completely degenerated and could not be identified after 60 hours (Figure 12).

Monocytes at zero & 6 hours stained with Hx & E revealed a kidney-shaped nucleus surrounded by pale basophilic cytoplasm (Figures 1,2). While, at 12, 24 & 48 hours the nuclei appeared to degenerate, the cytoplasm became vacuolated, and the cell's borders became indistinct (Figures 4-6). Moreover, at 60 hours more signs of degeneration with an irregular indistinct outline and faint basophilic cytoplasm appeared (Figure 7). Semithin sections at zero & 6 hours showed monocytes in normal structure with kidney-shaped nuclei (Figures 8,9). At 12 hours, the nuclei turned irregularly shaped (Figure

10), and at 24 hours the cell outline became irregular (Figure 11). Then, at 48 hours the nuclei became small-sized and rounded (Figure 12). Finally, no monocytes could be identified at 60 hours (Figure 12).

Lymphocytes stained with Hx & E at zero, 6 & 12 hours did not show any morphological changes. Small lymphocytes appeared in a round nucleus surrounded by a thin rim of basophilic cytoplasm (Figures 1,2,4) while, large lymphocytes had a large, indented nucleus surrounded by abundant basophilic cytoplasm (Figures 1,2,4). At 24, 48 & 60 hours, the nuclei became irregular in shape and the cell outline turned indistinct (Figures 5-7). Semithin sections with toluidine blue at zero & 6 hours, revealed that the small lymphocytes had a normal morphological appearance (Figures 8,9). After 12, 24 & 48 hours, the nuclei of some cells became irregular in shape (Figures 10-12). While at 60 hours, lymphocytes were the only cells that could be recognized in their large round nucleus (Figure 12).

Electron Microscopy (E.M)

When we examine the ultrastructure of the blood W.B.C, we cannot identify all types of cells at all time intervals, because of their low percentage in blood.

At zero and 6 hours, neutrophil contained a bilobed nucleus with peripheral clumps of heterochromatin surrounded by electron-lucent cytoplasm which contained variable-sized granules (Figure 13). At 6, 12 & 48 hours, the granules became inapparent (Figures 14,15,17).

Eosinophil appeared at 12 hours with a heterochromatic, oval nucleus and the surrounding cytoplasm contained many granules of varying morphology and electron-density (Figure 15). Moreover, the monocyte appeared at 24 hours with an irregularly shaped fragmented nucleus, and its cytoplasm contained large numbers of mitochondria (Figure 16).

Lymphocytes, at zero, 6 & 12 hours appeared with irregular, large, heterochromatic nuclei (Figures 13-15). At 24 hours, some mitochondria were observed in the cytoplasm (Figure 16). At 48 & 60 hours the nuclei were highly folded, heterochromatic and the outline were highly irregular (Figures 17,18).

pH level estimation

Plasma pH levels were measured at different time intervals as shown in (Figure 18). There was significant difference (p<0.05) between pH level at zero time (7.273±0.021) and pH level at 6 hours (7±0.057). Again, there was significant difference (p<0.01) between pH at zero time and pH at 12 hours (6.9±1). Moreover, there was highly significant difference (p<0.001) between pH level at zero hours and its level at 24 hours (6.85±0.03), at 48 hours (6.35±0.02) and at 60 hours (6.167±0.03).

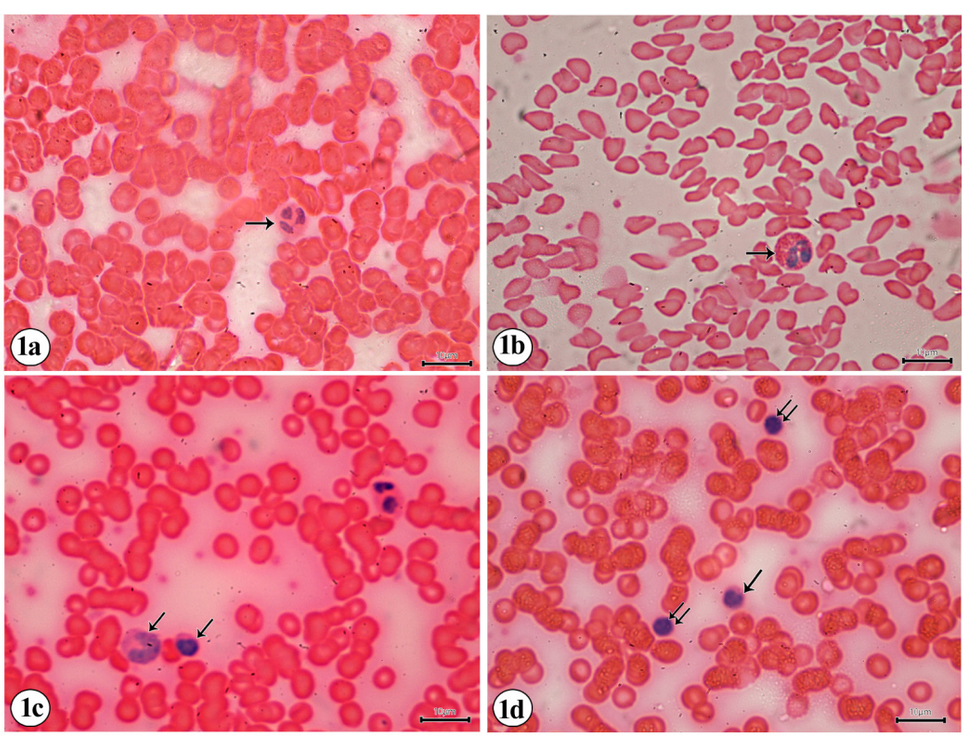

Fig. 1: A photomicrograph of peripheral blood films stained with H & E shows a normal morphological appearance of white blood cells at 0 hours post-storage **(a)** Neutrophil (↑) appear with three or four lobes of nuclear material joined by thinner nuclear strands **(b)** Eosinophil (↑) with a bilobed nucleus and their cytoplasm filled with numerous coarse acidophilic granules of uniform size. Observe the connecting chromatin threads between the two lobes of the nucleus. **(c)** The nucleus of the monocyte (↑) appears deeply indented. It appears lighter in staining than that of lymphocytes. The cytoplasm of monocytes is abundant and stains pale blue. **(d)** large lymphocyte (↑) with a slightly indented dense nucleus and two small lymphocytes (↑↑) with round dense nuclei surrounded by a thin rim of cytoplasm. (X 1000).

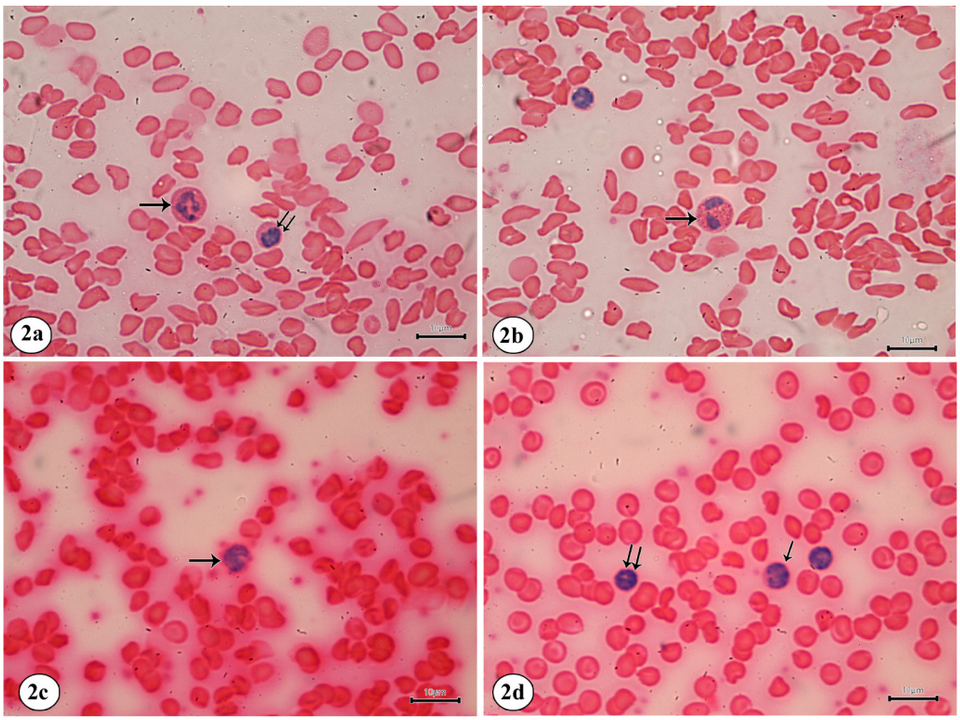

Fig. 2: A photomicrograph of peripheral blood films stained with H & E shows morphological changes of white blood cells at 6 hours post-storage **(a)** Neutrophil (↑) appears with a multilobed nucleus and large lymphocyte **(↑↑) (b)** Eosinophil (↑) with bilobed nucleus **(c)** Monocyte (↑) with kidney-shaped nucleus **(d)** large (↑) and small lymphocytes (↑↑) with normal shaped nuclei. (X 1000).

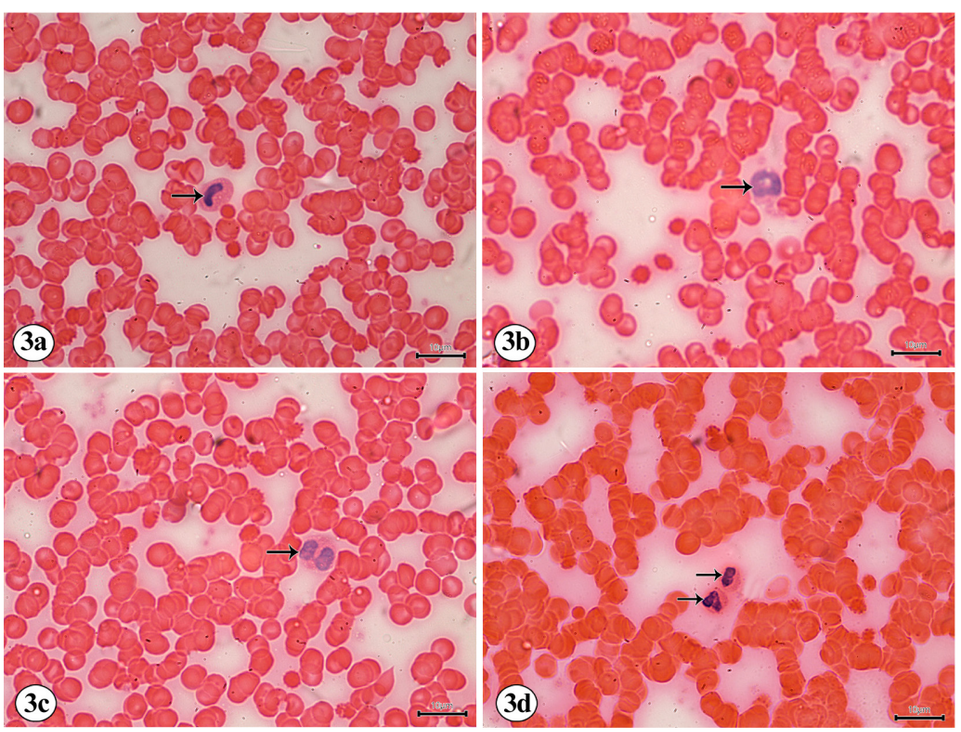

Fig. 3: A photomicrograph of peripheral blood films stained with H & E shows morphological changes of neutrophils at 12 hours post-storage **(a)** Band shape nucleus (↑). **(b)** Large ring shape nucleus (↑) lacking recognizable cytoplasm **(c)** Nucleus forms of two lobes (↑) connect by a distinct thin filament (glasses shaped nucleus) **(d)** Hypo lobulated nucleus (↑) with scanty cytoplasm and indistinct cytoplasmic border. (X 1000).

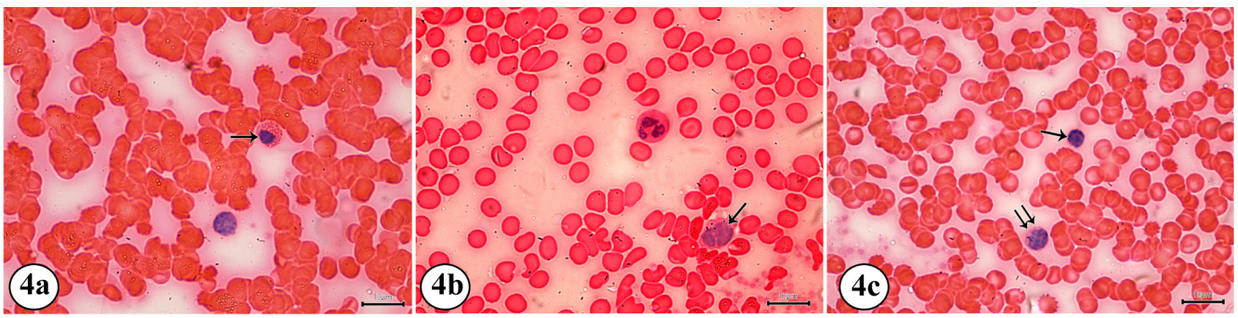

Fig. 4: A photomicrograph of peripheral blood films stained with H & E shows morphological changes of white blood cells at 12 hours post-storage **(a)** Eosinophil (↑) shows shrinkage with nuclear condensation and their cytoplasm appears filled with numerous acidophilic granules of uniform size. **(b)** Monocyte (↑) appears with indented shape nucleus with irregular indistinct borders and pale cytoplasm **(c)** small (↑) and large Lymphocytes (↑↑) appear normal without morphological changes. (X 1000).

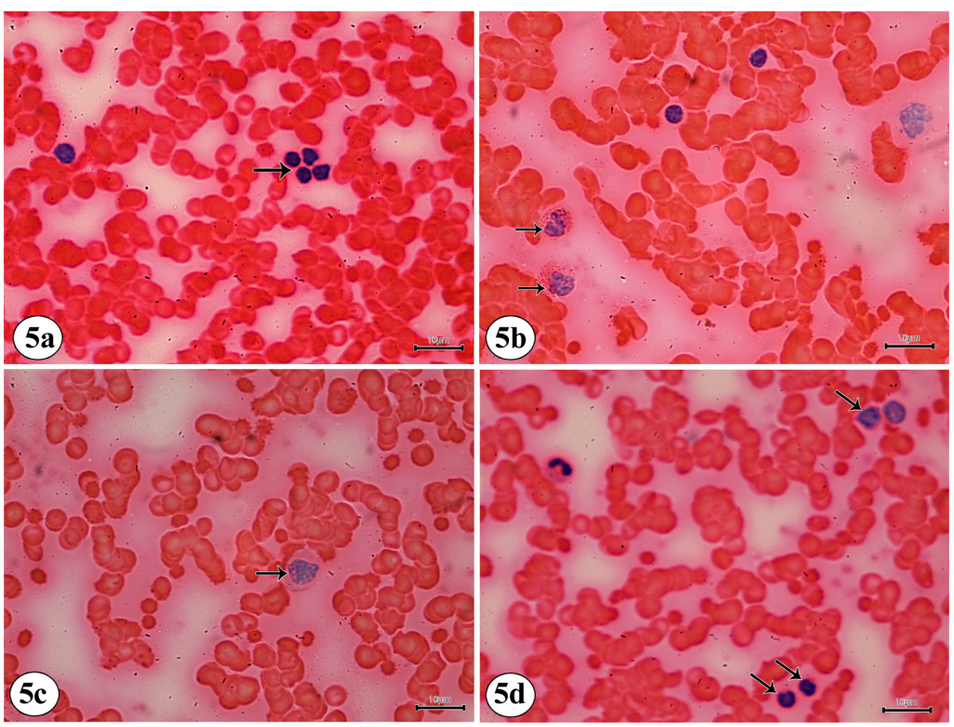

Fig. 5: A photomicrograph of peripheral blood films stained with H & E shows morphological changes of white blood cells at 24 hours post-storage **(a)** neutrophil (↑) with Large unconnected dark nuclear lobes with minimal amount of cytoplasm **(b)** Eosinophil (↑) with a degenerated nucleus and its cytoplasm filled with numerous acidophilic granules **(c)** Monocyte (↑) with nuclear degeneration and indistinct cell border **(d)** Lymphocytes (↑) with an irregular shaped nucleus and another with an oval-shaped nucleus. (X 1000).

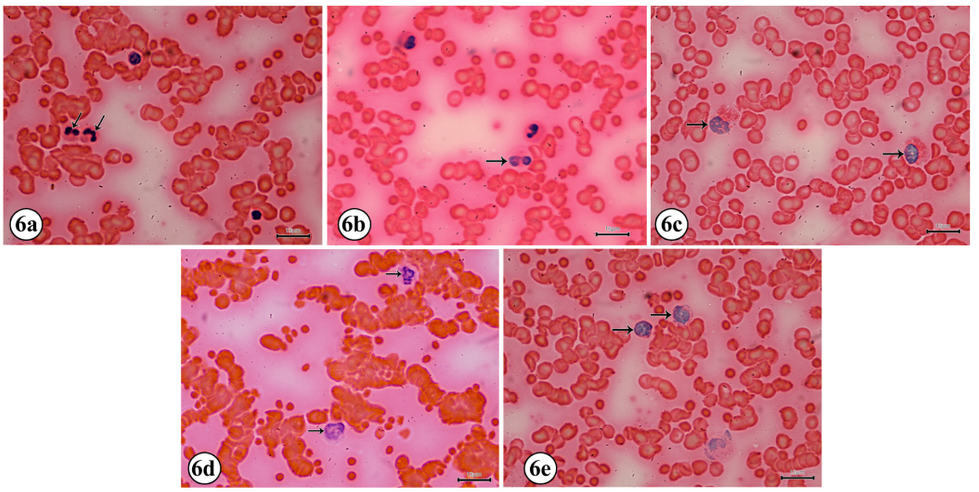

Fig. 6: A photomicrograph of peripheral blood films stained with H & E shows morphological changes of white blood cells at 48 hours post-storage **(a)** Neutrophil (↑) appears with irregular pyknotic nucleus **(b)** Neutrophil (↑) appears with a bilobed nucleus, indistinct cell border and scanty acidophilic cytoplasm **(c)** Eosinophil (↑) with nuclear degeneration and its nucleus is about to be extruded, and another eosinophil with a degenerated fragmented nucleus. **(d)** Monocytes (↑) with nuclear degeneration and irregular outline **(e)** lymphocytes (↑) with an irregular nucleus and indistinct cell border. (X 1000).

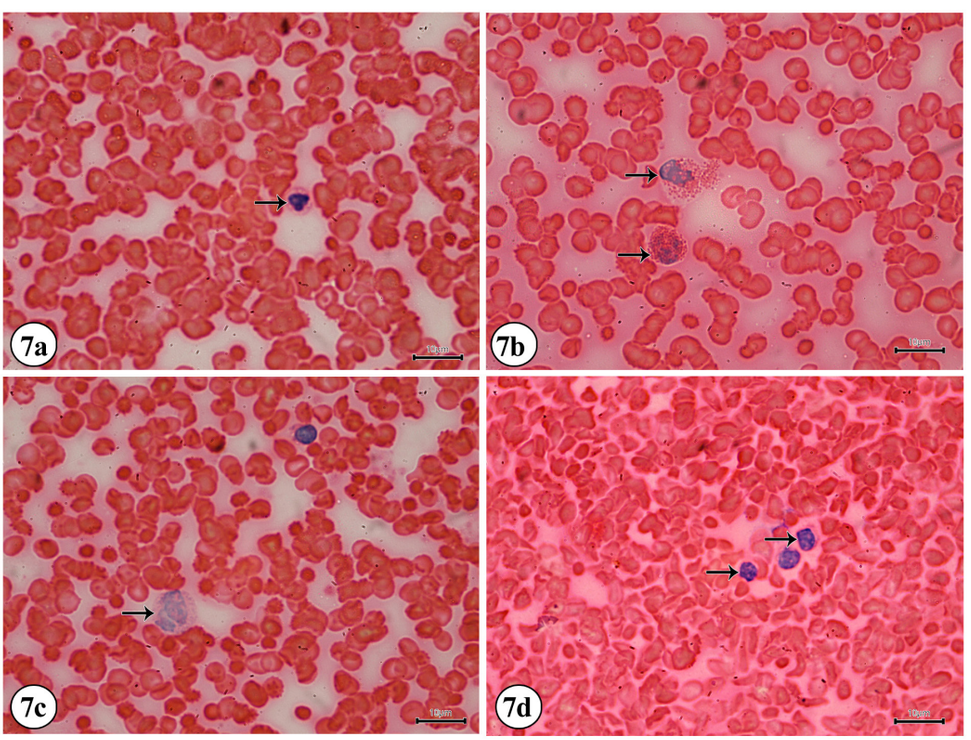

Fig. 7: A photomicrograph of peripheral blood films stained with H & E shows morphological changes of white blood cells at 60 hours post-storage **(a)** Neutrophil (↑) with a dark lobulated irregular nucleus and faint vacuolated cytoplasm. **(b)** Eosinophils (↑) appear with degenerated fragmented nuclei and acidophilic granules extruded outside the cell. **(c)** Monocyte (↑) appears with an irregular degenerated nucleus, irregular indistinct outline, and faint basophilic cytoplasm. **(d)** lymphocytes (↑) with irregular degenerated nucleus and indistinct cell border. (X 1000).

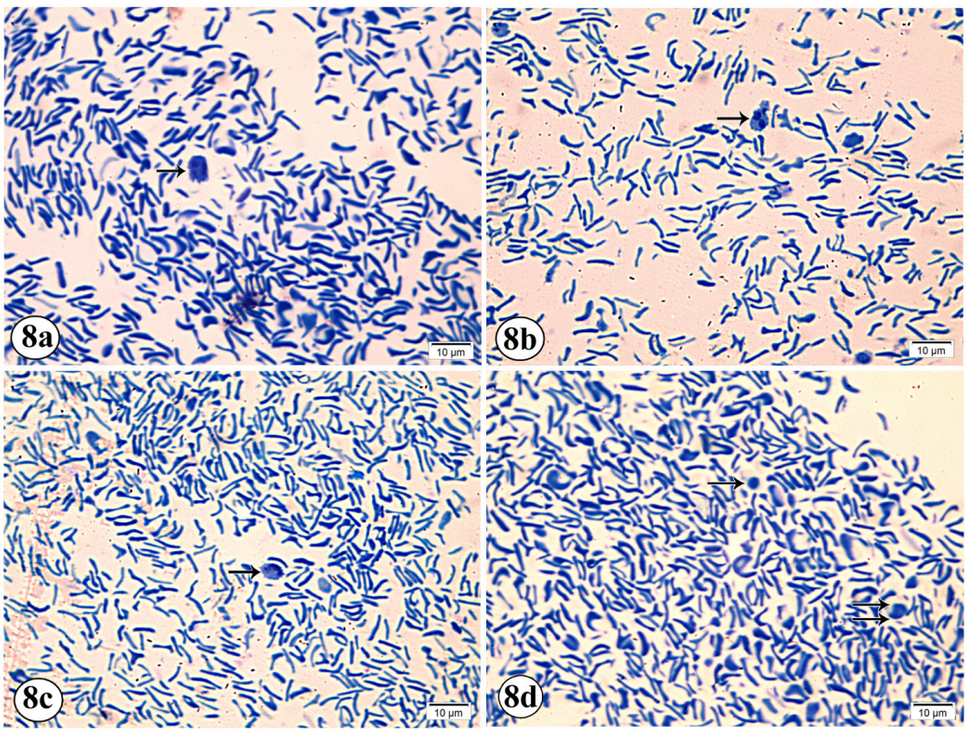

Fig. 8: Semithin sections of peripheral blood films show a normal morphological appearance of white blood cells at 0 hours post-storage **(a)** Neutrophil (↑) appears with multilobed nucleus **(b)** Eosinophil (↑) appears with bilobed nucleus **(c)** Monocyte (↑) appears with kidney-shaped nucleus **(d)** Small lymphocyte (↑) appears with a spherical shaped nucleus and large lymphocyte (↑↑) appears with large, indented nucleus. (X 1000).

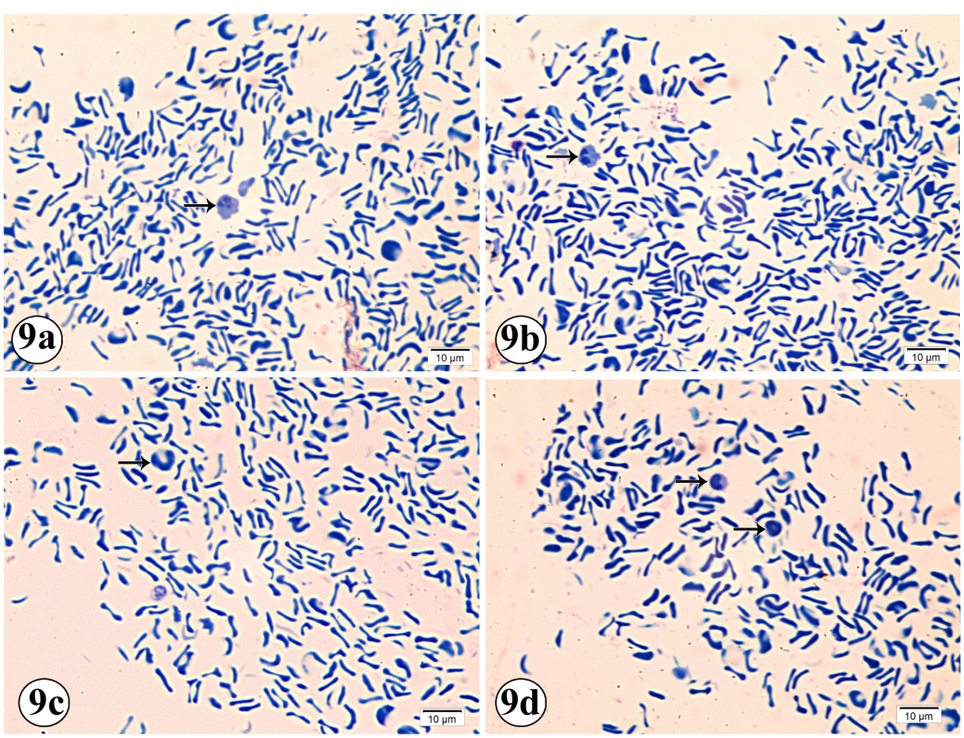

Fig. 9: Semithin sections of peripheral blood films stained with toluidine blue show a normal morphological appearance of white blood cells at 6 hours post-storage **(a)** Neutrophil (↑) appears with multilobed nucleus **(b)** Eosinophil (↑) appears with bilobed nucleus **(c)** Monocyte (↑) appears with kidney-shaped nucleus **(d)** Small lymphocytes (↑) appear with indented shaped or circular dense nuclei. (X 1000).

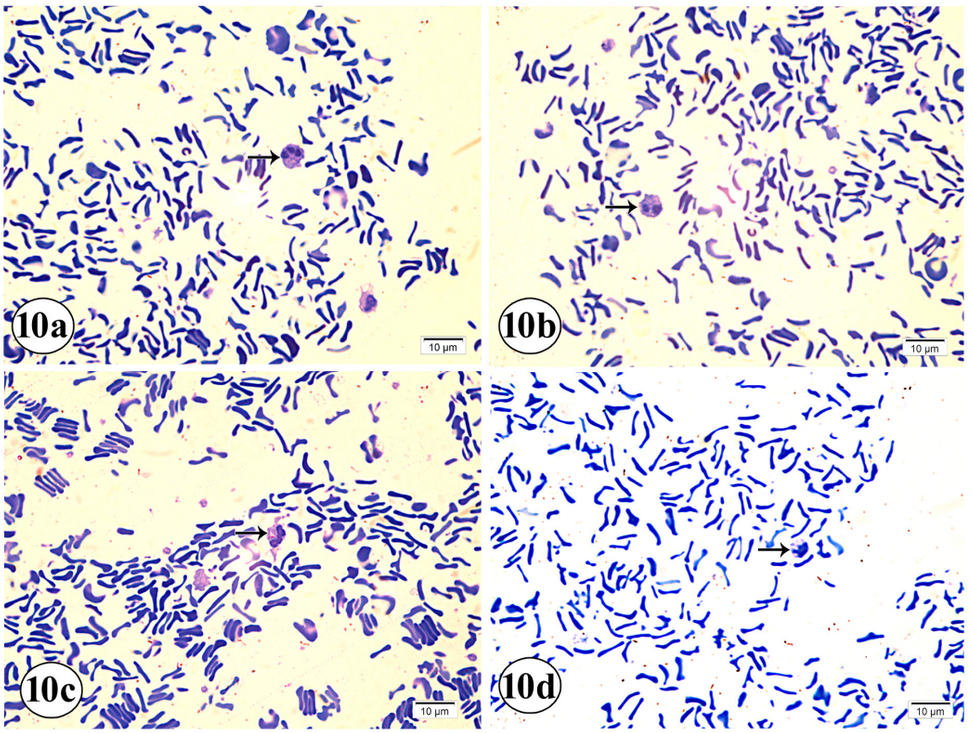

Fig. 10: Semithin sections of peripheral blood films stained with toluidine blue show morphological changes of white blood cells at 12 hours post-storage **(a)** Neutrophil (↑) with multilobed nucleus **(b)** Eosinophil (↑) with bilobed nucleus **(c)** Monocyte (↑) with irregularly shaped nucleus **(d)** Small lymphocyte (↑) with an irregular small dense nucleus. (X 1000).

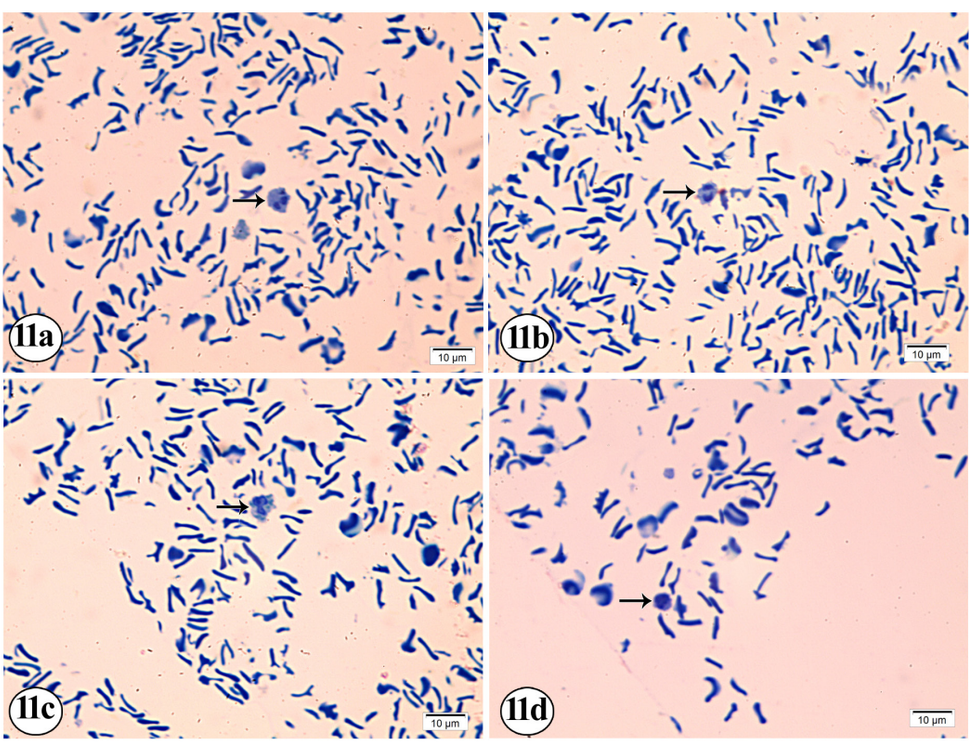

Fig. 11: Semithin sections of peripheral blood films stained with toluidine blue show morphological changes of white blood cells at 24 hours post-storage **(a)** Neutrophil (↑) with irregular shape pyknotic nucleus and scanty cytoplasm **(b)** Eosinophil (↑) with eccentric pyknotic nucleus **(c)** Monocytes with a degenerated nucleus and irregular outline **(d)** Lymphocyte (↑) with irregular shape nucleus. (X 1000).

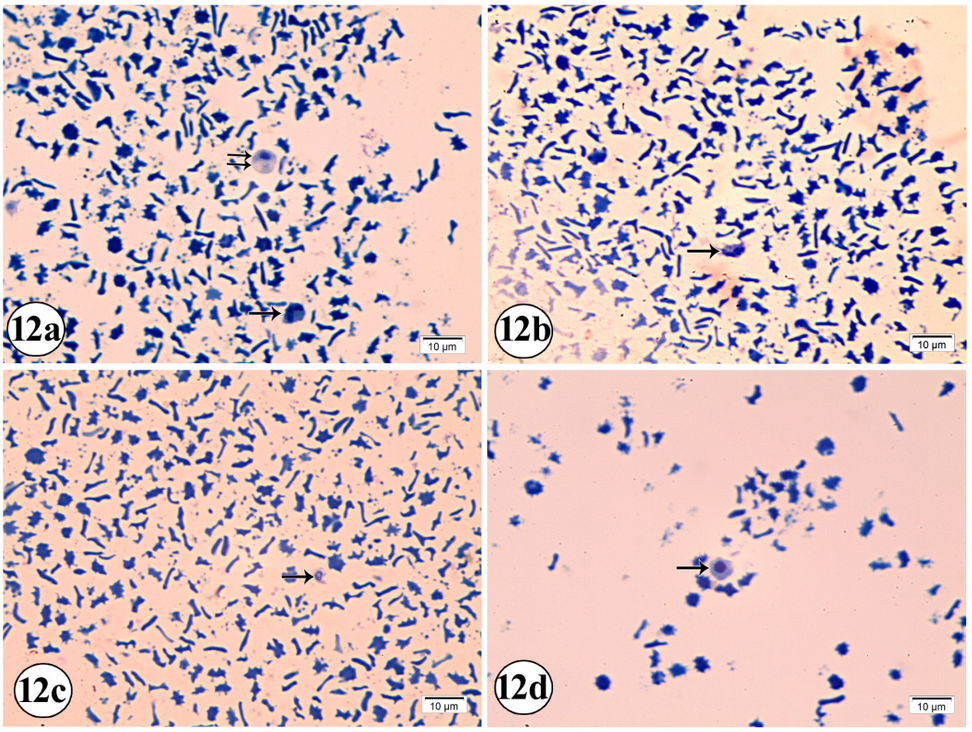

Fig. 12: Semithin sections of peripheral blood films stained with toluidine blue show morphological changes of white blood cells at 48 hours post-storage **(a)** Neutrophil (↑) with eccentric band-shaped nucleus and monocytes appear (↑↑) with degenerated small eccentric round nucleus **(b)** Eosinophil (↑) with a degenerated nucleus and irregular outline **(c)** Lymphocyte (↑) with a degenerated nucleus and irregular outline **(d)** At 60 hours: all WBCs are completely degenerated and cannot be identified except lymphocyte (↑) with a round central nucleus. (X 1000).

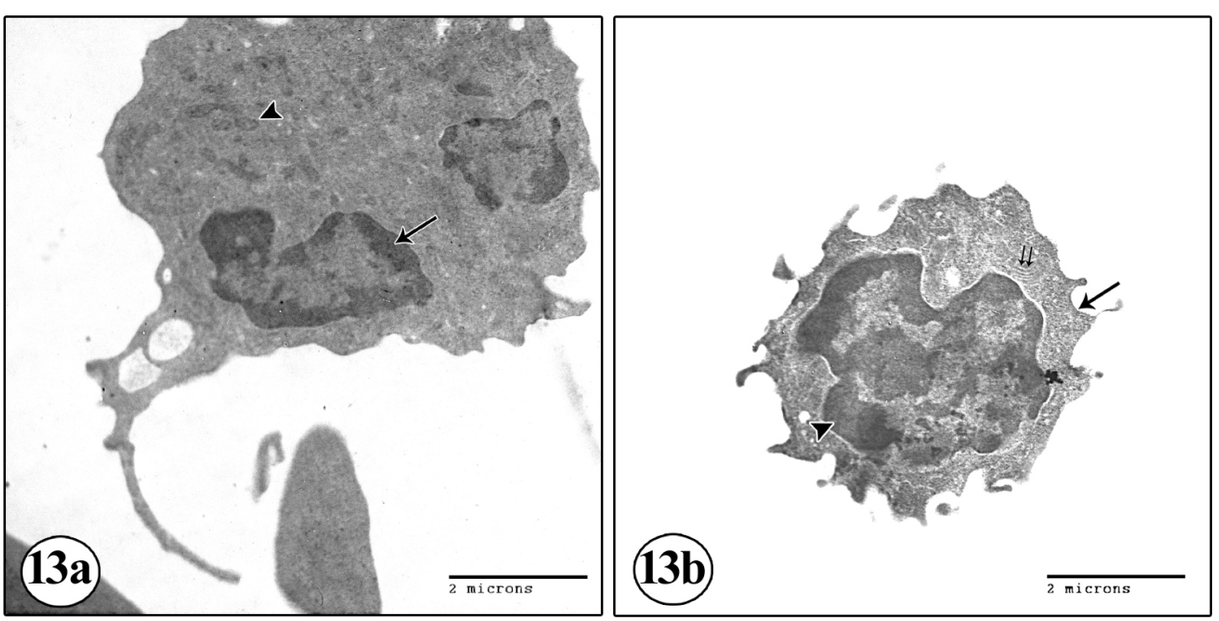

Fig. 13: Electro photomicrograph of **(a)** Neutrophil at 0 times shows Two nuclear lobes with clumps of electron-dense clumps of heterochromatin at the nuclear periphery (↑). Most of the cytoplasm is occupied by many granules in variable sizes (arrow head) **(b)** Lymphocytes at 0 time shows a nucleus with irregular outline (↑) and heterochromatin condensation mainly (arrow head) along the nuclear membrane. A Golgi complex with elongated tubules can be observed (↑↑). (X 10000).

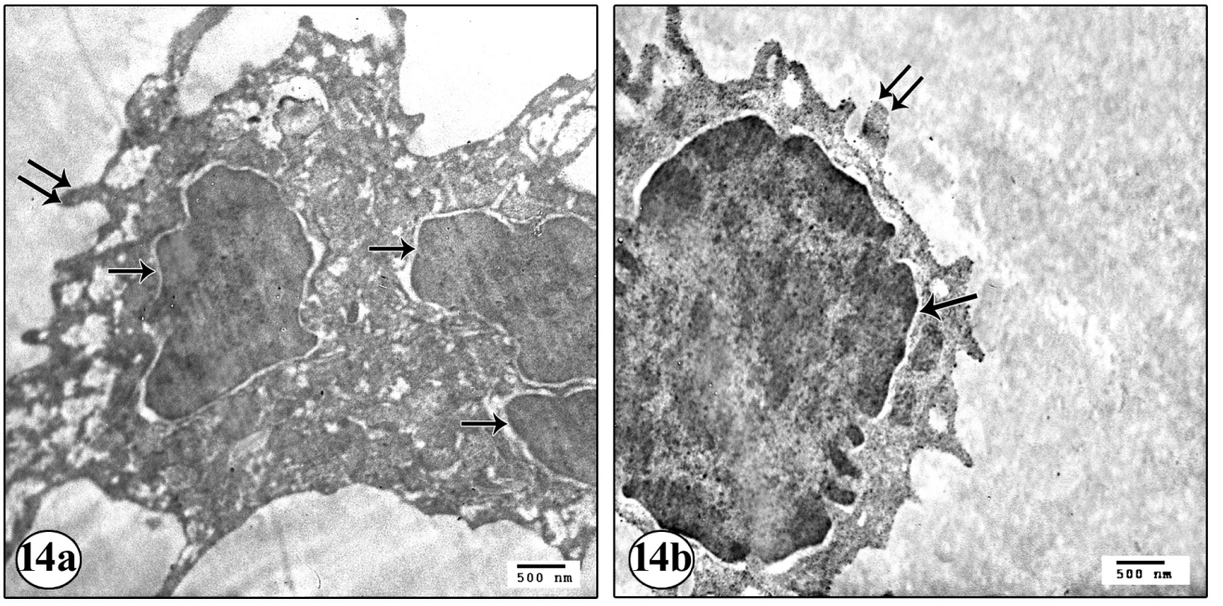

Fig. 14: Electro photomicrograph of white blood cells at 6 hours post-storage shows **(a)** Neutrophil with three nuclear lobes (↑) and irregular outline (↑↑), while the granules are inapparent **(b)** Lymphocyte with heterochromatic nucleus (↑) and irregular outline (↑↑). (X 14000).

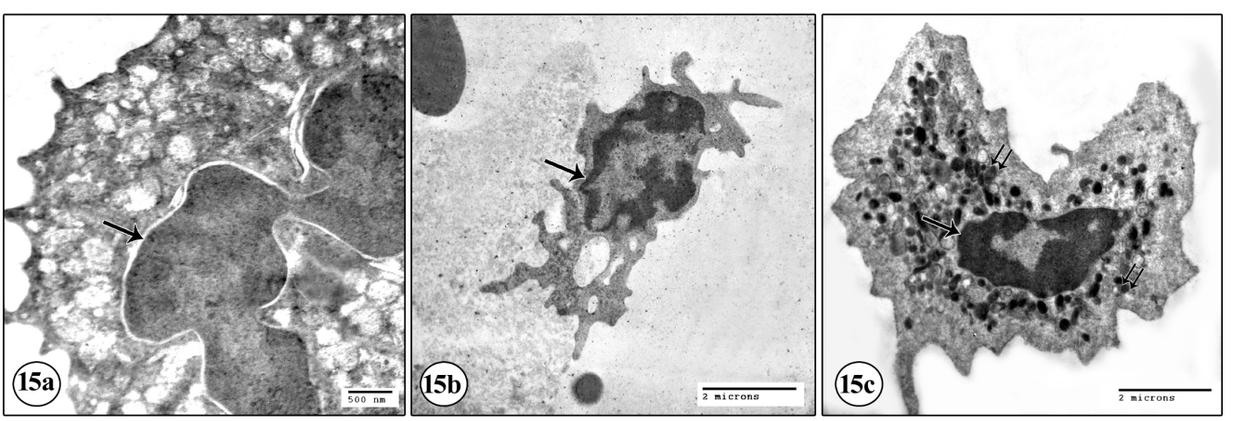

Fig. 15: Electro photomicrograph of white blood cells at 12 hours post-storage shows **(a)** Neutrophil with segmented shape nucleus (↑) with condensed heterochromatin. (x19000) **(b)** lymphocyte with irregular shape nucleus (↑) and irregular outline. (x10000) **(c)** Eosinophil with oval eccentric nucleus (↑) with condensed heterochromatin. In the cytoplasm, many granules (↑↑) of varying morphology and electron-density with a crystalloid structure in their interior. (X 10000).

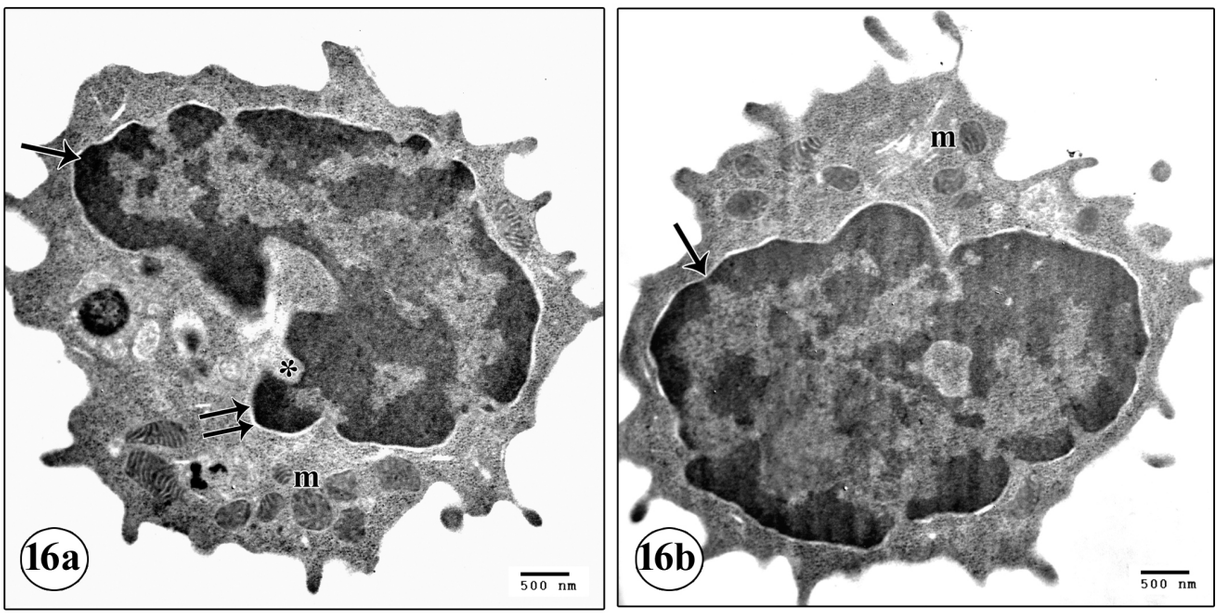

Fig. 16: Electro photomicrograph of white blood cells at 24 hours post-storage shows **(a)** Monocyte with irregular shape nucleus (↑) and irregular outline, its cytoplasm contains large numbers of mitochondria (m). Notice part of the nucleus is fragmented (↑↑) and attached by a narrow stalk (*) to the main nucleus. **(b)** Lymphocyte with irregular heterochromatic nucleus (↑) and the cytoplasm contains some mitochondria (m). (X 14000).

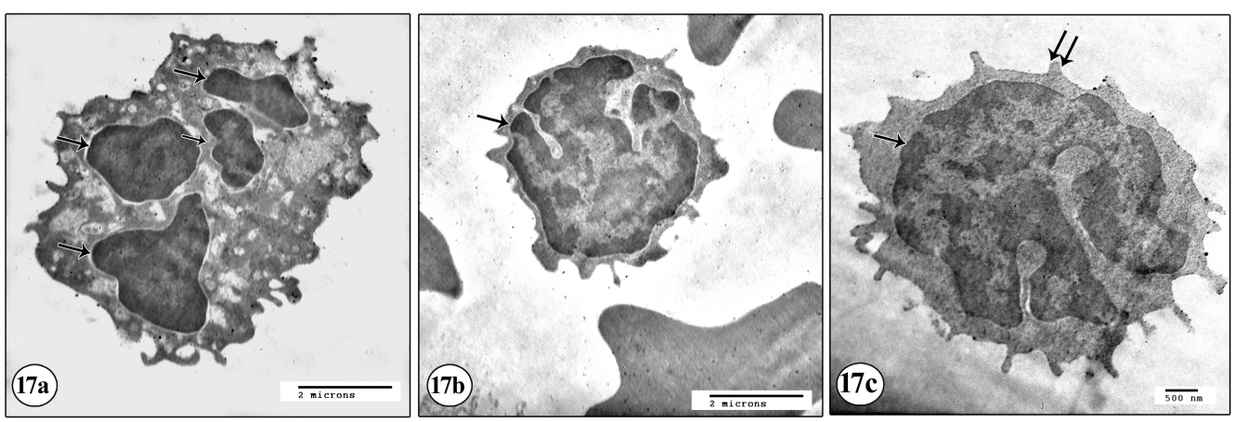

Fig. 17: Electro photomicrograph of white blood cells at 48 hours post-storage shows **(a)** Neutrophil with multilobed heterochromatic nucleus (↑). (x14000) **(b)** Lymphocytes with irregular folded heterochromatic nucleus (↑). (x10000) **(c)** At 60 hours: Lymphocyte with irregular folded heterochromatic nucleus (↑) and irregular outline (↑↑). (X 14000).

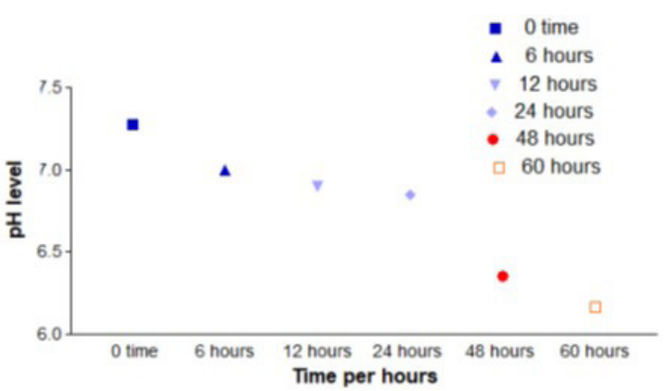

Fig. 18: Plasma pH levels at different time intervals.

DISCUSSION

In this study, the histological and ultrastructural changes of different white blood cells that occur with time interval from (0-60 hours), as indicators for the age of in *vitro*-storage blood sample, were examined by light microscope (LM) stained with Hx & E, semithin sections stained with toluidine blue, and the ultrastructure of WBCs were studied using a transmission electron microscope (TEM).

We use untreated whole blood to study blood samples in *vitro*. The blood clotting will occur within 30 seconds, so we added anticoagulants to prevent early clotting. No evidence was reported about the effect of anticoagulants on the processes associated with the aging of blood cells[9].

During laboratory storage of blood, leukocytes break down and release several enzymes and chemicals such as proteases and hydrogen peroxide. These proteases released by WBCs during storage have been reported to cause RBC lysis during storage[10]. Degenerative changes occur when blood is allowed to stand in the laboratory before films are made. These changes are not due to the presence of an anticoagulant as also occur in defibrinated blood. Certain changes take place when blood is allowed to stand in *vitro* at room temperature, the red cells start to swell, the leukocytes and platelet counts gradually fall. It is best to count leukocytes and especially platelets within 2 hours[11].

Concerning the morphological changes in different WBCs at different post-storage intervals, it was observed that neutrophils had normal architecture at zero- and 6-hours post-storage as degeneration started at 12-hours and progressed at 24-, 48-, and 60-hours. Degenerative changes include pyknosis, and irregularity in the nuclear shape, vacuolation, pallor, and decreased amount of cytoplasm. At 60 hours most cells were completely degenerated and could not be discriminated from other WBCs. By examination of the ultrastructure of neutrophils by E.M, it was noticed that the neutrophils still had normal ultrastructure with multilobed euchromatic nuclei, and granular cytoplasm till 6-hours. Degenerative changes were observed after 12-hours and progressed gradually at 24-and 48-hours till they became completely unidentifiable at 60-hours. Eosinophils had normal histological structure till 6-hours post-storage after those degenerative changes started to appear at 12-, 24-, and 48-hours then they became completely degenerated and unrecognised after 60-hours. Also, monocytes started to show degenerative morphological changes at 12-, 24-, 48-, and at 60-hours most of them completely degenerated. It was observed that lymphocytes were the most resistant cells as they were the only cells that could be detected at 60-hours by semithin sections. Also, obvious ultrastructural changes in different WBCs at different time intervals could not be detected.

In agreement with our study, Babapulle and Jayasundera found that the WBCs were normal in the first 6 hours, while some became abnormal between 6 and 72 hours, then all cells became morphologically abnormal and could not be identified after 72 hours[12]. Also, Dokgöz *et al*. (2013) found that lymphocytes were still identifiable after 120 h[13]. Also, Penttilä, and Laiho (1981) reported that lymphocytes were more resistant to autolysis than other types of WBCs. They recorded those normal stained lymphocytes were seen in up to 270 hours post-mortem[14].

In contrast with Rajesh Bardale who observed that neutrophils were detected up to 2024 hrs, lymphocytes up to 30 hrs, eosinophils up to 21 hrs, and monocytes up to 18 Hrs after death[6], the current study observed that neutrophils, eosinophils, and monocytes could be detected up to 48-hours while lymphocytes could be detected up to 60-hours post-storage. In other researches, they observed pyknosis after the first 6 h and cytoplasmic and nuclear vacuolation after 12 h in neutrophils, eosinophils, and monocytes. Nuclear fragmentation started after 18 h in neutrophils and eosinophils and after 24 h in monocytes. Disintegration was observed between 48and 96 h in neutrophils, and between 48 and 72 h in eosinophils and monocytes. In lymphocytes, nuclear swelling and indistinctness in the cytoplasmic cell membrane were observed after 24 h, while pyknosis, nuclear fragmentation, and disintegration were observed after 36, 72, and 96 h respectively[13]. Also, Laiho and Penttilä (1981) found that well-preserved monocytes and neutrophils were seen in the peripheral blood after over 200 hours, from cadavers with known post-mortem intervals[15].

The histological changes in WBCs observed in our study after different post-storage intervals can be explained by the metabolic changes that occur in the stored blood. Immediately, sudden fall in the concentration of oxygen occurs due to the absence of circulation, this results in the stoppage of the citric acid cycle and switch to anaerobic metabolism. Accumulation of lactic acid and an increase in NADH concentration resulted from anaerobic glycolysis[16]. Also, they can be explained by the fact that after storage the blood circulation ceases, and thereby cells cannot produce energy in the form of ATP, so the cells die, and the cell membrane permeability changes. Once membrane permeability is compromised, breakdown of cellular homeostasis occurs, and ions flow in and out uncontrollably[17].

Besides, the morphological changes observed on the present study could be explained by the change of the surrounding media of WBCs which was the change of pH of blood from normal to acidic (less than 7) with pass of time. The present study revealed that pH level of the in *vitro* blood sample was significantly decreased with the passing of time.

Post-storage biochemical changes include changes in the blood pH, and concentrations of lactic acid, ammonia, hypoxanthine, formic acid, NADH, and uric acid[16]. In stored blood, the concentration of the ammonia is decreased[18].

Donaldson and Lamont (2013) found that after 96 hours there is a slight decrease in the PH of the human blood stored in a tube from 7.4 to 7.1. They explain this as due to the

absence of glucose stores in *vitro* blood in a tube, so there is no fuel for anaerobic metabolism and no accumulation of lactate, and the PH is not significantly affected. While, in *vivo*, autolysis, and anaerobic metabolism result in the accumulation of metabolites as lactic acid, carbon dioxide, dihydrogen phosphate ions, bicarbonate, and hydrogen ions, so the blood PH is rapidly decreased[16].

CONCLUSIONS

The use of post-storage morphological changes of WBCs by LM in determination of the age of the blood sample is useful and easily applied. All WBCs have normal morphology in the first 6 hours, and the degenerative morphological changes start after 12 hours and progress gradually until they become completely identifiable except the lymphocytes which are the only cells that can be identified after 60 hours. The use of EM for determination of the post-storage time is not recommended, because it is difficult to perform, expensive and only very few or no WBCs can be detected in the sections. Also, EM specimens needs to be repeated several times to detect a specific WBCs, and this is not practical. Determination of the age of blood may help in forensic field in determination of the post-mortem intervals. So, it is recommended that, in blood transfusion it is preferring to use fresh blood or stored up to 6 hours. Further studies should consider to study relations between post-storage and post-mortem changes in all blood components.

CONFLICT OF INTERESTS

There are no conflicts of interest.

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الملخص العربى

التغيرات النسيجية لخلايا الدم البيضاء في التخزين المختبري للدم البشري على فترات زمنية مختلفة

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المقدمة: يعد تحديد التغيرات في عينات الدم موضوعًا مهمًا. الوقت المنقض من تغيير الشكل الطبيعي لكرات الدم البيضاء إلى فترة عدم تحديد الهوية مفيد في تحديد عمر تخزين الدم في المختبر والذي يمكن أن يساعد في تحديد فترات ما بعد الوفاة.

الهدف من البحث: أجريت هذه الدراسة لتوضيح التغيرات النسيجية والتركيبية التي تحدث في خلايا الدم البيضاء في عينات المختبر على فترات زمنية مختلفة باستخدام الفحص المجهري الضوئي والفحص المجهري الإلكتروني.

المواد و الطرق: تم اجراء الفحص المجهري الضوئي باستخدام الهيماتوكسلين و الايوزين، و التوليدين الأزرق و كذلك الفحص المجهري الالكتروني علي فترات (٠، ٦، ١٢، ٢٤ ، ٤٨ ، ٦٠ ساعة) لعينات الدم في المختبر المتروكة في درجة حرارة الغرفة.

النتائج: بالنسبة للتغيرات الميكروسكوبية في كرات الدم البيضاء المختلفة في فترات مختلفة، لاحظنا أن الخلايا متعادلات الاصطباغ كان لها بنية طبيعية عند صفر و٦ ساعات، وبدأت ملاحظة التنكس بعد ١٢ ساعة وازداد بعد ٢٤- ، ٤٨- ، و٦٠ ساعة. شملت التغيرات التنكسية عدم انتظام الشكل النووي، ظهور فجوات، بقع باهتة، وانخفاض في كمية السيتوبلازم. بعد ٦٠ ساعة تتحلل معظم الخلايا تمامًا ولا يمكن تمييز خلايا الدم البيضاء المختلفة عن بعضها. من خلال فحص التركيب الدقيق للخلايا متعادلات الاصطباغ بواسطة المجهر الالكتروني، لاحظنا أن التغييرات التنكسية قد لوحظت بعد ١٢ ساعة وتزداد تدريجيًا بعد ٢٤ و٤٨ ساعة حتى تصبح غير قابلة للتحديد تمامًا بعد مرور ٦٠ ساعة. أظهرت الخلايا حامضيات الاصطباغ تغييرات تنكسية بعد مرور ١٢ و٢٤ و٤٨ ساعة ثم أصبحت متدهورة بشدة وغير مميزة تماما بعد ٦٠ ساعة. أيضًا، بدأت الخلايا الكبيرة في إظهار التغيرات الميكروسكوبية التنكسية بعد ١٢ و٢٤ و٤٨ ساعة وبعد ٦٠ ساعة يتدهور معظمها تمامًا. لاحظنا أن الخلايا الليمفاوية هي الخلايا الأكثر مقاومة لأنها الخلايا الوحيدة التي يمكن اكتشافها بعد ٦٠ ساعة.

الخلاصة: خلصنا إلى أن التغيرات الميكروسكوبية لكرات الدم البيضاء لعينات الدم في المختبر بعد مرور فترات زمنية مختلفة يمكن أن تعطي مؤشرا على تحديد عمر عينه الدم وقد يساعد في تحديد العمر بعد الوفاة.