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Platelets count in apparently healthy Sudanese blood donors in Gezira state (Sudan)

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ABSTRACT

To detect Platelets count in apparently healthy male donors, to establish safety for both donors and recipient and also to transfuse safe blood and blood products. To perform platelets count for donors using automated machine (Blood cell counter). Venous blood samples were taken from 500 apparently healthy males donors and the platelets count was measured using an automated cell counter (sysmex KN21), accompanied by peripheral blood films were assessed to detect any abnormalities. The study revealed that the mean values of the platelets 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. The study revealed thata significant number of donors with low & high platelets count

Key words: Platelets, Thrombocytosis, Thrombocytopenia

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 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 from transfusion of allogeneic blood multiple layers of safety are needed [7]. 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 [7].

Platelets: Are small, irregularly-shapeda nuclear 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. 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.[1]

Thrombus formation: The function of platelets is the maintenance of hemostasis. 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. 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 WF 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 [4].

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[7]. 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[5].

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 [3].

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) [2].

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 [8].

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 [2].

Platelet Count: There is a slight diurnal variation of about 5% this occurs during the course of a day as well as from day to day.Within the wide normal reference range, there are some ethnic differences, and in healthy West Indians and Africans platelet counts may on average be 10–20% lower than those in Europeans living in the same environment. There may be a sex difference; thus, in women, the platelet count has been reported to be about 20% higher than in men. A decrease in the platelet count may occur in women at about the time of menstruation. There are no obvious age differences; however, in the first year after birth the platelet count tends to be at the higher level of the adult normal reference range. Strenuous exercise causes a 30–40% increase in platelet count the mechanism is similar to that which occurs with leucocytes [6].

MATERIALS 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 population of 4 millions. 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 . Hemoglobin 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.
- Hemoglobin : 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.

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 platelets count.This analysis was conducted at the Wad Medanni central blood bank, department of pathology (medical laboratory) and the central laboratory of 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 hemoconcentration. 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[5].

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 white blood count. It is calibrated by a standardized commercially prepared calibrators[5].

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[5].

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[5].The aim of this study was to detect the platelets count 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.

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

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- 1 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	9×10^9 /L	689×10^9 /L	215.15	68.367

Microscopic examination:
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 transmitted diseases. Careful donor selection contributes vitally to the safety of both donor and recipient. The mean platelet counts were 215.15×10^9 /L +/-68.367 with minimum count 9×10^9 /L and maximum 689×10^9 /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-2 Comparative Hematological mean values for Sudanese males with, Western Values, Pakistani

Sudanese	Western	Pakistani
215.015×10^9 /L	262×10^9 /L	255×10^9 /L

CONCLUSION

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 significant number of donors with low & high platelets count.
3- The blood transfusion services in Sudan should follow the standard international guidelines and the donor questionnaire form should be properly completed.

4- Donors with abnormal platelets counts should be deferred from donation and referred to a physician for further examination and management.

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