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THE HAEMATOLOGICAL ABNORMALITIES IN APPARENTLY HEALTHY SUDANESE BLOOD DONORS ATTENDING CENTRAL BLOOD BANK IN WAD MADANI, GEZIRA STATE, SUDAN

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ABSTRACT

Objective: To detect the haematological abnormalities in apparently healthy male donors to establish a safety for both donors and recipient and also to transfuse safe blood and blood products. **Material and Methods:** Venous blood samples were taken from 500 apparently healthy males donors and the haematological parameters were measured using an automated cell counter (sysmex KN21), accompanied by peripheral blood films were assessed to detect any haematological abnormalities. For haemoglobin estimation copper sulphate method is routinely performed in Wad medanni blood bank. Although this method is not properly performed. **Results:** In this study it was found that a considerable numbers of donors were anaemic, leucopenic, thrombocytopenic, few numbers with leucocytosis and thrombocytosis, high eosinophil percentage and lymphocyte percentage with reactive forms also detected. **Conclusion:** Copper sulphate method for haemoglobin estimation is not satisfactory, complete blood count and peripheral blood smears should be examined, detection of malaria is recommended to achieve success in blood transfusion services. The study revealed that significant number of anaemic donors and some thrombocytopenic and leucopenic donors.

KEYWORDS: White blood cells, Hemoglobin, Red blood cells, Platelets.

INTRODUCTION

The modern transfusion medicine is concerned with proper selection and utilization of blood components. Safe and efficient blood transfusion practice depends on elimination of clerical errors within the laboratory. Consideration also given to the patients clinical history particularly with respect to pervious transfusion, pregnancy and drugs and a satisfactory pre-transfusion testing to ensure donor-recipient compatibility are essential.

About 5% of the general population donates blood. Almost all donations are from volunteers. The first step in the donation process, registration, makes a record of the donor who can be contacted in the future, if necessary. The information requested include, name, sex, date of birth, telephone number, the donor must also sign a consent.[3]

Very little whole blood is used, this enables each product to be stored under ideal conditions, prolonging its life and making available the appropriate product for a particular clinical situation to allow proper selection and utilization of blood components.

The blood components, red cells, platelets, granulocytes, fresh frozen plasma and cryoprecipitate are made directly from a unit of whole blood. The major goal of transfusion medicine practice has been to reduce the risk of transfusion transmitted infection to as low level as possible. In order to approach the desired level of zero risk from transfusion of allogeneic blood multiple layers of safety are needed.[1] Methods used in attempting to maximize safety from donated allogeneic units, include donor selection criteria, donor medical history, the confidential unit exclusion (CUE) option, donor deferral registries, laboratory testing of donated units and modification of the blood units after collection either by leucocyte removal or physicochemical procedures for pathogen inactivation. All blood donors are asked about their medical history to help determine if they can safely donate blood without experiencing any negative health effects.[2]

White blood cells

The name "White blood cell" derives from the fact that after centrifugation of a blood sample, the white cells are found in the *Buffy coat*, a thin layer of nucleated cells between the sedimented red blood cells and the blood plasma, which is typically white in color. The scientific

term *leukocyte* directly reflects this description, derived from Greek *leukos* - white, and *kytos* - cell.[5]

White blood cells, or leukocytes, are cells of the immune system defending the body against both infectious disease and foreign materials. Several different and diverse types of leukocytes exist, but they are all produced and derived from a multipotent cell in the bone marrow known as a hematopoietic stem cell. Leukocytes are found throughout the body, including the blood and lymphatic system.[5]

Types white blood cells

There are several different types of white blood cells. They all have many things in common, but are all different. One primary technique to classify them is to look for the presence of granules, which allows the differentiation of cells into the categories granulocytes and a granulocytes:[5]

Granulocytes (polymorph nuclear leucocytes):
leukocytes characterized by the presence of differently staining granules in their cytoplasm when viewed under light microscopy. These granules are membrane-bound enzymes which primarily act in the digestion of endocytosed particles. There are three types of granulocytes: neutrophils, basophils, and eosinophils, which are named according to their staining properties.[5]

A granulocytes (mononuclear leucocytes)

leukocytes characterized by the apparent absence of granules in their cytoplasm. Although the name implies a lack of granules these cells do contain non-specific azurophilic granules, which are lysosomes. The cells include lymphocytes, monocytes, and macrophages.[5]

Leucopoiesis

The blood granulocytes are formed in the bone marrow from a common precursor cell (stem cells). In the granulopoietic series progenitor cells, myeloblasts, promyelocytes form a proliferative or mitotic pool of cells while the metamyelocyte, band and segmented granulocytes make up a post mitotic maturation compartment.[5]

The bone marrow normally contains more myeloid cells than erythroid cells in the ratio of 2:1 to 12:1, the largest proportion being neutrophil and metamyelocytes. In the stable or normal state, the bone marrow storage compartment contain 10 -15 times the number of granulocyte found in the peripheral blood. Following their release from the bone marrow granulocyte spend only 6 – 10 hours in the circulation before moving into the tissue where they perform their phagocytic function. It has been estimated that they spend an average 5 – 6 days in the tissues before they are destroyed during defensive actions or as the result of senescence.

Control of leucopoiesis

The granulocytes series arises from bone marrow progenitors cells which are increasingly specialized. Many growth factors are involved in this maturation process including interleukin-1(IL-1), IL-3, IL-5, IL-6, IL-11, granulocyte-macrophage-colony-stimulating factor(GM-CSF), G-CSF and monocyte CSF.

The growth factor stimulate proliferation and differentiation and also affect the function of the mature cells on which they act (e.g. phagocytosis, superoxide generation and cytotoxicity in the case of neutrophils phagocytosis, cytotoxicity and production of other cytokines by monocytes. Increased granulocytes and monocytes production in response to an infection is induced by increased productions of growth factors from stromal cells and T- Lymphocytes stimulated by endotoxin, IL – 1 or tumor necrosis factor TNF.[5]

Neutrophils

This cell has a characteristic dense nucleus consisting of between two to five lobes and pale cytoplasm with an irregular outline containing many fine pink – blue (azurophilic) or grey – blue granules.
The granules divided into:
A – primary – which appear at the promyelocytes stage.
B – secondary which appear at myelocytes stage and predominate in the mature neutrophil.

Both types of granules are lysosomal in origin, the primary contain myeloperoxidase, acid phosphates and other acid hydrolyses, the secondary contains collagenase, lactoferrin and lysozyme, the life span of neutrophil in the blood is only about 10 hours. (days in spleen and other tissue).[6]

Neutrophil precursors

The earliest recognizable precursor is the myeloblast a cell of variable size which has a large nucleus with fine chromatin and usually 2-5 nucleoli.

The cytoplasm is basophilic and no cytoplasmic granules are present, the bone marrow contains up to 4% of myeloblast. Myeloblast give rise by cell division to promyelocytes which are slightly larger cells and have developed primary granules in the cytoplasm.

These cells then produced myelocytes which have specific or secondary granules, the nuclear chromatin is now more condensed and nucleoli are not visible.

The myelocytes give rise by cell division to metamyelocytes, non dividing cells which have an indented or horseshoe shaped nucleus a cytoplasm filled with primary and secondary granules. Neutrophil forms between metamyelocytes and fully mature neutrophil are termed band, stab, or juvenile. These cells may occur in normal peripheral blood. They do not contain the clear fine filamentous distinction between nuclear lobe which is seen in mature neutrophil.[6]

Eosinophils
These cells are similar to neutrophil, except that the cytoplasmic granules are coarser and more deeply red staining and there are rarely more than 3 nuclear lobes.

Eosinophil myelocytes can be recognized but earlier stages are indistinguishable from neutrophil precursors. The blood transit time for eosinophils is longer than for neutrophils. They enter inflammatory exudates and have a special role in allergic responses, defense against parasites and removal of fibrin formed during inflammation.[5]

Basophils
These cells are only occasionally seen in normal peripheral blood. Basophils are chiefly responsible for allergic and antigen response by releasing the chemical histamine causing inflammation. The nucleus is bi- or tri-lobed, but it is hard to see because of the number of coarse granules which hide it. They are characterized by their large blue granules.[5]

Monocytes
These are usually large than other peripheral blood leucocytes and possess a large central oval or indented nucleus with clumped chromatin. The abundant cytoplasm stains blue and contain many fine vacuoles, giving a ground – glass appearance.

The monocytes precursors in the marrow (monoblast, promonocytes) are difficult to distinguish from myeloblast and monocytes. Monocytes spend only a short time in the marrow and after circulating for 20 to 40 hours, leave the blood to enter the tissue where the mature and carry out the principle function. Their extravascular lifespan after their transformation to macrophages may be also long as several months or even years.[5]

Lymphocytes
Lymphocytes are immunologically competent cells that assist the phagocytes in defense of the body against infection and other foreign invasion. In postnatal life, the bone marrow and thymus are the primary lymphoid organs in which lymphocytes develop. The secondary lymphoid organs in which specific immune responses are generated are the lymph nodes, spleen and lymphoid tissues of the alimentary and respiratory tracts.

By their appearance under light microscope there are two broad categories of lymphocytes namely the large granular lymphocytes. Most but not all large granular lymphocytes are more commonly non as natural killer cells.

The small lymphocytes are the T- cells and B- cells. The formation of lymphocytes is known as lymphopoiesis, B-cells mature in the bone marrow and circulate in the peripheral blood until they undergo recognition of antigen. While T- lymphocytes migrated to the thymus where they differentiate into mature T cells during passage from the cortex to the medulla. They live weeks to several years, which are very long compared to other leucocytes. T&B cells are the major cellular component of the adaptive immune response.

T cell are involved in cell mediated immunity where as B cells are responsible for humoral immunity.[5] The function of the T&B cell is to recognize specific antigen, during processes known as antigen presentation. B cell responds to pathogens by producing large quantity of anti bodies which then neutralize foreign objectives (Bacteria, Viruses). In response to pathogens some T cells called helper T cells produce cytokine that direct the immune response while other T cells called cytotoxic T cell produce toxic granules that induce the death of pathogen infected cells.[5] Natural killer cells (NK) are cytotoxic CD 8+ cells that lack the T- cell receptor (TCR) and also they are a part of innate immune system and play a major role in defending the host from both tumor and virally infected cells. N.K distinguish infected cells and tumors from the normal and infected cells by recognizing alteration in level of a surface molecule called major histocompatibility complex class I. They are large cells with cytoplasmic granules and typically express surface molecules CD16 (Fc receptor), CD56 and CD57. NK cells are activated in response to family of cytokines called interferons. Activated NK cells release cytotoxic granules (cell killing) which then destroy the altered cells. They were named (NK cell) because of the initial notion that they don't require prior activation in order to kill cell which are missing Major Histocompatibility Complex I (MHC I).

Red cells
Human erythrocytes
In health, the red blood cells vary relatively little in size and shape. In well-spread, dried, and stained films the great majority of cells have round, smooth contours and diameters within the comparatively narrow range of 6.0–8.5 mm. As a rough guide, normal red cell size appears to be about the same as that of the nucleus of a small lymphocyte on the dried film. The red cells stain quite deeply with the eosin component of Romanowsky dyes, particularly at the periphery of the cell in consequence of the cell's normal biconcavity. A small but variable proportion of cells in well-made films (usually less than 10%) are definitely oval rather than round, and a very small percentage may be contracted and have an irregular contour or appear to have lost part of their substance as the result of fragmentation (schistocytes). According to Marsh, the percentage of "pyknocytes" (irregularly contracted cells) and schistocytes in normal blood does not exceed 0.1% and the proportion is usually considerably less than this, whereas in normal, full-term infants the proportion is higher, 0.3–1.9%, and in premature infants it is still higher, up to 5.6%. Adult humans have roughly $2–3 \times 10^{13}$ red blood cells at any given time (women have about 4 to 5 million erythrocytes per microliter (cubic millimeter) of blood

and men about 5 to 6 million. People living at high altitudes with low oxygen tension will have more). In humans, haemoglobin in the red blood cells is responsible for the transport of more than 98% of the oxygen; the remaining oxygen is carried dissolved in the blood plasma. The red blood cells of an average adult human male store collectively about 2.5 grams of iron, representing about 65% of the total iron contained in the body. Erythrocytes consist mainly of haemoglobin, a complex metalloprotein containing heme groups whose iron atoms temporarily link to oxygen molecules (O_2) in the lungs and release them throughout the body. Oxygen can easily diffuse through the red blood cell's cell membrane.[9]

Haemoglobin in the erythrocytes also carries some of the waste product carbon dioxide back from the tissues; most of the carbon dioxide is however transported as bicarbonate dissolved in the blood plasma. Myoglobin, a compound related to haemoglobin, acts to store oxygen in muscle cells. The colour of erythrocytes is due to the heme group of haemoglobin. The blood plasma alone is straw-colored, but the red blood cells change colour depending on the state of the haemoglobin: when combined with oxygen the resulting oxyhaemoglobin is scarlet, and when oxygen has been released the resulting deoxyhaemoglobin is darker, appearing bluish through the vessel wall and skin.[6]

The red blood cell functions

When erythrocytes undergo shear stress in constricted vessels, they release ATP which causes the vessel walls to relax and dilate. When their haemoglobin molecules are deoxygenated, erythrocytes release S-nitrosothiols which also acts to dilate vessels, thus directing more blood to areas of the body depleted of oxygen. Erythrocytes also play a part in the body's immune response: when lysed by pathogens such as bacteria, their haemoglobin releases free radicals that break down the pathogen's cell wall and membrane, killing it.[6]

The red blood cell membranes and surface proteins

The membranes of red blood cells play many roles that aid in regulating immune recognition and deformability. There are two main types of proteins on the surface:

- Band 3.
- Glycophorins such as glycophorin C.

The blood types of humans are due to variations in surface glycoproteins of erythrocytes.

Life cycle of red blood cells

The process by which red blood cells are produced is called erythropoiesis. Erythrocytes are continuously produced in the red bone marrow of large bones, at a rate of about 2 million per second.

In the embryo, the yolk sac is the main site of red blood cell production. Form 6-7 months of fetal life the liver and spleen are the main organs involved and they continue to produce blood cells until about 2weeks after birth. The bone marrow is the most important site from 6 to 7months of fetal life. During normal childhood and adult life, the marrow is only source of new blood cells. The production of red blood cells are stimulated by the hormone erythropoietin (EPO), synthesised by the kidney. After leaving the bone marrow, the developing cells are known as reticulocytes; these comprise about 1% of circulating red blood cells. Erythrocytes develop from committed stem cells through reticulocytes to mature erythrocytes in about 7 days and live a total of about 100-120 days. The aging erythrocyte undergoes changes in its plasma membrane, making it susceptible to recognition by phagocytes and subsequent phagocytosis in the spleen, liver and bone marrow. Much of the important breakdown products are recirculated in the body.[6]

Red blood cell metabolism

The heme constituent of haemoglobin are broken down into Fe^{3+} and biliverdin. The biliverdin is reduced to bilirubin, which is released into the plasma and recirculated to the liver bound to albumin. The iron is released into the plasma to be recirculated by a carrier protein called transferrin. Almost all erythrocytes are removed in this manner from the circulation before they are old enough to haemolyze. Hemolyzed hemoglobin is bound to a protein in plasma called haptoglobin which is not excreted by the kidney.

Total red blood cell - The number of red cells is given as an absolute number per litre.
Haemoglobin- The amount of haemoglobin in the blood, expressed in grams per decilitre.

Hematocrit or packed cell volume (PCV) - This is the fraction of whole blood volume that consists of red blood cells.[5]

Red blood cell indices

1. The mean corpuscular volume (MCV) - Is the average volume of the red cells, measured in femtolitres. Anemia is classified as microcytic or macrocytic based on whether this value is above or below the expected normal range.
2. Mean corpuscular hemoglobin (MCH) - Is the average amount of hemoglobin per red blood cell, in picograms.
3. Mean corpuscular hemoglobin concentration (MCHC) - Is the average concentration of hemoglobin in the cells.
4. Red blood cell distribution width (RDW) - Is a measure of the variation of the RBC population.[5]

Haemoglobin

Haemoglobin is a protein that is carried by red cells. It picks up oxygen in the lungs and delivers it to the peripheral tissues to maintain the viability of cells and return carbon dioxide for the tissues to the lungs. Each red cell contain approxiately 640 million haemoglobin molecules. Each molecule of normal adult haemoglobin

(HbA) the dominant haemoglobin in blood after age of 3-6 months, consist of four polypeptide chains, alpha2 beta2 each with its own haem group. The molecular weight of Hb A IS 68000. Normal adult blood also contains small quantities of two other haemoglobins Hb F and Hb A2. These also contain alph chains, but with gamma and delta chains respictively, instead of beta.[5]

Platelets

Are small, irregularly-shaped anuclear cells (i.e. cells that do not have a nucleus containing DNA), 2-4 µm in diameter, which are derived from fragmentation of precursor megakaryocytes. The average lifespan of a platelet is between 8 and 12 days.

Platelets play a fundamental role in hemostasis and are a natural source of growth factors. Platelets release a multitude of growth factors including platelet-derived growth factor (PDGF), a potent chemotactic agent, and transforming growth factor-β, which stimulates the deposition of extracellular matrix. Both of these growth factors have been shown to play a significant role in the repair and regeneration of connective tissues. Other healing-associated growth factors produced by platelets include basic fibroblast growth factor, insulin-like growth factor-1 (IGF-1), platelet-derived epidermal growth factor, and vascular endothelial growth factor. Local application of these factors in increased concentrations through PRP (platelet-rich plasma) has been used as an adjunct to wound healing for several decades.[10] Platelets are produced in blood cell formation (thrombopoiesis) in bone marrow, by budding off from megakaryocytes. The physiological range for platelet count is 150-400 x 10^{9} per litre. Around 1 x 10^{11} platelets are produced each day by an average healthy adult. The lifespan of circulating platelets is 7 to 10 days. This process is regulated by thrombopoietin, a hormone usually produced by the liver and kidneys. Each megakaryocyte produces between 5,000 and 10,000 platelets. Old platelets are destroyed by phagocytosis in the spleen and by Kupffer cells in the liver.[8]

Thrombus formation

The function of platelets is the maintenance of haemostasis. This is achieved primarily by the formation of thrombi, when damage to the endothelium of blood vessels occurs. On the converse, thrombus formation must be inhibited at times when there is no damage to the endothelium.

The inner surface of blood vessels is lined with a thin layer of endothelial cells that, in normal hemostasis, acts to inhibit platelet activation by producing endothelial-ADPase, noradrenaline, and PGI2. Endothelial-ADPase clears away ADP, a platelet activator, from platelet surface receptors.[8]

Endothelial cells produce a protein called von Willebrand factor, a cell adhesion ligand, which helps endothelial cells adhere to collagen in the basement membrane. Under physiological conditions, collagen does not pass into the bloodstream. However vWF is secreted constitutively into the plasma by the endothelial cells that produce it, or otherwise is stored within the endothelial cells or in platelets. When endothelial damage occurs, platelets come into contact with exposed collagen and vWF, causing a reduction in secretion of endothelium platelet inhibitors.[8] The inner surface of blood vessels is lined with a thin layer of endothelial cells. Under this is a layer of collagen. When the endothelial layer is injured, the collagen is exposed. Then the platelets adhere to collagen and become activated. They are also activated by thrombin (primarily through PAR-1) and ADP receptors (P2Y1 and P2Y12) expressed on platelets. They can also be activated by a negatively-charged surface, such as glass.[8] Platelet activation further results in the scramblase-mediated transport of negatively-charged phospholipids to the platelet surface. These phospholipids provide a catalytic surface (with the charge provided by phosphatidylserine and phosphatidylethanolamine) for the tenase and prothrombinase complexes.[8]

Shape changes of platelets

Activated platelets change their shape to become more spherical, and pseudopods form on their surface. Thus they assume a stellate [star-like] shape.

Granule secretion

Platelets contain alpha and dense granules. Activated platelets excrete the contents of these granules into their canalicular systems and into surrounding blood. There are two types of granules:

1- dense granules (containing ADP or ATP, calcium, and serotonin)

2- α-granules (containing platelet factor 4, transforming growth factor-β1, platelet-derived growth factor, fibronectin, B-thromboglobulin, vWF, fibrinogen, and coagulation factors V and XIII).[8]

Thromboxane A2 synthesis

Platelet activation initiates the arachidonic acid pathway to produce TXA_2. TXA_2 is involved in activating other platelets.

Adhesion and aggregation

Platelets aggregate, or clump together, using fibrinogen and vWF as a connecting agent. The most abundant platelet aggregation receptor is glycoprotein (GP) IIb/IIIa; this is a calcium-dependent receptor for fibrinogen, fibronectin, vitronectin, thrombospondin, and von Willebrand factor (vWF). Other receptors include GPIb-V-IX complex (vWF) and GPVI (collagen). Activated platelets will adhere, via glycoprotein (GP) Ia, to the collagen that is exposed by endothelial damage. Aggregation and adhesion act together to form the platelet plug. Myosin and actin filaments in platelets are stimulated to contract during aggregation, further reinforcing the plug. Platelet aggregation is stimulated by ADP, thromboxane, and α2 receptor-activation, but

inhibited by other inflammatory products like PGI2 and PGD2. Platelet aggregation is enhanced by exogenous administration of anabolic steroids.[5]

Justification

1. Though complete blood count is available test for detection of haematological abnormalities, it is not currently used in pre-donation evaluation.
2. The current practice for estimation of haemoglobin level by copper sulphate method for blood donors is unreliable.

Objectives

General objective

To detect the haematological abnormalities in apparently healthy Sudanese blood donors in Gezira state.

Specific objectives

1. To perform complete blood count for donors using automated machine (sysmex).
2. To document any abnormalities of the various haematological parameters detected in apparently healthy blood donors.

MATERIAL AND METHODS

Study area

The study was carried out in the Central blood bank, Wad Madani teaching hospital. Wad Madani is the capital of Gezira state, it is considered one of the largest states in Sudan with an area of 35.304 km and population of 4 millions. The Central Blood Bank provide blood donation services to 4 governmental hospitals and other special hospitals in Wad Madani. About 1600 to 1700 donors attend the central blood bank monthly. Different types of blood components (whole blood, packed red cells, platelets, fresh frozen plasma) are prepared from whole blood using large refrigerated centrifuges. All donors are selected according to the accepted criteria for donation including age, weight, physical and medical examination and screening for viral infections (hepatitis B, C and HIV) and the test for syphilis. Haemoglobin level assessment is performed by copper sulphate method and donors are reported as fit for donation if a drop of blood sinks in a copper sulphate solution, of a certain specific gravity.

Study population

Apparently healthy male donors attending the Central Blood Bank (500 donors).

Selection criteria

Donors were selected according to the accepted criteria for donation.

1. Age between 18- 60 years.
2. Weight: 50 Kg (110 pounds) and more.
3. Haemoglobin : 12.5 g/dl.- 17.5 g/dl

Donors were selected with clinical examination (abdominal, cardiopulmonary), pulse and blood pressure were measured, VDRL, hepatitis B,C and HIV were screened.[4]

Exclusion criteria

1. All donors should be clinically in a good health, subject with any disease symptoms and signs should be excluded.
2. Any person taking medications.

Study design

Descriptive, prospective cross sectional study was conducted in Wad Madanni central blood bank, during the period from 16\03\2009 to 26\12\2009.

Methods

Sample collection

A total of 500 apparently healthy adult male donors were screened for complete blood count and peripheral blood smear also were examined. This analysis was conducted at the Wad Madani central blood bank, department of pathology (medical laboratory) and the central laboratory of Wad Madani teaching hospital. Venous Blood samples were taken from an antecubital vein by a 5ml syringe. The site of collection was cleaned using 70% alcohol and left to dry. An elastic tourniquet was applied if needed to the arm for a period not exceeding one minute to avoid haemoconcentration. 2.5 ml of blood was taken into a container with 0.05ml (K2 EDTA) as an anticoagulant with a concentration of 1.5- 2.2 mg/ml and then the sample gently mixed. The blood samples were tested within 2 hours of sample collection using an automated blood cell counters (sysmex KN21 analyzer) with a flow cytometry using a laser light to perform full blood count haemoglobin concentration (Hb), red blood cells count (RBCs) haemotocrit (Hct), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), white blood counts (WBCs) and platelet counts (PLTs). It is calibrated by a standardized commercially prepared calibrators.

Making a blood film

Manual spreading of blood films using frosted glass slides were performed. The frosted glass slides were clean and free of grease. A drop of blood was placed near one end of the slide and spreader was applied at an angle of 45, in front of the drop of blood making a thin blood film using a cover glass as spreader and allowed to dry. Then they were labelled with the donor number and date of sample collection. The films were then fixed in absolute methanol for 10-20 minutes. The films were placed horizontally on the staining rack and flooded with Leishman's stain and left for 4 minutes. A double volume buffer was added with gentle blowing over the surface without touching the film surface. The films were left for another 8 minutes and then washed off with buffered distilled water. The back of the slide was cleaned using cotton dipped in alcohol and then left to dry.[7]

Examination of the blood films

The identification of the specimen was checked and matched with the corresponding full blood count (FBC) report. The films were examined macroscopically to confirm adequate spreading followed by microscopic examination. A low power field (10 objective) to assess the quality of the stain and (40 objective) to determine the suitable area for blood film examination. The manual differential white blood cells were compared with the automated differential white blood cells. The morphology of the red cells regarding the staining character, shape, size of the cells and the presence of nucleated red blood cells are evaluated. The platelets were examined and assessment of their number, size, morphology and presence of aggregate were also evaluated.[7]

Statistical analysis: The results were analyzed using statistical software package of social sciences (SPSS**)** version 17 and descriptive data were expressed as means.

Ethical clearance: Ethical clearance was obtained from the University of Gezira ethical committee and blood bank authority. Verbal informed consent was obtained from all donors.

RESULTS

This prospective descriptive cross sectional study was conducted in wad Madani central blood bank, during the period from 16\03\2009 to 26\12\2009. The aim of this study was to detect the haematological abnormalities in 500 apparently healthy male donors selected according to the accepted criteria for donation including age, weight, physical and medical examination. All donors were subjected to screening for viral infections (hepatitis B, C and HIV) and the test for syphilis. Haematological parameters were estimated using automated cell counter (sysmex KN 21). The various haematological parameters including white blood count, platelet count, haemoglobin concentration, red blood cell count, haemotocrit, mean cell volume, mean cell haemoglobin, mean cell haemoglobin concentration and manual differential white blood counts were performed.

Table-1: Age distributions of 500 apparently healthy male donors.

Age	Number of donors	Frequency	%
17-25 years	8	204	40.8
26-35 Years	11	228	45.6
36-45 years	9	68	13.6

The age mean value was found to be 28.33 years+/- 5.97 standard deviation with a minimum value 17 years and maximum value 45 years.

Table-2: Mean, minimum, maximum values and standard deviation(SD) for the age in 500 apparently healthy Sudanese male donors.

	Number of donor	Minimum Value	Maximum Value	Mean Value	Standard Deviation
Age	500	17 years	45 years	28.33	5.97197

The white cells count mean value was found to be 5.64 +/- 1.7 standard deviation with a minimum value 2.1 and maximum value 14.1, with 77 cases ranged from 2.1 to 3.9, 372 cases ranged from 4 to 8, 46 cases ranged from 8.1 to 11 and 5 cases ranged from 11.1 to 14.1. The differential white blood count was performed manually based on counting 100 cells and expressed as percentage. This manual differential white blood cells were correlated with the automated differential white blood cells.

Table-3: Mean, minimum, maximum values and standard deviation(SD) for the white blood count values in 500 apparently healthy Sudanese male donors.

	Number of sample	Minimum Value	Maximum Value	Mean value	Standard Deviation
WBCs	500	2.1 x 10^9 /L	14.1 x 10^9 /L	5.696	1.7989

The neutrophil count mean value was found to be 54.43 +/- 11.576 standard deviation with a minimum value 12% and maximum value 80%, the lymphocyte count mean value was found to be 41.20 +/- 11.497standard deviation with a minimum value 16% and maximum value 88%. The eosinophil count mean value was found to be 3.18 +/- 2.269 standard deviation with a minimum value 1% and maximum value 16%, the monocyte count mean value was found to be 2.07 +/- 1.496 standard deviation with a minimum value 1% and maximum value 10% and the basophil count mean value was found to be 1.22 +/- 0.441 standard deviation with a minimum value 1% and maximum value 2%.

Table-4: Mean, minimum, maximum values and standard deviation (SD) for the neutrophil % values in 500 apparently healthy Sudanese male donors.

	Number of sample	Minimum Value	Maximum Value	Mean value	Standard Deviation
Neutrophils	500	12%	80%	54.43	11.576

Table-5: Mean, minimum, maximum values and standard deviation (SD) for the lymphocyte % values in 500 apparently healthy Sudanese male donors.

	Number of sample	Minimum Value	Maximum Value	Mean value	Standard Deviation
Lymphocytes	500	16%	88%	41.20	11.497

Table- 6: Mean, minimum, maximum values and standard deviation (SD) for the eosinophils % values in 500 apparently healthy Sudanese male donors.

	Number of sample	Minimum Value	Maximum Value	Mean value	Standard Deviation
Eosinophils	500	1%	16%	3.18	2.269

Table-7: Mean, minimum, maximum values and standard deviation (SD) for the monocyte % values in 500 apparently healthy Sudanese male donors.

	Number of sample	Minimum value	Maximum Value	Mean value	Standard Deviation
Monocytes	500	1%	10%	2.07	1.496

Table-8: Mean, minimum, maximum values and standard deviation (SD) for the basophil % values in 500 apparently healthy Sudanese male donors.

	Number of sample	Minimum value	Maximum Value	Mean Value	Standard Deviation
Basophils	500	1%	2%	1.22	0.441

The mean haemoglobin level was found to be 14.509 g/dl +/- 1.2076 standard deviation with maximum value 17.8 g/dl and minimum value 10.1 g/dl, with 89 cases ranged from 10.1. g/dl to 13.4 g/dl, 360 cases ranged from 13.5 g/dl to 16 g/dl and 51 cases ranged from 16.1 g/dl to 17.8 g/dl and 30 donors (6%) with haemoglobin concentration less than 12.5 g/dl.

The mean level of mean corpuscular volume was found to be 85.08 +/- 5.7391 standard deviation with maximum value 104.3 and minimum value 65.3, with 79 cases ranged from 65.3 to 79.9, 398 cases ranged from 80 to 94.6 and 23 cases ranged from 95.2 to 104.3.

The mean level of mean corpuscular hemoglobin was found to be 28.244 +/- 2.1959 standard deviation with maximum value 34.4 and minimum value 19.1, with 99 cases ranged from 19.1 to 26.9 and 401 cases ranged from 27 to 34.8. The mean level of mean corpuscular hemoglobin concentration was found to be 32.218+/- 1.9002 standard deviation with maximum value 37.4 and minimum value 32.218. The mean hematocrit or packed cell volume level was found to be 43.625 +/- 3.775 standard deviation with maximum value 55.2 and minimum value 24.6 and haemotocrit found to be less than 39% in 45cases (9%) and in 5 cases (1%) more than 52%.

The mean total red blood cell level was found to be 5.1515 +/- 0.45432 standard deviation with maximum value 7.08× 10^{12}/l and minimum value 3.83× 10^{12}/l with 23 cases (4.6%) with mild erythrocytopenia (RBCs < 4.5× 10^{12}/l) and 5 cases (1%) with mild erythrocytosis (RBCs > 6.5× 10^{12}/l)

Table-9: Mean, minimum, maximum values and standard deviation (SD) for the red cells indices values in 500 apparently healthy Sudanese male donors.

	Number of sample	Minimum Value	Maximum Value	Mean value	Standard Deviation
RBCs	500	3.83	7.08	5.1515	.45432
Hb	500	10.1	17.8	14.509	1.2076
HCT	500	24.6	55.2	43.625	3.7750
MCV	500	65.3	104.3	85.08	5.7391
MCH	500	19.1	34.8	28.244	2.1959
MCHC	500	27.2	37.4	32.218	1.9002

The mean platelet level was found to be 215.15 +/- 68.367 standard deviation with maximum value 689 and minimum value 9, with 67 cases ranged from 9 to 149, 426 cases ranged from 150 to 387 and 7 cases ranged from 403 to 689.

Table- 10: Mean, minimum, maximum values and standard deviation (SD) for the platelets values in 500 apparently healthy Sudanese male donors.

	Number of sample	Minimum value	Maximum Value	Mean value	Standard Deviation
PLTs	500	9 x 10^9 /L	689 x 10^9 /L	215.15	68.367

Microscopic examination

Red blood cells

Anisocytosis was observed in 15 cases (3%) with 90 cases with microcytic- hypochromic (18%) and 10 cases with macrocytic RBCs. No rouleauax formation, autoagglutination or nucleated red blood cells detected in the smears.

White blood cells

Hypersegmented neutrophils (1-2) observed in 15 cases (3%) with lymphocytes predominance in 159 cases (31.8%) and reactive lymphocytes (2-10) in 105 cases (21%), eosinophil count more than 4% detected in 72 cases (14%) and 15 cases (3%) with monocyte count more than 8%. No lymphoblast, myeloblast, promyelocytes, myelocytes, metamyelocytes detected in the blood films.

Platelets

Isolated thrombocytopenia from mild, moderate to severe was observed in 67 cases (13.4%) with 7 cases (1.4%) with mild aggregation and giant forms.

DISCUSSION

The approach to the selection of blood donors is to ensure the safety of the donor and to obtain a high quality blood component that is as safe as possible for the recipient.

The steps that are taken before donation are donor selection, medical history, medical and physical examination and conducting a laboratory testing of donated blood to exclude the risk of acquiring a transmitted diseases. Careful donor selection contributes vitally to the safety of both donor and recipient. Complete blood count using automated analyzer (sysmex KN 21) were performed to 500 apparently healthy male donors prior to donation to prevent taking blood from anaemic donors. The minimal level of haemoglobin, haemotocrit and platelet in male blood donors are 12.5 g/dl, 39% and 150 respectively. All donors were screened for haemoglobin estimation using copper sulphate method and their haemoglobin reported as satisfactory for donation.

Erythrocytes

The mean haemoglobin values were 14.5 g/dl +/-1.2076, with minimum count (10.1 g/dl) and maximum count 17.8 g/dl. Haemoglobin less than 12.5 g/dl was obtained in 30 donors (6%) and they were reported as fit for blood donation using copper sulphate for haemoglobin estimation. Those 30 donors actually they are not fit for blood donation because their haemoglobin concentration must be more than 12.5 g/dl. Haemotocrit found to be less than 39% in 45cases (9%), with MCV less than 80 fl in 79 donors (15.8%) and donors with MCH less than 27 pg in 99 (19.8%) which indicate iron deficiency. MCV found to be more than 95 fl in 23 (4.6%) (may be suggestive of megaloblastic anaemia or other causes like smoking and alcohol).

Leucocytes

The mean values of white blood cells were found to be 5.696 +/-1.7989 with minimum count 2.1 x 10^9 /L and maximum count 14.1 x 10^9 /L and 77 donors (15.4%) presented with white blood count less than 4 x 10^9 /L (leucopenic donors).

White blood count more than 11 x 10^9 /L was observed in 5 donors (1%) dominated by neutrophils which suggestive of pyogenic infection. The neutrophil percentage less than 50% observed in 165 donors (33%) and 231 donors (46.2%) and neutrophil percentage more than 70% observed in 26 donors (5.2%) with lymphocyte percentage more than 40% observed acompenied by reactive lymphocytes most probably due to chronic infection.[11] The eosinophils percentage of more than 4% observed in 121 donors (24.2%), the bulk of this eosinophilia probably reflects asymptomatic parasitism (e.g. schistosomiasis). Monocyte percentage more than 8% observed in 6 donors (1.2%).[11]

Platelets

The mean platelet counts were 215.15 x 10^9 /L +/-68.367 with minimum count 9 x 10^9 /L and maximum 689 x 10^9 /L and 67donors presented with platelets count less than 150 which comprise 13.4% of the cases, may be due to asymptomatic parasitism (e.g. malaria), no prominent aggregation or giant forms detected in all cases of thrombocytopenia. Thrombocytosis occurred in 7donors with platelet count more than 400 (1.4% of cases) accompanied by low MCV and low MCH (suggestive of iron deficiency) which the one of causes of thrombocytosis.

Table-11: Comparative Haematological mean values for Sudanese males with, Western Values, Pakistani, Saudi and Basotho.

	Sudanese	Western	Pakistani	Saudi	Basotho
Hb	14.5	15.5	13.04	14.88	13.87
WBCs	5.7	7.5	8.25	6.043	4.94
Platelet	215.015 x 10^9 /L	262 x 10^9 /L	255 x 10^9 /L	-	-

CONCLUSION

1. The screening of Hb level by copper sulphate method only, will not revealed the true haematological status of the blood donors.
2. The study revealed that significant number of anaemic donors were not detected by estimation of Hb by copper sulphate method.
3. Other parameters were not looked for, therefore they will be missed.
4. Although incidental findings such as CLL, CML or pancytopenia were not detected, some donors with such condition may have been missed without performing Full Blood Counts.

Recommendations

1. The blood transfusion services in Sudan should follow the standard international guidelines and the donor questionnaire form should be properly completed.
2. Copper sulphate method for Hb estimation is not satisfactory, FBC and peripheral blood smears should be done.
3. Donors with abnormal peripheral blood picture and abnormal serological tests should be deferred from donation and referred to a physician for further examination and management.
4. Although Sudan is now considered to be an endemic area for malaria, the present effort will eventually lead to eradication of the disease. Screening blood donors for malaria will help in this effort and in the future will become an important factors in continuous control of the disease.

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