THE SUITABLE ANTICOAGULANT, TIME AND TEMPERATURE FOR BLOOD SAMPLE COLLECTION

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ABSTRACT

The blood is a connective tissue that composes of suspended cells in liquid matrix. In this study ,we discuss the effect of three factors on blood sampling during the collection of blood samples (anti-coagulants, temperature and duration of storage) . The delay of storage processing led to hemolysis of blood sample , increase of hemoglobin, decrease of red cells ,triglycerides, hematocrite, mean corpuscular volume, mean corpuscular hemoglobin and its concentration and increase number of white blood cells. The histological analysis of blood sample during long-term storage showed a slight difference in the shape and size of RBC,WBC and platelets as well as condensed nucleus bluish cytoplasm and basophilic segmented nucleus in neutrophils in addition to decrease in sodium ,calcium and chloride. The effect of temperature on blood sampling showed slight variation in shape and size of RBC , basophilic neutrophils and platelets aggregation and decrease of leukocyte number and K levels .

INTRODUCTION

The blood is a special type of connective tissue that consist of suspended cell in liquid matrix, blood cells are characterized by continuous movement from one place to other (1) . The total blood volume in most mammalian is about 7-8% of total body weight .

it is composed of the cellular component and blood plasma. The cellular components occupy 45%.The blood cell is divided into erythrocyte, leukocyte and platelets or thrombocyte . The Red color of blood is due to hemoglobin staining in RBC (2). The blood involved in 1- Body immunity.2-water balance 3-body thermo regular 4- bleeding stop 5- Nutrition such as oxygen ,liquid, nutrient, hormones and vitamins. Blood plasma is a transparent liquid material and represents about 55% from total blood volume .It is composed of water 90% and protein7%. Plasma plays a role in the transportation of nutrients which is necessary for cells and transportation of the metabolic product in the body.

1-1 The effect of anticoagulants on hematology

There are few studies of the anticoagulant on blood sample in animals. In human, they observed that heparin anticoagulant should not be used for blood smearing because it stained the back ground blue and clotted the leuckocytes, whereas EDTA may cause damage to blood cells(1). Researchers had noticed that EDTA anticoagulant may increase hemolysis via effecting on cell membranes(3). Reece et al compared three anticoagulant affecting the blood parameter in fish (Mugilcephalus) (2)as shown in Table(1).

Table (1): The effect of three anticoagulant on blood parameter in mugilcephalus in day one.

Parameter	EDTA	Sodium citrate	Heparin
RBC	2.55 ±0.41	2.26±0.76	2.34±0.91
HCT90	30.14±5.74	26.89±4.89	25.43±4.83
HB(g\dl)	7.34±1.18	6.13±1.83	6.54±1.91
WBC(x10^3\ML)	8.02±2.14	7.95±3.08	8.03±3.26
Lymphocyte%	96.8 ±2.60	90.10±2.62	91.40±3.80
Monocyte%	2.50±1.00	2.90±2.10	2.96±2.2.80
Neutrophile%	6.70±2.30	7.00±2.40	5.70±2.30
Esinophile	0.00±0.02	0.00±0.02	0.00±0.02
Basophile	0.00±0.02	0.00±0.02	0.00±0.02
TC (x10^3\M	22.36±3.87	24.59±3.65	24.99±3.14
MCV(FL)	119.84±22.66	130.67±44.23	117.93±32.27
MCH(pg)	28.89±1.88	27.88±5.16	28.99±3.74
MCHC(g\dL)	24.65±2.79	23.19±7.19	25.65±4.95

From Table (1) we can see that there are some significant effects of the three anticoagulant on some blood parameters. There are significant effect of these anticoagulants on Hb, HCT, TG, Lymphocytes, monocytes. From Table (1) above it is also shown that Hb and HCT values in EDTA anticoagulant are more higher as compared to sodium citrate and heparin .

1-2-The Effect of Duration on hematology

Sawant *et al* observed that the storage duration of blood was very important to avoid hemolysis(4) . Austic and Scott noticed that hemolysis of the RBC increases during the first week of duration(5) .They observed that there were differences in the effect of storage duration of blood on hematological parameters depending on species as shown in human RBCs deteriorate slowly during storage which rats RBCS have .

Sawant *et al* observed that RBC begins to swell while leukocyte and platelet counts fall offered collection of blood (4). Goossens *et al* also showed that nucleated RBC disappear during two days of duration (6).

Michael *et al* observed that sodium citrate creatinin, alkaline phosphatase, amylase decrease after one day of the collection, whereas Potassium and lactate dehydrogenase increase at one day after the collection , inorganic phosphate after four days, magnesium after seven days, uric acid after three days in a human. (7) .

Anita Tendulkar et al Noticed that hemoglobin (g\dl)were 14-25 at4 hours of the Donors,14-30 at 48hours and 14-32 at72hours, while WBC were 7-63 at 4hours, 7-60 at 48hours and 7-58 at 72hours. (8). Fazio et al Noticed that there were significant changes in RBCS,HCT,MCT and HB at 366 hours as in Table(2) as compared to zero hour in blood samples of rainbow trout(oncorhynchus mykiss). (9)

Table (2): The effect of time (0 h and 366 h)on blood parameter of rainbow trout *(Oncorhynchus mykiss)*

0	RBC	WBC	TC	HCT	HB	MCV	MCH	MCHC
0Hours	1.55±0.03	20.17±0.22	59.50±5.37	29.66±0.79	1.63±0.08	191.40±2.50	74.75±2.69	39.57±1.43
366Hours	1.47±0.03	20.22±0.17	43.95±2.56	26.63±0.77	1.48±0.05	148.90±2.28	65.03±1.02	34.52±1.02

Ibrahim studied the effect of storage duration on rabbits blood cells. He noticed that the Mean leukocyte count of 48hour storage was (7.055±0.09)as compared to the mean leukocyte count of 24hour(7.075±0.08)(no significant differences)according to histological changes(10).

He showed no any changes in RBC, WBC and platelets ,but there was a slight difference in the size ,shape and rouleau appearance in normal RBC with central pallor. There were also large condensed nucleus and bluish cytoplasm in lymphocytes, while eosinophile had a bilobed nucleus and cytoplsm granules. There were also basophilic segmented nucleus in neutrophil and also small nuclear fragments of purple staining granules in the platelets Fig1,Fig(2).

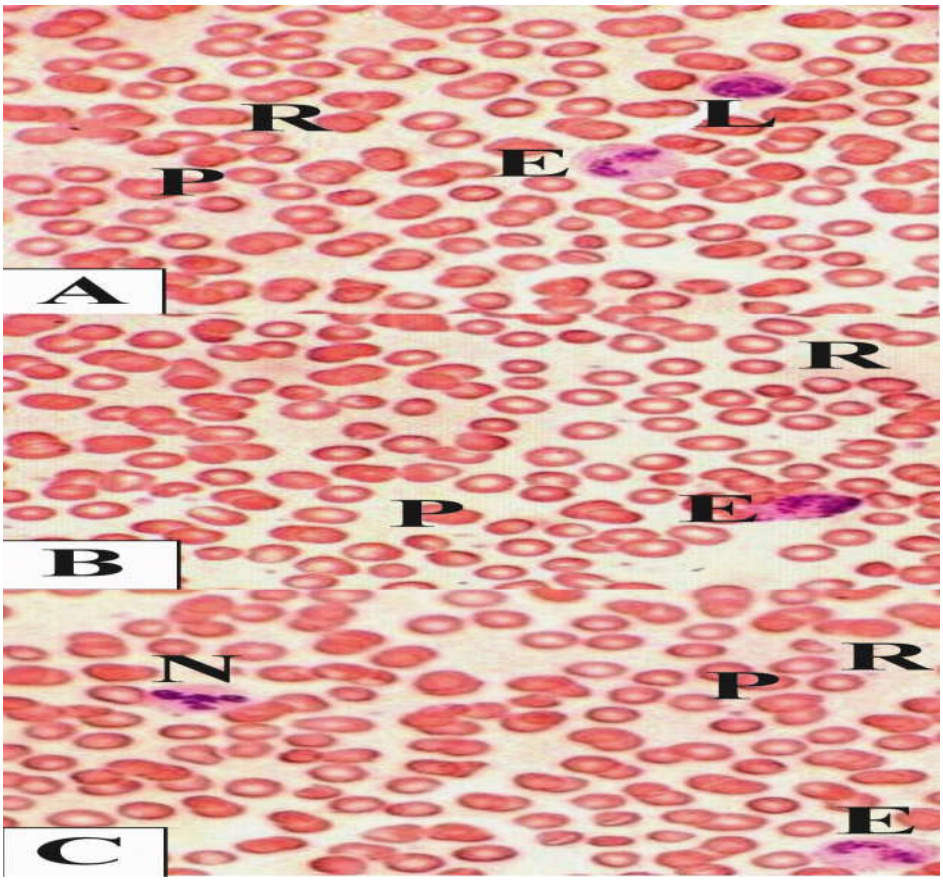
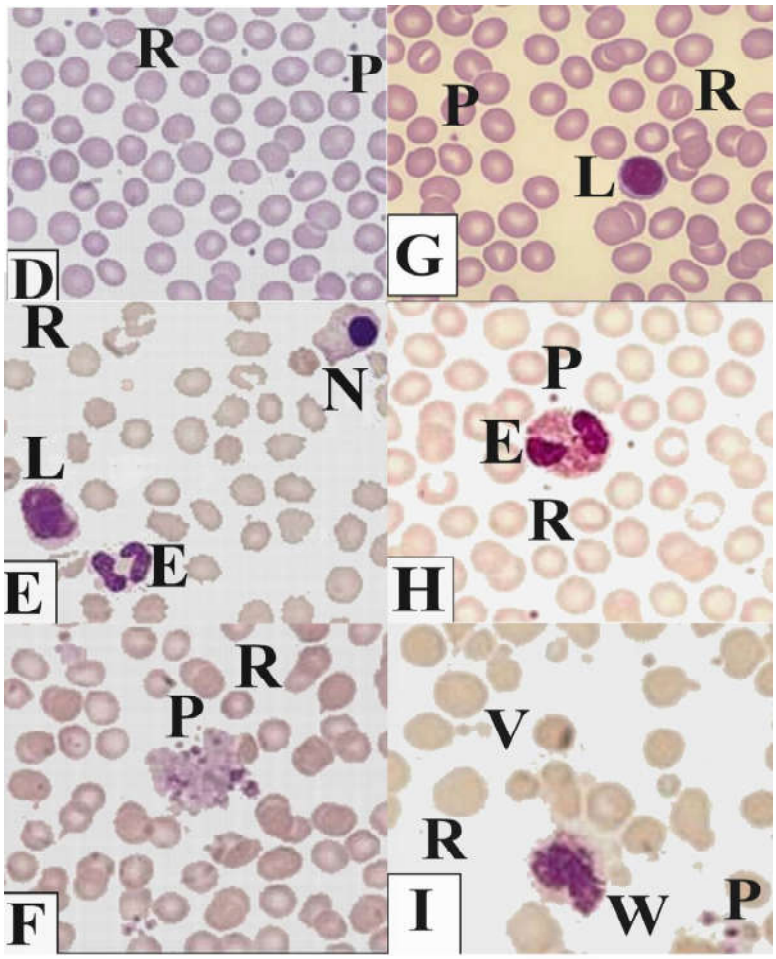


Fig (1):- Red cells with rouleau appearance. (R). Lymphocyte(L). Eosinophil (E) had bilobed nucleus with many cytoplasmic granules. Neutrophil (N) had basophilic segmented nucleus. The platelets (P). (Ibrahim K-I(2008)).



Fig(2):- red cells(R). Neutrophil (N). Platelets (P) aggregates. Early the central biconcave area of RBCs appeared slit like. Normal platelets (P) . vesicles (V). White cells (W) .(Ibrahim K-I(2008)).

In comparison of the complete blood count after taking blood immediately and at incubation for 48hours by studying on human blood ,there was no significant difference in leukocytes, hemoglobin and platelets befor and after incubation for 24 hours, significant difference in RBC,HCT,MCV,MCH as well as MCHC as shown in table - 3 **(11).**

Table (3): The effect of time (0 after incubation immediately and after 48 h) on blood parameter.

Blood parameters	Before incubation for 48	After incubation for 48
WBC	6.45±1.94	6.41±1.93
RBC	4.69±0.71	4.66±0.71
HB	14.08±2.15	14.07±2.16
HCT	40.54±5.56	41.81±5.60
MCV	87.04±8.22	90.37±8.55
MCH	30.29±3.86	30.44±3.84
MCHC	30.27±3.86	34.69±1.70
Platelets	226.41±7.70	226.75±7.45

1-3-The effect of Temperature on hematology.

Ibrahim observed that the heat blood sample at storage or processing is a risky factor related to hemolysis via effecting the membrane deformity , .also he showed a histological study on rabbits blood sample that at 4C˚few variation in the shape and size of RBC is realized while at 24C˚,the RBC became as a spiky appearance but at 36 C˚ observed central biconcave region of RBC(10). Reece *et al* In the Leukocyte picture, at 4 C˚ , showed that there were no significant changes, while at 24 C˚ and at 36 C˚ the WBC count were significantly decreased at 36 C˚.

It was found that at4 C˚ no morphological changes in lymphocytes, eosinophil's and neutrophils , but at 24 C˚ the neutrophils became as homogenous mass. At 36 C˚ the Leukocyte became weak basophilic and similar to the size of platelets. Similar to the platelets morphology, there were no morphological changes at 4 C˚, but at 24 C˚ the platelets aggregate and avoid of granules (2).

.**Table (4):**The Effect of Temperature on K^{+}, Na^{++} and CL^{-} ion of the blood sample.

Temperature	K	Na	CL
9	3.83a	154.4a	117.6a
22	2.36b	156.3b	117.7a
30	1.82c	157.3b	117.6a

Table(4) above showed the effect of heat on the mean value of K,Na,CL shown significant reduction($p<0.01$)in k^{+}. Plasma (Na+) increase ($p<0.05$) when stored at 22C° as compared to9 C°, the difference was not significant between the sample of 22 C° or 30 C. ($C\bar{l}$) no significant difference among samples stored at 9,22 and30.

.DISCUSSION

2-1- The Effect of anticoagulants on hematology

Walencik, and Witeska, noticed that the anticoagulant which applied in the laboratory of human hematology are potassium and sodium salts of ethylene diamine tetra acetic acid (EDTA), Sodium citrate and heparin (3). Witeska, and Wargocka observed that the heparin is a common endogenous anticoagulant acting on vessels in vivo and Exo vivo when added to blood samples. Heparin acts by inhibiting the conversion of pro thrombin to active thrombin as well as it did not allow fibrinogen to change into fibrin . EDTA and sodium citrate act as inhibitors of thrombocyte aggregation via chelating factor to free ca++ ions(12).

Some researchers considered that heparin is the most suitable anticoagulants (3),while other considered that EDTA is anticoagulant of choice .(13).In human hematology there is a disadvantage to use heparin in blood smear due to bluish of back

ground staining and clotting W.B.C , while EDTA leads to damage of blood cell(1).From our literature , the HCT and HB in anticoagulant (EDTA) increase significantly as compared to heparin and sodium citrate .This may be attributed to erythrocyte swelling and hemolysis(12)

The swelling of erythrocyte may be due to elevation in PCO2and acidification which is caused by treatment with acidic EDTA(14) ,(15) . In fished in mammal blood observed erythrocyte swelling with EDTA anticoagulant(12). While observed decrease in hematocrite in human blood sample with EDTA as compared to heparin(16).The present literature showed that a high number of RBC were found in the blood sample of EDTA as compared to heparin and sodium citrate ,so it observed that EDTA is a better preservation of cell.

2-2-Effect of time on the hematology.

Many literature noticed that the delay of blood sample processing led to hemolysis of blood sample , increase of hemoglobin, decrease of RBC ,TC, hematocrit ,mean corpuscular volum, mean corpuscular hemoglobin and its concentration and increase number of WBC.The biochemical analysis of blood after delayed processing led to decrease in sodium citrate ,creatine, alkaline phosphate and amylase while potassium acetate and phosphate showed an increase as well as increase in magnesium.

The histological analysis of blood sample showed a slight difference in shape and size of RBC,WBC and platelets as well as condensated nucleus bluish cytoplasm and basophilic segmented nucleus in neutrophils.The decrease in Sodium ,Calcium and Chloride may be contributed to the low molecular substance in the erythrocyte ,while the increase in potassium and magnesium may be contributed to these ions moved to the membrane of RBC but those slight elevations are due to low decrease of erythrocyte.(17) . The increase in inorganic phosphatase is due to the hydrolysis of phosphate ester (18). The slight elevation in urea may be attributed to cleavage of ammonia from basic amino acid which cause slight urea interference and reaction of glutamate dehydrogenase .

The amount of alkaline phosphatase amylase depends on the PH value ,so to the determination of these enzymes is dependent on the amount of these substance .

(19). The instability of dehydrogenase 3, dehydrogenase 4and dehydrogenase5 in the cold state led to decrease in lactate dehydrogenase activity (20). The decrease of RBC, triglycerides, hematocrit, hemoglobin, mean corpuscular volume and concentration is due to the degeneration of erythrocyte due to long –duration . The long periods storage of blood sample cause the dilatation of the pores of the membrane of RBC which allow water to enter the cells . The decrease in HB value caused change in MCH and MCHC value (9). The slight difference in shape and size was due to decrease negative changes on the red blood cells membrane that caused by high concentration of positively changed protein in the blood plasma such as fibrinogen and immunoglobuline(21).Moreover, the spiky appearance was explained by the changes in the distribution of phospholipids in the membrane and lipoprotein of the blood plasma(22), (23) and enzymes which led to lysis of RBC.

2-3-Effect of temperature on hematology

The present review showed a slight variation in the shape and size of RBC. These explained by inhibition of negative changes on the membrane of RBC that is due to the elevation of the concentration of positively changed proteins in the plasma (21) . The slight variation in shape and size of RBC that showed in this study may be attributed to the changes in the distribution of phospholipids in the membrane of RBC via the mature RBC cannot produce lipids (22),as well as the phospholipid that were lost in storage (20)Our review noticed decreased leukocyte at storage which is due to breaking that led to the release of chemicals and enzyme (H_2O_2 and proteases which cause red blood cell lysis (23).

The decreased leukocyte in the present study was similar to the study of (24) in wester. Many leukocyte derived lipids mediators, histamines and cytokines which can lead to the destruction of leukocytes (25). The homogenous mass of neutrophils during heat and characterization of basophilic was not consistent with(24) .The platelet aggregation may contributed to the activation of the platelets by agonists thrombin ,ADP and collagen ,that led to activate fibrinogen receptors which allow platelets cross – linkeded(6)

The result of the present study noticed a decreased potassium in storage due to the cyclic adenosine monophosphate –mediated potassium transport into the erythrocyte

was activated by the production of catechol a – mines (26).Meyer *et al* Noticed that catecholamine affects the distribution of Na, K and CL, that theirs function for the volume –regulatory response of red cells in anisomotic conditions, as well as the $Na^{+,}$ K and CL transport is activated by exposure to c – AMP (27).

مضاد التخثر و درجة الحرارة والوقت الملائمين لجمع عينات الدم

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الخلاصة

يعد الدم من الانسجة الرابطة المتكونة من خلايا معلقة في مادة سائلة . هذه الدراسة بحثت تأثير ثلاثة عوامل على نماذج الدم وهي(مانعات التخثر ودرجات الحرارة ووقت الخزن).من خلال استعراض المراجع بينت النتائج بأن بعض الباحثين سجلوا بان الهيبارين هو مانع التخثر المفضل بينما اخرين لاحظوا بان اي دي تي أي هوالافضل.ان تأخير فحص الدم يؤدي الى تحلل نموذج الدم،وزيادة في الهيموغلوبين ونقص في كريات الدم الحمر،والدهون الثلاثية، وزيادة في عدد كريات الدم البيض .الفحص النسجي لنموذج الدم خلال الخزن الطويل بين اختلاف بسيط في شكل وحجم كريا الدم الحمر والبيض والصفائح الدموية بالاضافة الى ظهور سايتوبلازم ازرق ونواة كثيفة علاوة على نواة مقطعة قاعدية للخلايا المتعادلة ،اضافة الى قلة في كالسيوم وصوديوم والكلور. ان تأثير الحرارة على نموذج الدم اظهرت اختلاف بسيط في شكل وحجم الكريات الدم الحمر ،وخلايا متعادلة قاعدية ،وتجمع للصفائح الدموية وقلة في عدد الخلايا البيض ومستوى البوتاسيوم. .

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