Sudan University of Sciences and Technology
Colleges of Graduates Studies
Faculty of Medial Laboratory Sciences

Effect of Blood Storage at Temperature (4C°) on Complete Blood Count Parameters

تأثير تخزين الدم في درجة حرارة (4 درجة مئوية) على معملات تعداد الدم الكامل

A thesis submitted in partial fulfillment of the Requirement of MSc Degree in Medical Laboratory Sciences: Hematology and Immunohematology.

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الآية

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قال تعالى:

((وَابْتَغِ فِيمَا آتَاكَ اللَّهُ الدَّارَ الْآخِرَةَ ۖ وَلَا تَنْسَ نَصِيبَكَ مِنَ الدُّنْيَا ۖ وَأَحْسِنْ كَمَا أَحْسَنَ اللَّهُ إِلَيْكَ ۖ وَلَا تَبْغِ الْفَسَادَ فِي الْأَرْضِ ۖ إِنَّ اللَّهَ لَا يُحِبُّ الْمُفْسِدِينَ ﴿٧٧﴾))

صدق الله العظيم

سورة القصص

Dedication

To My mother

The reason of what I become today. Thanks for your great support and continuous care.

To My father

Whom I hold his name will all honor

To My sisters

For endless love, support and encouragement.

And

To everyone struggle to support me ….

Acknowledgments

Allah enabled me to conduct this study by his blessing therefore thanks for my god **Allah** firstly and lastly.

In addition, I would like to thank my supervisor: **Dr. Abdalla Musa Abdalla Mohamed** who encourage, guidance and support me from the initial to the final level enabled me to develop this research.

To my truly **great friends** who have made available their support in a number of ways.

Also I would like to acknowledge the members of khalifa medical laboratory for their assistance in collection and analysis of specimen.

Abstract

Background: Complete blood counts are done to monitor overall health, to screen for some diseases, to confirm a diagnosis of some medical conditions, to monitor a medical condition, and to monitor changes in the body caused by medical treatments. Most blood counts today include a WBC Count and leukocyte differential count (LDC) (that is, not just the total WBC count but also the count of each WBC type, such as neutrophils, eosinophils, basophils, monocytes, and lymphocytes). More sophisticated modern analyzers can provide extended differential counts.

Objectives: This study aimed to assess the effect of Storage at Temperature ($4C^0$) on Complete Blood Count Parameters.

Materials and Methods: This is cross sectional study conducted in Khartoum state during the period from March 2019 to January 2021. It included 70 samples, complete blood count conducted to all 70 samples freshly(base line) and after 24hours storage at Temperature ($4C^0$). Sysmex xp-300 hematological analyzer was used to estimate CBC. The statistical analysis was performed using SPSS version 21.

Results: the results of study shows that the mean level of HB ,HCt, MCV,MCH, MPV, PDW and PLCR were significantly increased after storage for 24hrs compared to base line results (12.6 ± 2.1; 12.7 ±2.1, p value= 0.000), (39.08 ±0 .76; 39.4 ± 0.78, p value= 0.000), (84.7 ± 5.3; 84.9 ± 5.4, p value= 0.000), (27.4 ± 2.1; 27.5 ± 2.1, p vale= 0.001) , (9.5 ± 0.95; 10.7 ± 0.99, p value= 0.000),(11.7± 1.8, 13.8±2 ,P vaue= 0.000),(22± 6.9: 31.1± 7.5,p value0.000) respectively, while the mean level of RDW and Platelets were significantly decreased after storage for 24hrs compared to initial results (13.6 ± 1.7; 13.9 ± 1.7, p value= 0.000), (318.8 ± 119.9; 305.2 ± 118.4 , p value =0.000).

In contrast, RBCs, MCHC,WBCs, Lymph %, Lymph Count, Neut %, Neut count, MXD%, MXD count and PCT show no difference between base line and 24hrs reading (4.57± 0.7: 4.58± 0.7,p value= 0.708),(32.27 ± 1.21: 32.25± 1.25,p value= 0.758),(7.22± 2.6: 7.09± 2.6,p value= 0.180),((2.23 ± 0.76; 2.22 + 0.78, p value=0.703), (57.3 ± 12.5; 132.4 ± 639.1, p value = ±0.327), (4.3 ± 2.3; 4.1 ± 2.1, p value= 0.125), (10.23 ± 3.9; 11.5 ± 6, p value= 0.195), (0.702 ± 0.28; 0.705 ± 0.36, p value= 0.947), (0.30 ± 0.10; 0.59 ± 2.2, p value= 0.278) respectively.

Conclusion: This study conclude that, the level of Hb, HCt, MCV, MCH, PDW and PLCR were increased , the level of RDW and Platelets were decreased while RBCs, MCH, WBCs and differential leukocytes were not affected.

المستخلص

الخلفية: يتم إجراء تعداد الدم الكامل لمراقبة الصحة العامة، والكشف عن بعض الأمراض، وتأكيد تشخيص ومراقبة بعض الحالات الطبية، ورصد التغيرات في الجسم التي تسببها العلاجات الطبية. تتضمن معظم تعداد الدم اليوم تعداد كريات الدم البيضاء وعدد الكريات البيض التفاضلي (أي ليس فقط إجمالي عدد كرات الدم البيضاء ولكن أيضًا عدد كل نوع من كريات الدم البيضاء، مثل الخلايا المتعادلة، والحمضية، والقاعدية ، والخلية الوحيدة ، والخلايا الليمفاوية). يمكن أن توفر أجهزة التحليل الحديثة الأكثر تطورًا أعدادًا تفاضلية ممتدة.

الأهداف: هدفت هذه الدراسة الى تقيم تأثير التخزين عند درجة حرارة (4 درجة مئوية) على تعداد الدم الكامل.

المواد والطرق: هذه دراسة مقطعية أجريت في ولاية الخرطوم خلال الفترة من مارس 2019 إلى يناير 2021. وشملت 70 عينة ، تم إجراء تعداد دم كامل لجميع العينات طازجة وبعد 24 ساعة من التخزين عند درجة حرارة (C4). تم استخدام محلل الدم سيسمكس اكس بي_300 لتقدير تعداد الدم الكامل. تم إجراء التحليل الإحصائي باستخدام الإصدار 25 من الحزمة الإحصائية للعلوم الاجتماعية.

النتائج: أظهرت نتائج الدراسة أن هنالك زيادة ذو دلالة معنوية في متوسط مستوى الهيموقلوبين ، الحجم الكروي الوسطي، متوسط هيموقلوبين الخلية، متوسط حجم الصفائح الدموية، عرض توزيع الصفائح الدموية ونسبة الخلايا الكبيرة بالصفائح الدموية بعد التخزين ل24ساعة مقارنة بالنتائج الأولية (12.6 ± 2.1; 12.7 ±2.1, p value= 0.000), (39.08 ±0 .76; 39.4 ± 0.78, p value= 0.000), (84.7 ± 5.3; 84.9 ± 5.4, p value= 0.000), (27.4 ± 2.1; 27.5 ± 2.1, p vale= 0.001) , (9.5 ± 0.95; 10.7 ± 0.99, p value= 0.000),(11.7± 1.8, 13.8±2 ,P vaue= 0.000),(22± 6.9: 31.1± 7.5,p value0.000) على التوالي.

كذلك بينت الدراسة أن هنالك انخفاض ذو دلالة معنوية في متوسط مستوى عرض توزع كريات الدم الحمراء والصفائح الدموية بعد التخزين ل24ساعة مقارنة بالنتائج الأولية (13.6 ± 1.7; 13.9 ± 1.7, p value= 0.000) ، (318.8 ± 119.9; 305.2 ± 118.4, p value =0.000).

في المقابل، لم تظهر الدراسة اي فرق في متوسط كريات الدم الحمراء، متوسط تركيز الهيموقلوبين في الخلية ، مستوى كرويات الدم البيضاء، نسبة اللمفاويات، عدد الليمفاويات، نسبة الخلايا المتعادلة، عدد الخلايا المتعادلة، نسبة الخلايا المختلطة، عدد الخلايا المختلطة والبليتلكريت بعد التخزين ل24 ساعة مقارنة مع النتائج الأولية (4.57± 0.7: 4.58± 0.7,p value= 0.708),(32.27 ± 1.21: 32.25± 1.25,p value= 0.758),(7.22± 2.6: 7.09± 2.6,p value= 0.180),((2.23 ± 0.76; 2.22 + 0.78, p value=0.703), (57.3 ± 12.5; 132.4 ± 639.1, p value = ±0.327), (4.3 ± 2.3; 4.1 ± 2.1, p value= 0.125), (10.23 ± 3.9; 11.5 ± 6, p value= 0.195), (0.702 ± 0.28; 0.705 ± 0.36, p value= 0.947), (0.30 ± 0.10; 0.59 ± 2.2, p value= 0.278)على التوالي.

الخلاصة: خلصت هذه الدراسة إلى أن هناك زيادة في مستوى مستوى الهيموقلوبين ، الحجم الكروي الوسطي، متوسط هيموقلوبين الخلية، متوسط حجم الصفائح الدموية، عرض توزيع الصفائح الدموية ونسبة الخلايا الكبيرة بالصفائح الدموية ، وان هنالك نقصان في عرض توزع كريات الدم الحمراء والصفائح الدموية، بينما لم يظهر متوسط كريات الدم الحمراء، متوسط تركيز الهيموقلوبين في الخلية ، مستوى كرويات الدم البيضاء، نسبة

اللمفاويات، عدد الليمفاويات، نسبة الخلايا المتعادلة، عدد الخلايا المتعادلة، نسبة الخلايا المختلطة، عدد الخلايا المختلطة والبليتلكريت اي تغير.

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List of Abbreviations

Symbol	Meaning
CBC	Complete blood count
EDTA	Ethylene-diamine-tetra acetic acid
MCV	Mean cell volume
MCH	Mean cell hemoglobin
MCHC	Mean cell hemoglobin concentration
MPV	Mean platelets Volume
PCT	Plateletcrit
PDW	Platelets distribution widith
PLCR	Platelets large cells ratio
PLTs	Platelets
RBC	Red blood cell
RDW	Red cell distribution width
WBC	White blood cell

CHAPTER I
INTRODUCTION

Chapter I

Introduction

A complete blood count (CBC), also known as a full blood count (FBC), is a blood panel requested by a doctor or other medical professional that gives information about the cells in a patient's blood, such as the cell count for each cell type and the concentrations of various proteins and minerals. A scientist or lab technician performs the requested testing and provides the requesting medical professional with the results of the CBC (Beverly George-gay et al, 2003). Complete blood counts are done to monitor overall health, to screen for some diseases, to confirm a diagnosis of some medical conditions, to monitor a medical condition, and to monitor changes in the body caused by medical treatments (Mayo Clinic, 2014).

In many countries, centralized laboratories are equipped with modern automated analyzers that are capable of processing large volumes of hematological tests in an efficient and timely manner. Literature of most of the manufacturers of automated analyzers often cite that blood specimens kept at either room temperature (RT) or at 4 _ 2°C (refrigerated) for up to 24 hr, generally yield reliable results for CBC and automated differential leukocyte count (Gulati GL et al, 2002). The frequency of performing the CBC in tropical countries have tremendously increased due to the high incidence of conditions such as dengue, which has a high mortality and morbidity. Due to this, a large number of specimens are transported for CBC analysis from collecting centers to a centralized laboratory where a delay of 1–3 days could occur. Therefore, it is vital to know the storage conditions of the sample.

Storage at RT may cause ethylene diamine tetra acetate (EDTA) changes and quantitative effects of storage on blood as cellular elements are known to have limited stability in EDTA (Turhan T, et al, 2011). The reliability of the hematocrit (Htc) and

the platelet count (PLT) is of paramount importance in the management of the fatal dengue infection. As blood tests are commoner than testing other biological fluids, using standard methods for sample collection, incubation, and the role of environmental factors that affect the blood indices should be considered. When such a specimen arrives at the laboratory, the staff will need to decide whether to accept it or reject it; if accepted, whether to perform all of the ordered tests or only those appropriate based on the age of the specimen (Gulati GL et al, 2002).

In this backdrop, this study was aimed at assessing the changes in various components of CBC with different storage temperatures at different incubation time periods as this would be helpful in determining which parameters can change with time and the conditions of the storage and the best temperature to store the sample, if a delay is anticipated.

1.2 Rationale

The complete blood count (CBC) is one of the most common tests requested by physicians. The results of this test are affected by different factors such as temperature and time of incubation.

Recent studies have examined the combined effects of both temperature and storage time on blood analysis results. This study seeks to solely assess the changes in various parameters of automated CBC resulting after storage of blood at refrigerator for 24 h and compare it with the result of CBC performed immediately after sample collection.

1.3 Objectives

3.1 General objective

To assess the effect of Storage and Temperature (4ºC) on Complete Blood Count Parameters in healthy Sudanese subjects in Khartoum state.

3.2 Specific objectives

1-To measure complete blood count on study population.

2- To compare the mean level of automated CBC results before and after Storage.

CHAPTER II
LITERATURE REVIEW

CHAPTER II

Literatures review

2.1 Blood

Blood is a fluid connective tissue constituting about 7% of our total body weight (About 5 liters in the human) (Gartner and Hiatt, 2006).

The function of blood is the exchange of respiratory gases, transport oxygen from the lung to tissue and deliver carbon dioxide from tissue leading the lung to be exchange and excreted, blood also transport metabolic wastes to the lung, kidney skin and intestine from removal, blood is also responsible for maintaining acid base balance (Young *et al*., 2006).

Blood elements include erythrocytes or red cells, leukocytes or white cells, and platelets. Red blood cells (RBCs) are the most numerous blood cells in the blood and are required for tissue respiration. RBCs lack nuclei and contain hemoglobin, an iron containing protein that acts in the transport of oxygen and carbon dioxide. White blood cells (WBCs) serve an immune function and include a variety of cell types that have specific functions and characteristic morphologic appearances. In contrast to mature red cells, WBCs are nucleated and include neutrophils, lymphocytes, monocytes, eosinophils, and basophils. Platelets are cytoplasmic fragments derived from marrow megakaryocytes that function in coagulation and Hemostasis (Greer *et al*., 2013).

Plasma which makes up 45-55% of blood volume, plasma is made up of 90% water, 7-8% soluble proteins (albumin maintains bloods osmotic integrity, others clot, etc), 1% electrolytes, and 1% elements in transit. One percent of the plasma is salt, which helps with the pH of the blood. The largest group of solutes in plasma contains three important proteins to be discussed. There are albumins, globulins, and clotting proteins (Hoffbrand *et al*., 2006).

2.1.1 Blood function

Blood is main transportation vehicle of the body. It is carries oxygen and nutrients to tissues and waste products of metabolism e.g carbon dioxide and urea to lung and kidneys. Most of the hormones are also carried from the endocrine gland to target organs. Blood also have important hemostatic functions exemplified in following. Blood circulation helps to distribute heat around the body from metabolically active and warmer organs, e.g liver and gut to peripheral organs. Buffer in the blood like

hemoglobin, plasma, protein, bicarbonate and other help to keep hydrogen concentration of extracellular fluid constant at a ph 7.4 . Blood play a vital protective function against infection by virtue of it is leukocyte and antibody (immunoglobulin) in the plasma. Furthermore, injury to blood vessels is followed by blood clotting, which stops further loss of blood (Sukkar *et al;* 2000).

2.2 Hemopoiesis (blood cell formation)

Is the process by which immature precursor cell (pluripotential stem cell) develop into mature blood cell.

2.2.1 Site of hemopoiesis

Formation of blood cell occur in different anatomical sites during the course of development from embryonic to adult life (Metcalf and Moore, 2012). Production of blood cells commences in the yolk sac of the embryo, but then shift to the liver, and to lesser extent to the spleen, so that these organs become the dominant sites of production between the second and seventh month of gestation. The liver and spleen are superseded by the bone marrow, which serves as the only important sites of blood cell production after birth. An exception is lymphocyte production, which occurs substantially in other organs, in addition to the bone marrow, in adult life. Haemopoietic tissue fills the entire cavity with in the bones of the newborn, but with increasing age become localized in the cavities of the upper shafts of the femur and humerus, the skull, pelvis, spine and bone of the thorax. The total volume of haemopoietic tissue in adult is 1-2litres. This tissue is referred to as red marrow because of its macroscopic appearance; the remaining bone marrow in the more peripheral regions of the skeleton contain predominantly fat and termed yellow marrow. Yellow marrow also occupies a volume of 1-2 liters and serves as Are serve space into which haemopoietic tissue can expand in response to an
increased demand for blood cell production. Only in pathological situation does significant haemopoietic activity occur in the liver, spleen and other sites during adult life when it is referred to as extra medullary haemopoiesis (Orkin, S. H *et al*, 2008).

2.2.2 Erythropoiesis

Process by which red blood cells are formed; it is stimulated by decreased O_2 in circulation, which is detected by the kidneys, which then secrete the hormone called erythropoietin. This hormone stimulates proliferation and increased differentiation of

red cell precursor, which activate erythropoiesis in the haemopoietic tissue, ultimately producing red blood cells (Ciesla, 2007).

Red cells are produced by proliferation and differentiation of precursors whose Dominant representatives in the bone marrow are the erythroblast. Erythroblast are referred to as normoblast when their morphological feature are with in normal limits. During the course of differentiation the size of erythroblast, progressively decrease, and the character of the nucleus and cytoplasm changes as the cells proceed toward the point where proliferative capacity is lost and hemoglobin become the predominant protein in the cytoplasm (Greer *et al*., 2003).

2.2.2.1 The erythroid series

1-The pro-erythroblast

It is the least mature of the morphologically identifiable members of the erythroid series. It has diameter of (14-20) μm and a round outline with several round nucleoli in the nucleus, which occupies most of the cell. The chromatin in the nucleus consist of a network of fine red -purple strands. A characteristic feature is that the peripheral cytoplasm is more basophilic than in the myeloblast, which is the corresponding member in the maturation sequence of the granulocytic series. Pro-erythroblast undergo rapid division and give rise to basophil erythroblasts (Keohane *et al*., 2016).

2.The basophilic erythroblast:

It is round cell with a diameter of (12-16) μm, and more basophil cytoplasm than the proerythroblast. It is also undergoes rapid proliferation. The nucleus occupies a relatively large proportion of the cell, but differs from the nucleus of the proerythroblast by having coarser and more basophilic chromatin strands (Lang, 2008).

3. The polychromatic erythroblast

It is a round cell between (12-14) μm in diameter. The characteristic polychromatic appearance of the cytoplasm is derived from the mixture of the basophilic ribonucleic acid (RNA) and acidophilic hemoglobin. Nuclear chromatin is in coarse deeply basophilic clumps, and proliferative activity ceases after in this stages (Frank *et al*; 2011).

4. Orthochromatic erythroblast

Constitute the next and final stage of maturation of the nucleated red cell series. They are small and have a diameter between (8-12) μm. The nucleus is relatively small with

a homogenous blue –black appearance. Active hemoglobin synthesis occurs in cytoplasm which contain mitochondria and ribosomes. The ribosomal RNA imparts a basophilic tint to the cytoplasm is predominantly acidophilic due to the presence of large amount of haemoglobin. The nucleus is extruded from the orthochromatic erythroblast to form the reticulocyte (Palis and Segel, 2000).

5 .Reticulocytes

They have the same biconcave discoid shape as mature red cells, although they have a slightly greater volume and diameter than the latter. Reticulocyte cytoplasm is similar in staining characteristics to that of orthochromatic erythroblast, which are distinguished from mature red cells by a diffuse basophilic hue. When stained with vital stains such as new methylene blue reveals deeply stained granules or chain of granules .Reticulocyte lose their mitochondria and ribosome over the course of a few days, and in doing so lose the basophilic tint and evolve into the mature erythrocyte. Red cell normally enter the blood at the stage of reticulocyte or of the mature erythrocyte (Davis and Bigelow, 2000).

6. Mature red blood cell

Mature erythrocytes are unique among the cells of human tissues in that they normally lack nuclei and cytoplasmic structures such as lysosomes, and mitochondria. It is biconcave shape, which allow maximum flexibility to transverse the smallest capillaries, which have a diameter of only 5μm .The red cell membrane, is composed of specialized protein (cytoskeleton) which responsible for red cell shape, and outer lipid bilayer which responsible for provide hydrophobic skin.

Defect in both red cell membrane and lipid bilayer lead to change in shape and red cell destruction .lifespan of the red cell(120 days) (Martin and Peter ;2008) .

2.2.3 Hemoglobin

Hemoglobin, which is contained in red blood cell, the main function of red cell is to carry O_2 to Tissues and return carbon dioxide (CO_2) from tissue to lung in order to achieve this gaseous exchange they contain specialized protein called hemoglobin (Hoffbrand *et al;* 2004).

2.2.3.1 Hemoglobin structure

Each RBC contain approximately 640 million hemoglobin molecule, each molecule of normal adult hemoglobin (Hb A) which is the dominant hemoglobin in the blood after age of3-6 month consist of four polypeptide chain 2α and 2β each with it is own

heam group .The molecular weight of HbA is 68000. Normal adult blood also contain small quantity of two other hemoglobin, HbF and HbA2 .These also contain α chain but with γ and δ respectively instead of β. The major switch from fetal hemoglobin to adult hemoglobin occur 3-6 month after Birth (Steinberg, 2001).

2.2.3.2 Hemoglobin synthesis

Occur largely in mitochondria by series of biochemical reaction commencing with the condensation of glycine and succinyl Co enzyme. Under the reaction of the key rate limiting enzyme δ amino levulinic acid (ALA) synthetase. Pyridoxal phosphate (Vit B6) is Co enzyme for this reaction which is stimulated by erythropoietin .Ultimately, protoporphyrin combine with iron in ferrous state (Fe^{2+}) to form heam, each molecule of which combines with globulin chain made on polyribosome .A tetramer of four globin chains each with it is own heam group a pocket is then formed to make up hemoglobin molecule (Sidell *et al*.,2006).

2.2.4 Leucopoiesis

It is the process of formation of white blood cells. The leukocyte may be divided into two broad groups: the phagocytes and immunocytes. Granulocytes, which include three types of cell: neutrophils (polymorphs), eosinophil's, and basophiles together with monocytes comprise the phagocytes. Only mature phagocyte and lymphocyte are found in normal peripheral blood. The lymphocyte, their precursor cells and plasma cells make up the immunocyte population. The function of phagocytes and immunocytes in protecting the body against infection is closely connected with two soluble protein systems of the body: immunoglobulin and complement (Hoffbrand *et al*; 2006).

2.2.4.1 Granulopoiesis

Is the process of granulocyte formation. The predominant white blood cell, or leukocyte in the circulation is mature granulocyte (Basu *et al*., 2004).

2.2.4.1.1 Granulopoiesis series

1.Myeloblast

Is relatively large cell (15-20) μm in diameter, with round to oval nucleus, which occupies a large Proportion of the cell. There are no typical granules in the moderately basophilic cytoplasm. Nuclear chromatin is arranged in a fine network of red-purple strands with occasional small aggregates. Nucleoli are typically prominent while two or three is the usual number, there may be up to six nucleoli (Mitchell *et al.*,2007) .

2. Promyelocyte

It is larger thanmyeloblast, loose chromatin with nucleoli and dark blue cytoplasm with large granules distributed throughout the cytoplasm (Young *et al*.,2006).

3. The myelocyte

It has prominent cytoplasmic granules, and the area of cytoplasmic relative to the nucleus is greater than in the promyelocyte .The cytoplasm is also less basophilic, nucleoli are no longer present, and the chromatin appears more aggregated than in The promyelocyte (Young *et al*.,2006).

4. Metamyelocyte

The nucleus becomes indented and assumes a kidney –shaped appearance. Granules are prominent in the cytoplasm.

Band or stab form

The degree of indentation of nucleus is greater than 50% of nuclear diameter. Cytoplasmic granules are identical to those in the mature segmented form (Hoffbrand and Moss, 2011).

6. Neutrophil (polymorph)

This cell has a characteristic dense nucleus consisting of between two and five lobes, and a pale cytoplasm with an irregular outline containing fine pink-blue granules or grey- blue granules. The granules are divided into:

a- Primary, which appear at the promylocyte stage.

b- Secondary (specific) which appear at the myelocyte stage and predominant in the mature neutrophil.

Both types of granules are lysosomal in origin. The life span of neutrophils in the blood is only 6-10 hours. Neutrophils are the most comemon circulating form of granulocyte and play essential role in phagocytosing and killing invading microorganism (Bruce, 2002).

2.2.4.2 Lymphopoiesis

Is process by which lymphocyte formed. Lymphocytes pass through a series of developmental changes in the course of evolving into various lymphocyte subpopulation . The developmental process in certain instances involves migration of immature precursors to other organs such as the thymus, where inductive effects on differentiation are mediated via locally produced factors. Mature lymphocytes are engaged in extensive recirculation through the exravascular and vascular

compartments .This is important in facilitating the recognition of foreign antigens by lymphocytes, and it naturally assist the recognition by lymphocytes of foreign antigens to which the individual has been previously exposed. Cell –mediated and antibody-mediated immune response involve a complex sequence of events in which lymphocyte subsets interact with other subsets of lymphocytes (Birbrair and Frenette,2016).

2.2.4.3.1 lymphopoiesis series

1. Lymphoblasts

Are slightly smaller than the myeloblasts which they resemble , except that the ratio of the diameter of the nucleus to that of the cell is greater and the number of the nucleoli per nucleus is fewer than in the myeloblast .Lymphoblast are actively dividing cells (Gillian, 2011).

2. prolymphocyte

Differ fromblast by subtle changes such as slightly more clumped chromatin, a lessening of nuclear prominence and changes in sickness of nuclear chromatin (Bernadette; 1995)

3. The large lymphocyte

Is between (12-16) μm in diameter, round in outline, nucleus is round or slightly indented, chromatin is more clumped and the cytoplasm is more abundant than in the lymphoblast which is usually pale blue. Some granules may be present in the cytoplasm, but are fewer than in the granulocyte (Farlex, 2012).

4. Small lymphocytes

Are between (9-12) μm in diameter, smaller than segmented granulocytes, cytoplasm usually forms only a thin medium to deeply basophilic rim encircling a round or marginally indented nucleus which contains deeply staining heavily clumped chromatin (Miller, 2003).

2.2.5 Thrombopoiesis

Platelet are formed in the bone marrow by megakaryocytes, and are subsequently released into the vascular compartment where they play an essential role in hemostasis.

2.2.5.1 The megakaryocytic series

1. Megakaryoblast

The most immature stage of platelet development, which resembles the myeloblast in it, is basic feature. These cell amounts to less than 8% of the total megakaryocytic population (Sun, L *et al*, 2006).

2. The promegakaryocyte

Is the next stage in the sequence of maturation, and is larger than it is precursors because it has undergo endore duplication. Endore duplication is nuclear replication without division of the series .Promegakaryocytes make up about 25% of megakaryocytes , and have deeply basophilic cytoplasm containing some basophilic granules. The nucleus may be lobulated and the chromatin is more deeply (Chang, Y *et al*, 2007).

3. Mature megakaryocytes

It is extremely large cell with accentric placed single lobulated nucleus and low nuclear to cytoplasmic ratio with coarsely clumped chromatin, the cytoplasm stains light blue and contains many small red-purple granules (Hoffbrand *et al;*2006).

4. Platelet

Are small, anucleate, terminal stage of development of the megakaryocytic series. They are discoid and have a diameter of 1-4μm. The cytoplasm stains light blue and contains small red-purple granules, which are centrally located in platelet in blood films. its released into the circulation to prevent leakage of blood . The life span is about 1 week (Emmanuel; 1993).

2.3 complete blood cell count (CBC)

Complete blood cell count is a very common test that use to evaluate the three major type of cell in blood, red blood cell, white blood cell and platelet (Lewis *et al* ;2006).

2.3.1 Haemoglobin estimation

The hemoglobin concentration of a solution may be estimated by several methods: by measurement of its color, by its power of combining with oxygen or carbon monoxide or by its iron content. The methods to be described are all color or light-intensity matching techniques, which also measure to a varying extent, any methaemoglobin (Hi) or sulphaemoglobin (SHB) ,that may be present. Also Hb can be determined accurately by spectrophotometry. The blood is diluted in a solution contain potassium cyanide and potassium ferricyanide and the absorbance of the solution is then

measured in a spectrometer at wavelength of 540nm and then Hb is calculated with special formula (Lewis *et al;*2006).

2.3.2 Red blood cell estimation

Red cell and other blood cells can be counted in systems based on either a aperture impedance or Light –scattering technology. Because large number of cell Can be counted rapidly, there is a high level of precision. Consequently electronic counts have rendered the RBC and red cell indices derived from it (the MCV and MCH) of much greater clinical relevance than was possible when only a slow and imprecise manual RBC was available (Lewis *et al*; 2006).

2.3.3 Haematocrit

The haematocrit or packed red cell count (PCV) refer to the proportion of the volume of red cells relatives to the total volume of the blood. High –speed centrifugation in the microhaematocrit procedure used to sediment the red cells yields highly reproducible result .The values do not correspond strictly to those obtained by electronic automated devices which derive a result from a formula which involves multiplying the red cell count by the mean red cell volume, the microhaematocrit procedure is of value in providing a reliable and simple means for rapid determination by clinician of the red cell content of the blood (Keohane *et al*.,2016).

2.3.4 Red cell indices

Red blood cell indices use to help diagnose the cause of anemia or a condition in which there are too few red blood cell. (Hutchison *et al;* 2011).

2.3.4.1 Mean corpuscular volume (MCV)

The mean volume of red cells was formerly determined by dividing the total volume of red cells (derived from the PCV) by the number of red cells in that particular sample of blood. Automated electronic-particle counting devices have revolutionized the estimation of the MCV. Most devices measure the electrical impedance caused by red cell as it passes through the counting mechanism and the extent of the impedance provide an accurate indication of the volume of each cell.

The MCV derived by this means therefore provides a reliable index of the average size of red cells, which is a guide of considerable importance to the nature of the disorder underlying an abnormality in the hemoglobin level. A subnormal MCV is indicative of microcytosis, and an elevated MCV indicative of macrocytosis (Frank *et al;*2011).

2.3.4.2 Mean corpuscular hemoglobin (MCH)

The mean amount of haemoglobin per red cell (MCH) is also rapidly and reliably estimated by automated electronic counting devices by dividing the total amount of haemoglobin by the number of red cells in a sample of blood. A subnormal MCH occurs in microcytosis, but is even lower when microcytosis occurs in conjunction with a subnormal concentration of hemoglobin in the red cell, as in thalassemia minor or iron deficiency (Cheesbrough, 2006).

2.3.4.3 MCHC (Mean Cell Hemoglobin Concentration)

This test is measure the average concentration of haemoglobin with in the red cell and it is used to evaluate and manage blood disorder (Fischbach and Dunning ;2004).

It is derived by dividing the concentration of haemoglobinin g/dl by the volume of red cell in ml/dl. Both measurement are readily and reliably obtained by manual methods, and the result is expressed in g haemoglobin/dl packed red cells.

A subnormal MCH is usually indicative of an abnormality where interference with the synthesis of hemoglobin is greater than that of other constituents of red cells, as in thalassemia or iron deficiency. Elevated value reflect dehydration of the erythrocytes, and one of the relatively few important clinical causes of this phenomenon is spherocytosis (Kern, 2002).

2.3.4.4 red blood cells distribution width (RDW_CV and RDW_SD)

Red cell distribution width is derived from pulse analysis and can be expressed as standard deviation (SD) in (fl) or as coefficient variation(CV) (%) of measurement of red cell volume RDW is usually increased in iron deficiency anemia and normal in thalassemia trait.(Lewis *et al;*2006)

2.3.5 White blood cell count (WBCs)

Is determined in whole blood by using lytic reagent which lysis RBCs while WBC is intact. Fully automated instrument perform WBCs by using impedance or light scattering technology (Alberts and Bruce, 2005).

2.3.6 platelet count

Platelet can be counted in whole blood using the same techniques of electrical or electro-optical detection as are used for counting red cell .Also platelet can be counted by flow cytometer by labeling plt fluorescently with specific monoclonal antibody or combination of antibodies.(lewis *et al;*2006)

2.3.6.1 Mean Platelet Volume (MPV)

The same technique that are used to size red cells can be applied to platelets. The calculated MPV is very dependent on the technique of measurement .When MPV is measured by impedance technology it has been found to vary inversely with plt count in normal subjects. MPV is higher in myeloproliferative disorders and lower in thrombocytopenia caused by megaloblasticanaemia or bone marrow failure (Lewis *et al*;2006).

2.3.6.2 Platelet Distribution Width (PDW)

Is a measure of platelet anisocytosis and plt crit which is the product of the MPV and Platelets count. (Lewis *et al*; 2006).

2.4 Factors affect on CBC

Over and under filled tubes: all tubes with anticoagulant must be filled to the correct blood ratio in order to obtain accurate result, Hemolysis: which caused by use of very small bore needle, forcing blood syringe to an evacuated tube, and improper shaking of tubes ,Tourniquet: improper use and left for longer time and Instrument: Calibration and monitoring of the analyzer (Gulati and Hyun; 1986)

2-4-1 Adapting to Climate Extremes

Humans and many other mammals have unusually efficient internal temperature regulating systems that automatically maintain stable core body temperatures in cold winters and warm summers. In addition, people have developed cultural patterns and technologies that help them adjust to extremes of temperature and humidity. In very cold climates, there is a constant danger of developing hypothermia, which is a life-threatening drop in core body temperature to subnormal levels. The normal temperature for humans is about 98.6° F (37.0°C.). However, individual differences in metabolism, hormone levels, physical activity, and even the time of day can cause it to be as much as 1° F (.6°C.) higher or lower in healthy individuals. It is also normal for core body temperature to be lower in elderly people. Hypothermia begins to occur when the core body temperature drops to 94° F. (34.4° C.). Below 85° F. (29.4°C.), the body cools more rapidly because its natural temperature regulating system (in the hypothalamus) usually fails. The now rapid decline in core body temperature is likely to result in death. However, there have been rare cases in which people have been revived after their temperatures had dropped to 57-60° F.(13.9-15.6°C.) (Dennis ;2012).

Previous studies have shown that decreasing in the core body temperature to less than 38.9 0C with in 30 minutes of presentation improve survival.(Hubbard *et al*;1995).

When core body temperature in excess of 40 0C lead to heat stroke with central nervous system (CNS) dysfunction.(Knochel *etal;* 1994)and (Khogali and Weiner 1980).

The life threatening illness result from failure od thermoregulatory mechanism coupled with an exaggerated acute phase response , causing an elevation in core body temperature and producing multi organ dysfunction .(Shapiro and Seidman 1990) The thermoregulatory control of human skin blood flow is vital to the maintenance of normal body temperatures during challenges to thermal homeostasis. Sympathetic neural control of skin blood flow includes the noradrenergic vasoconstrictor system and a sympathetic active vasodilator system, the latter of which is responsible for 80% to 90% of the substantial cutaneous vasodilation that occurs with whole body heat stress. With body heating, the magnitude of skin vasodilation is striking: skin blood flow can reach 6 to 8 L/min during hyperthermia (Charkoudian, 2003).

2.5 previous study

In USA, Brent L and his Colleges studied the effect of storage at 4ºC on complete blood coun on 2015. They found that three directly measured parameters, RBC, hemoglobin, and platelet values, were essentially unchanged after storage at 4° C. In contrast, while a statistically significant decrease *(P* <.0008) in the WBC count was observed with storage at 4^{0}C.

Study done in Turkey 2011 by Turan Turhan et al. their results found that Refrigerated storage caused a decline in WBC and platelet counts. MPV was increased in the specimens whereas MCHC was decreased.

Naif Taleb Ali (2017) in khartoum recently confirmed that storage caused significant difference in Hematocrit, mean cell volume, MCHC, red cell distribution width, Lymphocytes, granulocyte %, and platelets indices.

In study conducted by Biswajita Rautaray in 2016, her finding concludes Storage of samples at 4 °C, increased the stability of most parameters. CBC parameters, namely WBC, platelet count, haematocrit, MCV, RET% and MCHC, as well as Differential parameters, namely percentages of neutrophils, were more stable when stored at 4 °C. However, some DIFF parameters, namely percentages of eosinophil, basophils and monocytes, had lower stability.

Another study conducted by Gene L. et al in 2002 their results reviled that Blood specimens stored at room temperature for more than 1 day (up to 3 days or possibly longer) were found to be acceptable with some limitations for CBC but not for the differential.

CHAPTER III
MATERIALS AND METHODS

Chapter III

Materials and Methods

3.1 Study design

This was observational cross- sectional study.

3.2 Study area

The study was conducted at khalifa Al Tayeb Laboratory in Khartoum state during the period from March 2019 to January 2021.

3.3 Study population

Seventy Sudanese volunteers were enrolled in this study. The volunteers consisted of purportedly healthy volunteers without a medical history of diseases.

3.4 Ethical consideration

The Sudan University of Sciences and Technology, College of the Medical Laboratory Science approved this study; also, an informed consent was obtained from each participant before sample collection. The participants were issued that the results will be kept highly confidently and will be used for research purpose only.

3.5 Methods

3.5.1 Blood collection

Three milliliters (ml) of blood were collected from anticubital vein from each participant, under aseptic conditions, after cleaning the area around the vein with 70% alcohol, then were poured into EDTA blood container and mixed well before processing. CBC was carried out on the blood samples immediately upon collection to obtain the baseline value (BV) and there after 24-h duration of storage at 4ºC.

3.5.2 Principle of blood analyzer (Sysmex xp-300)

There are two methods are used in the analyzer : Coulter method and colorometric method:

1-Blood cells (, RBCs, WBCs and PLts are counted and sized by the Coulter method. This method is based on the measurement of changes in electrical resistance produced by a particle, which in this case is blood cell, suspended in a conductive diluents as it passes through an aperture of known dimensions .An electrode is submerged in the liquid on both sides of the aperture to create an electrical pathway. As each particles passes through the aperture a transitory change in the resistance between the electrodes is produced. This change produces a measurable electrical pulse. The

number of pulses generated signals the number of particles that passed though the aperture. The amplitude of each pulse is proportional to the volume of each particle .Each pulses is amplified and compared to the internal reference voltage channels which only accepts the pulses of a certain amplitude.

2-Hemoglobin is determined by colorimetric method .The WBC ⁄ HGB dilution is delivered to the WBC bath where it is bubble mixed with a certain amount of lyse ,which converts hemoglobin to HB complex that is measurable at 525 nm .An LED is mounted on one side of the bath and emits a beam of light, which passes through the sample and a 525nm filter, and then measured by photo-sensor that is mounted on opposite side. The signal is then amplified and the voltage is measured and compared to the blank reference reading amplified and the voltage is measured and compared to the blank reference reading.

3.5.3 Procedure

1-The instrument was checked up for the sufficient of reagents, also electric power supply was connected.

2-The power was switched at the back of the analyzer at the (ON) position to turn on the analyzer. The power indicator light illuminated and the screen displayed (Initializing). The analyzer sequentially initializing the file, hardware and fluidic system and whole initialized process last about 3-4 minutes .When the initialization is finished the analyzer is automatically enter count screen.

3-Sample were mixed well and entered to the probe sample then aspirate pressed, when LCD screen was displayed analyzing the sample was removed and the result were printed out.

3.5.4 Quality control

The quality control for CBC in our laboratory internal control is done after every 50samples batch. Bring the control from refrigerator and allow its to cool at room temperature for 15 minutes, then read it in instrument and compare the results to the reading of control in the sheet to see whether the instrument in or out control.

3.5.5 Data analysis

Data were analyzed by using Statistical Package for Social Science (SPSS) program version 21 by paired t. test and expressed as mean ± SD, with p value set at 0.05 to be considered statistically significant.

CHAPTER IV
RESULTS

Chapter IV

Results

4.1 Demographic Data

In seventy samples (thirty_five were males and thirty_five were females) aged (25_50) years collected in this study, Complete Blood Count was estimated for all samples using Sysmex **xp-300** hematological analyzer. The CBC was done on fresh sample and after 24 hour at 4 C.

4.2 Results

The results found that there were significant increase in the mean ± SD of hemoglobin, Hematocrit, MCV and MCH in 24hrs stored sample 12.7 ± 2.1, 39.4 ± 0.78 , 84.9 ± 5.4 and 27.5 ± 2.1 when compared to base line sample 12.6 ± 2.1, 39.08 ±.76, 84.7 ± 5.3 and 27.4 ± 2.1 with p value (0.000), (0.000), (0.000) and (0.001) respectively. Also there was significant decrease in the mean ± SD of RDW-CV and RDW-SD in 24hrs sample 13.6 ± 1.7, 44.9 ± 4.4 when compared to base line sample 13.9 ± 1.7 ± 44.1 ± 4.9 with p value (0.000) and (0.036) respectively, whereas RBCS and MCHC show no difference between two periods (p value= 0.708 and 0.758respectively) (table 3.1).

WBCS and Differential Leukocytes including Lymphocytes count, Lymphocytes % Count, Neutrophils %, Neutrophils count, Mixed %, Mixed Count show No effect between base line and 24hrs stored sample with p value (0.180), (0.578), (0.70), (0.327), (0.125) (0.195) and (0.947) (table 3.2).

The mean ± SD of Platelets show significant decrease while MPV, PDW and PLCR show significant increase on 24 hours 305.2 ± 118.4, 10.7 ± 0.99, 13.8 ± 2, and 31.1 ± 7.5 in comparison to fresh sample 318.8 ± 119.9, 9.5 ± 0.95, 11.7 ± 1.8 and 22 ± 6.9 with p value (0.000), (0.000), (0.000), (0.000) respectively. in contrast PCT is not affected p value = 0.278 (table 3.3).

Table 3.1: The mean and STD of RBCs, Hematocrit and RBCS indices and relation with time

	Base line	24 hrs.	p. value
Hb	12.6 ± 2.1	12.7 ± 2.1	0.000
RBCs	4.57 ±0.7	4.58 ± 0.7	0.708
Hematocrit	39.08 ±.76	39.4 ± 0.78	0.000
MCV	84.7 ± 5.3	84.9 ± 5.4	0.000
MCH	27.4 ± 2.1	27.5 ± 2.1	0.001
MCHC	32.27 ± 1.21	32.25 ± 1.25	0.758
RDW-SD	44.9 ± 4.4	44.1 ± 4.9	0.036
RDW-CV	13.9 ± 1.7	13.6 ± 1.7	0.000

Table 3.2 The mean and STD of WBCs and differential leukocyte counts and relation with time

	Base line	24 hrs.	p. value
WBCS	7.22 ± 2.6	7.09 ± 2.6	0.180
Lymph %	32.4 ± 10.7	32.7 ± 10.5	0.578
Neut%	57.3 ± 12.5	132.4 ± 639.1	0.327
MXD%	10.23 ± 3.9	11.5 ± 6	0.195
Lymph #	2.23 ± 0.76	2.22 + 0.78	0.703
Neutro#	4.3 ± 2.3	4.1 ± 2.1	0.125
MXD#	0.702 ± 0.28	0.705 ± 0.36	0.947

Table 3.3: The mean and STD of RBCs, Hematocrit and RBCS indices and relation with time

	Base line	24 hrs.	p. value
PLTs	318.8 ± 119.9	305.2 ± 118.4	0.000
MPV	9.5 ± 0.95	10.7 ± 0.99	0.000
PDW	11.7 ± 1.8	13.8 ± 2	0.000
PLCR	22 ± 6.9	31.1 ± 7.5	0.000
PCT	0.30 ± 0.10	0.59 ± 2.2	0.278

CHAPTER V

DISCUSSION, CONCULSIONS, RECOMMENDATIONS

CHAPTER V

Discussion, conclusions and recommendations

5.1 Discussion

There is substantial evidence from in vitro studies documenting the change that haematological parameters undergo during storage. It was observed that hematological parameters analyze at 4°C increase and decreased.

In This study, the mean level of HB, HCt, MCV, MCH, MPV, PDW and PLCR were significantly increased after storage for 24hrs. At 4ºC compared to initial results, p value was (0.000), (0.000), (0.000), (0.001), (0.000), (0.000) and (0.000) respectively. These findings show agreement with study done by (Obeidi et al, 2012) who found significant increase in Hb, HCT, MCV and MCH depending on temperature and storage duration and (Ali N T, 2017) who found significant increase in MPV, PDW and PLCR. Such a significant increase can be attributed to storage-related degenerative changes that occur in the RBCs and Platelets that lead to widening of the "pores" on the surface of the RBCs and PLTs, which permit ingress of water into the cells.

In addition, the study shows that the mean level of RDW-CV, RDW-SD and Platelets were significantly decreased after storage for 24hrs. At 4ºC compared to initial results, p value was (0.000), (0.036) and (0.000). These finding in consistent to study conducted By (Ali N T, 2017) who found significant decrease in RDW-CV, RDW-SD and platelets with significant increase in RDW. Platelets count decrease can be attributed to swelling and abrupt of PLTs and RDW decrease due to increase in MCV.

The study also display that, the mean level of RBCS, MCHC, WBCs, Lymph %, Lymph Count, Neut %, Neutr, MXD%, MXD count and PCT show no difference between initial and 24hrs reading, p value (0.708), (0.758), (0.180), (0.578), (0.327). (0.195), (0.703), (0.125), (0.947) and (0.278) respectively which in agreement to result of (Mingoas et al, 2020), (Turan Turhan et al, 2011) and (Ali N T, 2017), were that hemoglobin, MCH, RBC, Neut %, Neutr, MXD%, MXD count and PCT were essentially unchanged after storage at 4° C. In contrast, while a statistically significant Increase in Lymph % and Lymph Count, the was observed with storage at 4° C. The difference between these results and other results may be due environmental factors, different instruments use or technical difference.

5.2 Conclusion

This study conclude that, the level of Hb, HCt, MCV, MCH, MPV, PDW and PLCR were increased , the level of RDW and Platelets were decreased while RBCs, MCHC, WBCs and differential leukocytes were not affected.

5.3 Recommendations

1-Blood samples for complete blood count (CBC) test must be measured immediately as soon as possible after collection.

2-further studies with larger sample size should be done to establish my own values of complete blood count parameter.

3- CBC must be carried out at other environmental temperature and different exposure period to study the seasonal variation.

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APPENDIXES

Appendix (1)

Questionnaire paper

Appendix(A)

Sex: male ( ) female ( )

Age: ( )years

History of disease: yes ( ), No ( )

Treatment: .....

Laboratory Information:

Parameters	Base line reading	24 hrs reading
Hb		
RBCS		
Hct		
MCV		
MCH		
MCHC		
RDW-SD		
RDW-CV		
WBCS		
Lymph %		
Neut%		
MXD%		
Lymph #		
Neutro#		
MXD#		
PLTs		
MPV		
PDW		
PLCR		
PCT		

Appendix(B)

Hematological analyzer instrument