World Journal of Advanced Research and Reviews
eISSN: 2581-9615 CODEN (USA): WJARAI
Cross Ref DOI: 10.30574/wjarr
Journal homepage: https://wjarr.com/

(RESEARCH ARTICLE)

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Evaluation of hemoglobin concentration, red blood cell indices and microscopic examination of red blood cell in Sudanese blood donors in Gezira state (Sudan) - 2023

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World Journal of Advanced Research and Reviews, 2023, 18(03), 1028–1035

Publication history: Received on 01 May 2023; revised on 18 June 2023; accepted on 20 June 2023

Article DOI: https://doi.org/10.30574/wjarr.2023.18.3.1119

Abstract

Objective: To detect hemoglobin level and Red blood cell indices in apparently healthy Sudanese male, to establish safety for both donor and recipient. To perform hemoglobin estimation and Red blood cell indices for blood donors using automated machine (Blood cell counter).

Material and Methods: Venous blood samples were taken from 200 apparently healthy males donors and Hemoglobin level was measured using an automated cell counter (Sysmex KN21), accompanied by peripheral blood films were assessed to detect any abnormalities.

Results: The study revealed that the mean hemoglobin values were 14.5 g/dl ±1.2076, with minimum count (10.1 g/dl) and maximum count 17.8 g/dl. Hemoglobin 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 hemoglobin estimation. Those 30 donors actually they are not fit for blood donation because their hemoglobin concentration must be more than 12.5 g/dl.

Conclusion: The study revealed that a significant number of anemic donors were not detected by estimation of Hb by copper sulphate method.

Keywords: Hb; MCV; RBCs; HCT

1. 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 is 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.[1]

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

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risk of transfusion transmitted infection to as low level as possible. In order to approach the desired level of zero risk of 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 leukocyte 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. [1]

1.1. Red blood cells

Erythropoiesis is the generation of red blood cells carrying the respiratory pigment haemoglobin, for the transport of oxygen to the tissues. This process, from the erythroid commitment of multipotent haemopoietic stem cells (HSCs), through the maturation of erythroblasts, to the terminal differentiation of red blood cells, is governed by complex transcriptional and epigenetic programmes, in response to extracellular signaling.

Erythropoiesis normally maintains the steady state of an individual's red cell mass, producing 1011–1012 new cells per day to replace those that are lost through senescence or premature destruction.

Furthermore, erythropoiesis must be able to respond rapidly to erythroid stress such as haemorrhage and haemolysis. Perhaps unsurprisingly, this system is remarkably sensitive to systemic disease, with anaemia being a common manifestation of a wide

range of inherited and acquired clinical disorders. Understanding the basic biology of erythropoiesis provides a logical basis for the diagnosis and treatment of the inherited and acquired anaemias that are so frequently encountered in clinical practice. [2]

1.2. The origins of erythroid cells during development:

Both primitive (embryonic) and definitive (fetal/adult) HSCs endothelial cells are thought to arise from a common progenitor, the haemangioblast, which has the potential to form both blood and vessels. Erythropoiesis occurs in waves that emerge from several sites in the developing embryo, and begins at the same time as development of the circulatory system. Primitive erythropoiesis is first evident at around three weeks of gestation, and arises from the blood islands of the

extraembryonic yolk sac. A second wave of haemopoietic activity emerges from the yolk sac at approximately 4 weeks' gestation, and marks the onset of definitive erythropoiesis. Erythroid progenitors released into the circulation at this time pass

to the liver, which becomes the main site of erythropoiesis in the fetus. A final wave of haemopoietic activity occurs in the aorta–gonad–mesonephros (AGM) region, the placenta and the major vessels at approximately 4–6 weeks of gestation. By 10–12 weeks, haemopoiesis starts tomigrate to the bonemarrow, where blood formation becomes established during the last three months of gestation. [2]

Primitive and definitive erythropoietic cells are distinguished by their morphology, cytokine responsiveness, growth kinetics, transcription factor programmes, epigenetic programmes and patterns of gene expression. Importantly, the types of haemoglobin produced are quite distinct in embryonic (Hb Gower I $\zeta 2\varepsilon 2$, Hb Gower II $\alpha 2\varepsilon 2$ and Hb Portland $\zeta 2\gamma 2$), fetal (HbF $\alpha 2\gamma 2$) and adult (HbA $\alpha 2\beta 2$ and HbA2 $\alpha 2\delta 2$) erythroid cells. These specific patterns of globin expression provide critical markers for identifying the developmental stages of erythropoiesis.

It remains unclear whether primitive and definitive haemopoiesis in mammals have entirely separate origins or if they are both derived from common stem cells that arise during early development. Accurately defining the embryological origins of these cells is important for understanding arise in close association with endothelial cells. HSCs and the normal mechanisms that establish and maintain HSCs and how these programmes are subverted in common haematological disorders. [2]

1.3. Terminal maturation of committed erythroid cells:

Once the erythroid programme has been specified, the final phase of erythropoiesis involves the maturation of committed erythroid progenitors to fully differentiated red cells. The earliest recognizable erythroid precursor in the bone marrow is the

pronormoblast. Division of pronormoblasts leads to progressively smaller basophilic normoblasts, early polychromatic and finally late polychromatic/orthochromatic normoblasts. It has been estimated that, on average, four divisions occur within the morphologically recognizable proliferating precursor pool, so that each newly formed pronormoblast develops into 16 red cells. As a small amount of cell death (ineffective erythropoiesis) normally occurs, the average amplification is slightly less than 16-fold. The majority (60–80%) of pronormoblasts, basophilic normoblasts and early

polychromatic normoblasts are in cell cycle. By contrast, late polychromatic/orthochromatic erythroblasts are postmitotic, non-dividing cells. In the final stages of terminal maturation, the nucleus condenses further and is eventually extruded. This produces the mature reticulocyte, which has no nucleus, but retains a few mitochondria and ribosomes. The cytoplasm of reticulocytes is predominantly pink on Wright–Giemsa staining because of the high concentration of haemoglobin, but it has a greyish tint due to the presence of ribosomes. [2]

When stained supravitally, the ribosomes precipitate into basophilic granules or a reticulum. Reticulocytes continue to synthesize haemoglobin for 24–48 hours after leaving the bone marrow.

1.4. Red cell senescence and clearance:

Mature red cells live for approximately 120 days in the circulation under normal conditions, suggesting thatmechanisms exist to monitor their senescence and control their removal from the circulation. Since mature red cells have no nucleus, they lack the capacity to synthesize new cellular components. Their ability to maintain cellular integrity becomes compromised with age, and characteristic features of the ageing red cell include increased glycation of haemoglobin and deamination of cytoskeletal components such as protein 4.1. Microvesiculation, which represents an effective means of removing damaged or ineffective red cell components, results in the continual loss of small fragments of the red cell, producing cells that are more dense and less deformable in the microcirculation than their younger counterparts.

As well as these changes, however, specific cues for clearance of the aged red cell from the circulation are thought to exist. Phosphatidylserine and phosphatidylethanolamine are key constituents of the red cell membrane that are normally confined to the inner aspect of the lipid bilayer. This asymmetry is maintained by an ATP-dependent aminophospholipid translocase. In senescent cells, phosphatidylserine is found in the outer leaflet, where it is able to bind to macrophages in the liver and spleen and prompt erythrophagocytosis. Other senescence-related neo-antigens on the red cell surface may be generated by the clustering of membrane proteins such as band 3, thought to occur in response to oxidative change. Following erythrophagocytosis, the red cell components, including the iron from its haem groups, are recycled for subsequent red cell synthesis.

It is possible to compensate for a small decrease in the lifespan of mature red cells through increased Epo production and a reduction in apoptosis. Even when compensatory mechanisms are able to drive increased red cell synthesis, more significant reductions in red cell survival will lead to haemolytic anaemia, Systemic illness may also limit red cell survival, with the short red cell lifespan in uraemia and the anaemia of inflammation being well described, if not well understood.

In health, the red blood cells vary relatively little in size and shape. [2]

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. [3]

A small but variable proportion of cells in well-made films (usually less than 10%) are definitely an 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%. [3]

Adult humans have roughly 2–3 × 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. [4]

People living at high altitudes with low oxygen tension will have more). In humans, the hemoglobin 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 (Dacie 2006). Erythrocytes consist mainly of hemoglobin, 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 membrane. The 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 hemoglobin, acts to store oxygen in muscle cells. The color of erythrocytes is due to the heme group of hemoglobin.

The blood plasma alone is straw-colored, but the red blood cells change color depending on the state of the haemoglobin: when combined with oxygen the resulting oxyhemoglobin is scarlet, and when oxygen has been released the resulting deoxyhemoglobin is darker, appearing bluish through the vessel wall and skin. [5]

1.5. 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. [5]

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. [5]

1.6. 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.

1.7. 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. [6]

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 the only source of new blood cells.

The production of red blood cells is stimulated by the hormone erythropoietin (EPO), synthesized 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. [6]

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. Many of the important breakdown products are recirculated in the body. [7]

1.8. Red blood cell metabolism

The heme constituent of haemoglobin is 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. [7]

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. 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. [4]

1.9. Red blood cell indices

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. Mean corpuscular hemoglobin (MCH) - Is the average amount of hemoglobin per red blood cell, in picograms. Mean corpuscular hemoglobin concentration (MCHC) - Is the average concentration of hemoglobin in the cells. Red blood cell distribution width (RDW) is a measure of the variation of the RBC population. [4]

1.10. 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 contains approximately 640 million hemoglobin molecules. Each molecule of normal adult hemoglobin (HbA) the dominant hemoglobin in blood after the 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 hemoglobin Hb F and Hb A2. These also contain alpha chains, but with gamma and delta chains, respectively, instead of beta. [1]

2. Material and Methods

2.1. Material

2.1.1. 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 a population of 4 million. The Central Blood Bank provides 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. A 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.

2.1.2. Study population

Apparently healthy male donors attending the Central Blood Bank (200 donors).

2.1.3. Selection criteria

Donors were selected according to the accepted criteria for donation.

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

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

2.1.4. Exclusion criteria

- All donors should be clinically in a good health, subject with any disease symptoms and signs should be excluded.

- Any person taking medications

2.1.5. Study design

Descriptive, prospective cross sectional study was conducted in wad Madani central blood bank, during the period from March 2023 to April 2023.

2.2. Methods

2.2.1. Sample collection

A total of 200 apparently healthy adult male donors was screened for Hemoglobin level and red blood cells indices. This analysis was conducted at the Wad Madani central blood bank. 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 in 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 counter (sysmex KN21 analyzer) with a flow cytometry using a laser light to perform white blood count. It is calibrated by a standardized commercially prepared calibrators. [4]

2.2.2. 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. [4]

2.2.3. Examination of the blood films

The identification of the specimen was checked and matched with the white blood cells 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 red blood cells were examined and an assessment of their size, morphology. [4]

2.3. Statistical analysis

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

2.4. 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.

3. Results

The study included a total of 200 apparently healthy Sudanese male blood donors. The mean hemoglobin level was found to be 14.53g/dl+/- 1.211 standard deviation with maximum value 17.9 g/dl and minimum value 10.3 g/dl, with 35 cases ranged from 10.1.g/dl to 13.4 g/dl, 144 cases ranged from 13.5g/dl to 16 g/dl and 21 cases ranged from 16.1g/dl to 17.8g/dl and 13 donors (6.5%) with hemoglobin concentration less than 12.5g/dl. The mean level of mean corpuscular volume was found to be 86.01 ± 5.741 standard deviation with maximum value 103.9 and minimum value 64.3, with 32 cases ranged from 65.3 to 79.9, 159 cases ranged from 80 to 94.6 and 9 cases ranged from 95.2 to 104.3.

The mean level of mean corpuscular hemoglobin was found to be 28.254 ± 2.284 standard deviation with maximum value 35.5 and minimum value 19.8, with 40 cases ranged from 19.1 to 26.9 and 160 cases ranged from 27 to 34.8. The mean level of mean corpuscular hemoglobin concentration was found to be 32.315 ± 1.911 standard deviation with maximum value 37.8 and minimum value 27.7. The mean hematocrit or packed cell volume level was found to be 43.63 ± 3.783 standard deviation with maximum value 55.4 and minimum value 24.8 and hematocrit found to be less than 39% in 22 cases (11%) and in 3 cases (1.5%) more than 52%. The mean total red blood cell level was found to be 5.09 ± 0.4532 standard deviation with maximum value 6.88 × 10^{12}/l and minimum value 3.91× 10^{12}/l with 9 cases (4.5%) with mild erythrocytopenia (RBCs **<**4.5× 10^{12}/l) and 3 cases (1.5%) with mild erythrocytosis (RBCs **>** 6.5 × 10^{12}/l).

Table 1 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	200	3.91	6.88	5.09	.4523
Hb	200	10.3	17.9	14.53	1.211
HCT	200	24.8	55.4	43.63	3.783
MCV	200	64.3	103.9	86.01	5.741
MCH	200	19.8	35.5	28.254	2.284
MCHC	200	27.7	37.8	32.315	1.911

Microscopic examination: Anisocytosis was observed in 9 cases (4.5%) with 42 cases with microcytic- hypochromic (21%) and 8 cases with macrocytic RBCs (4%). No rouleauax formation, autoagglutination or nucleated red blood cells detected in the smears.

4. Discussion

The minimal level of Hb, HCT, MCV, MCH in blood donors are 10.3 g/dl and 24.8%, 64.3, 19.8 respectively, which were consistent with microcytic – hypochromic anemia, microscopic examination was performed and microcytic – hypochromic RBCs was observed in 21% of donors, most probably due to iron deficiency anemia. High Hb, HCT with erythrocytosis were detected in 3% of all samples which suggestive of erythrocytosis. High MCV count with microscopic macrocytes were detected in 8 donors may be nutritional or due to other causes (like smoking, alcohol and liver disease). The majority of the donors were within normal values of Hb and RBCs indices.

5. Conclusion

The study revealed that a significant number of microcytic – hypochromic anemia, some macrocytosis and few with high Hb, HCT and RBCs.

Complete blood count is very important and must be performed using automated machines as routine test before donation.

Compliance with ethical standards

Acknowledgments

Authors are grateful to the staff of the Wad Madani Central Blood Bank, MOH – Gezira state.

Disclosure of conflict of interest

The authors do have not any conflicts of interest in this case report and any financial resources.

Statement of ethical approval

Ethical approval was obtained from the University of Gezira ethical committee and blood bank authority.

Statement of informed consent

Informed consent and verbal permission were obtained from the donors before the submission of this article.

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