

# Hemoglobin level And Red Blood Cell Indices In Apparently Healthy Sudanese Blood Donors in Gezira state (Sudan)

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**Objective:** To detect hemoglobin level and Red blood cell indices in apparently healthy male donors, 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 500 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  $\pm$  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 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.

**Key words:** Hemoglobin (HB), packed cell volume (PCV), Red Blood Cells (RBC), Mean corpuscular volume (MCV), Mean corpuscular hemoglobin (MCH), Mean corpuscular hemoglobin Concentration (MCHC).

## 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 previous 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 consent. 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 of transfusion of allogeneic blood multiple layers of safety are needed (Dodd 2002). 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 (Dodd 2002).

### Red blood cells

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  $\mu\text{m}$ . 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 (Bain BJ -1996). 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 "pyknotocytes" (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% (Bain BJ -1996). Adult humans have roughly  $2\text{--}3 \times 10^{13}$  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 (Dacie 2006). 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 ( $\text{O}_2$ ) 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 (Amador 1975).

### 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 (Amador 1975).

### 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 (Daniel Catovsky - 2005). From 6-7 months of fetal life the liver and spleen are the main organs involved and they continue to produce blood cells until about 2 weeks after birth. The bone marrow is the most important site from 6 to 7 months 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. 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 (A.V. Hoffbrand – 2005).

### Red blood cell metabolism

The heme constituent of haemoglobin is broken down into  $\text{Fe}^{3+}$  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 (A.V. Hoffbrand – 2005). 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 is expressed in grams per deciliter. Hematocrit or packed cell volume (PCV) - This is the fraction of whole blood volume that consists of red blood cells (Dacie 2006).

### 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 (Dacie 2006).

## 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 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 (Dacie 2006).

## MATERIAL AND METHODS

### Study area

The study was carried out in the Central blood bank, Wad Medanni teaching hospital. Wad Medanni 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 Medanni. 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.

### 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.

- 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.

### 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.

## Study design

Descriptive, prospective cross sectional study was conducted in wad medanni 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 Hemoglobin level and red blood cells indices. This analysis was conducted at the Wad Medanni central blood bank, department of pathology (medical laboratory) and the central laboratory of the Wad Medanni 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 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 standardized commercially prepared calibrators (Dacie 2006).

### 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 labeled 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 (Dacie 2006).

### 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 objectives) to assess the quality of the stain and (40 objectives) to determine the suitable area for blood film examination (Dacie 2006). The red blood cells were examined and an assessment of their size, morphology.

### 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

Table (1) showed: The mean hemoglobin level was found to be 14.509g/dl $\pm$  1.2076 standard deviation with maximum value 17.8g/dl and minimum value 10.1 g/dl, with 89 cases ranged from 10.1g/dl to 13.4 g/dl, 360 cases ranged from 13.5g/dl to 16g/dl and 51 cases ranged from 16.1g/dl to 17.8g/dl and 30 donors (6%) with hemoglobin concentration less than 12.5g/dl. The mean level of mean corpuscular volume was found to be 85.08  $\pm$  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  $\pm$  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 $\pm$  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 $\pm$  3.775 standard deviation with maximum value 55.2 and minimum value 24.6 and hematocrit 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 $\pm$  0.45432 standard deviation with maximum value 7.08 $\times$  10<sup>12</sup>/l and minimum value 3.83 $\times$  10<sup>12</sup>/l with 23 cases (4.6%) with mild erythrocytopenia (RBCs >4.5 $\times$  10<sup>12</sup>/l) and 5 cases (1%) with mild erythrocytosis (RBCs <6.5 $\times$  10<sup>12</sup>/l).

## Microscopic examination

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

## DISCUSSION

The minimal level of haemoglobin, haematocrit in male blood donors are 12.5 g/dl and 39% respectively. All donors were screened for haemoglobin estimation using copper sulphate method and their haemoglobin reported as satisfactory for donation. The mean hemoglobin values were 14.5g/dl  $\pm$  1.2076, with minimum count (10.1 g/dl) and maximum count 17.8 g/dl. Haemoglobin less than 12.5g/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 12.5g/dl. Hematocrit 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 anemia or other causes like smoking and alcohol, liver disease).

## CONCLUSION AND RECOMMENDATIONS

1. The screening of Hb level by copper sulphate method only, will not reveal the true hematological status of the blood donors.
2. The study revealed that a significant number of anemic donors were not detected by estimation of Hb by copper sulphate method.
3. Copper sulphate method for Hb estimation is not satisfactory; Full Blood Count and peripheral blood smears should be done.

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