A STUDY ON CHANGES IN HEMATOLOGICAL PARAMETERS DUE TO MULTIPLE BLOOD DONATIONS

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IN

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LIST OF ABBREVATIONS USED

EDTA	Ethylene Diamine Tetra Acetate.
SD	Standard Deviation
WHO	World Health Organization
NACO	National AIDS Control Organization
RBC	Red Blood Cell
Hb	Hemoglobin
HCT	Hematocrit
MCV	Mean Corpuscular Volume
MCH	Mean Corpuscular Hemoglobin
MCHC	Mean Corpuscular Hemoglobin Concentration
RDW	Red cell Distribution Width
WBC	White Blood Cell
HIV	Human Immuno-deficiency Virus
HBOCs	Hemoglobin-Based Oxygen Carriers
PFBOCs	Perfluorocarbon-Based Oxygen Carriers

ABSTRACT

BACKGROUND

Blood is the living force of our body and currently there are no substitutes for it. People all over the world require blood and its products for various reasons every day. This constant demand of blood is fulfilled by the voluntary unpaid blood donors, who are the lifeline of a blood transfusion service. Hence, the health of these donors is of utmost importance.

The only possible adverse effect of blood donation is iron deficiency and anemia in the donors. So, it is important that the blood donors should undergo regular health check-ups to look for signs of anemia. Performing iron studies is not feasible in all the donors. So, there is a need to identify at risk and anemic donors with basic hematological investigations.

OBJECTIVE

To study the changes in hematological parameters among regular blood donors due to multiple blood donations by comparing them against the normal reference values.

MATERIALS AND METHODS

A cross-sectional study was carried out on the regular blood donors, fulfilling the inclusion criteria, donating at the blood bank at BLDE (DU) Shri B.M. Patil Medical College, Hospital and Research Centre, Vijayapur and at the voluntary blood donation camps organized by it.

Study period: 1st November, 2016 to 30th June, 2018.

Two ml of anticoagulated venous blood was taken from the donors before donation and analyzed for RBC count, HCT, Hb, MCV, MCH, MCHC, RDW, WBC count, Differential count and Platelet count using an automated hematology analyzer.

RESULTS

In the present study, out of the 132 blood donors screened, 52 (39.4%) donors were found to be anemic. Out of these, 44 donors showed mild anemia, 10 showed moderate anemia while 2 were severely anemic. A negative correlation was observed between RBC count, Hb, HCT, MCV, MCH, MCHC and platelet count with number of donations. Significant change was noted in RBC count, Hb and HCT parameters.

It was noted that the anemic donors in comparison to the non-anemic donors showed reduction in RBC count, Hb, HCT, MCH, MCHC and increase in RDW, with significant changes seen in Hb, HCT, MCH, MCHC and RDW parameters.

It was also observed that many non-anemic donors showed significant changes in other RBC parameters. Out of the 80 non-anemic donors, 32.5% donors had decreased RBC count, 35% showed low MCHC, 11.3% showed low HCT and 28.8% showed increase in RDW levels.

An unexpected finding of eosinophilia was also noted in 18.9% of the blood donors.

CONCLUSION

Prevalence of anemia is high among the regular blood donors, which is mainly due to iron deficiency in the donors. Hence, donor counselling, regular health check-up and post-donation free iron supplementation are the need of the hour to keep our blood donors healthy.

KEY WORDS:

Blood, Donation, Anemia, Regular donors.

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INTRODUCTION

Voluntary unpaid blood donation is considered a humanitarian as well as a life-saving act for the benefit of those in need. This noble act is capable of saving lives of millions of people worldwide. However, there is a severe dearth of this valuable resource and so, blood donors are encouraged to donate blood at regular intervals.

Therefore, these voluntary unpaid blood donors form the lifeline of a blood transfusion service. The primary responsibility of a blood transfusion service in such a scenario is to ensure that the act of blood donation is safe and causes no harm to the donor or the recipient and to provide a safe, sufficient and timely supply of blood and its products.

So, to uphold these standards, strict regulations have been established for the selection of blood donors that protects both the donor and the recipient. An acceptable frequency of donation is normally two to three times a year or every 12 weeks.[1,2]

Anemia is a recognized complication of regular blood donation as, a donation of 450 ml of blood, leads to a loss of 220-240mg of haem iron. So, a pre-donation hemoglobin cut-off value of 12.5g/dl is often recommended.[3–5]

Many studies have been done on the prevalence of anemia and the status of iron stores among regular blood donors.[2–8] However, performing iron studies on every blood donor is quite expensive and not feasible for detection of iron deficiency anemia. Moreover, little effort has been made to evaluate other non-RBC hematological parameters among regular donors. So, in the present study, the changes in hematological parameters among the regular blood donors were evaluated and an attempt was made to identify anemic and at-risk blood donors.

AIMS AND OBJECTIVES

To study the changes in hematological parameters among regular blood donors due to multiple blood donations by comparing them against the normal reference values.

REVIEW OF LITERATURE

People all over the world, belonging to all walks of life, require blood transfusions to survive every second of every day, making the need for blood omnipresent. According to WHO, around 1,30,000 women die each year during pregnancy or childbirth and approximately 99% of these deaths occur in developing countries due to haemorrhage which invariably requires blood transfusion.[9,10] The demand and reasons for blood vary from place to place some of which include [9,10]:

- Blood transfusions for accidents and injuries, the number for which is growing day by day
- Children with anemia due to malnutrition or infectious diseases and women with high-risk pregnancies and childbirth are affected in developing countries, due to chronic shortage of blood and its products
- Ever increasing demand for blood as developing countries expand their diagnostic and treatment options
- Even the developed countries with their advancement in treatment regimens and procedures require blood

Global Blood Requirement

According to WHO, at least 1% of the population should donate blood to meet a country's most basic requirement of blood,[10,11] while the Lancet commission on Global Surgery suggests a blood donation rate of no less than 15 units/1000 people/ year.[12,13] Globally, more than 80 million units of blood are collected per year. Approximately half of these donations take place in developed countries, where only 19% of the world population resides.[10,14,15] A closer look shows that the median blood

donation rate is 36.8 units/1000 people/year in high income countries while it is 11.7 and 3.9 in middle and low-income countries respectively.[13,14] These figures show the disparity in blood donation among developed and developing countries.

In India, the donation rate is about 4 units/ 1000 people/ year.[12] If the 1% WHO criteria is taken, then, India with a population of 1.2 billion requires at least 12.8 million units of blood annually.[11] According to the NACO data,[11] in the year 2015, a total of 11.6 million units of blood were collected, leading to a shortfall of about 1.2 million units of blood annually.

History of Transfusion medicine

Humans have known about blood since as early as 2500 B.C. Egyptians drew out the blood from the body, thinking it would cleanse the body of diseases. The ancient Greeks performed human dissections in 500 B.C. to understand the flow of blood throughout the body.[16] They believed that blood was formed in the heart and carried throughout the body through veins, while arteries transported air from the lungs.

It was William Harvey, a British physician, who described how blood circulates throughout the body in 1628 A.D. Shortly after that in 1665, the first recorded successful blood transfusion took place, where another British physician Richard Lower transfused blood from one dog to another.[17] However, the first successful human blood transfusion was carried out by James Blundell, a British obstetrician in the year 1818 for treatment of postpartum haemorrhage.[16,18,19] Nevertheless, blood transfusions, up to the early part of 20th century, were neither safe nor efficient.

A true breakthrough of sorts was made in the field of transfusion medicine by Karl Landsteiner in 1901, when he discovered the ABO blood group system and in 1939, along with Alexander Weiner, Philip Levine and R.E. Stenson, discovered the Rh blood group system.[20–22] This ushered in the era of immunohematology and safe blood transfusion as we know today.

It was in 1916 when Rous *et al*.[23,24] prepared an anticoagulant and preservative for blood storage, which was comprised of sodium chloride, isocitrate and dextrose. This led to use of stored blood during World War I and the first large scale blood bank was started in 1937 at Chicago.[21] Until this time, tubes, bottles and flasks made of glass were used for handling and storage of blood. It was in 1960s, that plastic bags made of polyvinyl chloride, replaced glass bottles allowing for a safer and easier collection of blood and reduced wastage.[23,25] These plastic bags had an additional advantage as they permitted removal of plasma following centrifugation for component preparation.[26]

Edwin Cohn developed the first cell separator in 1951. This apparatus allowed the separation of blood components into red cells, white cells, platelets and plasma.[27] This invention coupled with the use of plastic blood bags led to the concept of component preparation in blood banks.

With further improvement and refinement in blood collection and storage, apheresis was started in 1972, wherein one component of blood was collected and the rest was returned to the donor. The first HIV blood screening test was licensed and implemented by blood banks in 1985.[17] This led to regular screening of blood for transfusion transmissible infections like Hepatitis B, Hepatitis C, HIV, Syphilis and malaria.

Alternatives to human blood

Since blood is such a precious and scarce commodity, as evident by the statistics mentioned earlier, efforts are on to find a substitute for this life force. Active investigations are going on throughout the world to look for alternatives to human blood transfusion called as “blood substitutes”. Two different methodologies are being investigated for red blood cell substitute development. These are the hemoglobin-based oxygen carriers (HBOCs) and perfluorocarbon-based oxygen carriers (PFBOCs).[28] HBOCs are engineered human/ animal hemoglobin which are designed for oxygen delivery and longer intravascular circulation.[29–33] PFBOCs are aqueous emulsions of perfluorocarbon derivatives that dissolve substantial amount of oxygen.[34–37] Recent studies are now evaluating the role of nanotechnology in development of blood substitutes.[38]

However, these products are under various stages of preclinical and clinical trials and yet to be approved for use as a blood substitute. So, despite the numerous advances in science, technology and healthcare over the centuries, blood and its products remain indispensable, thus the need for healthy young blood donors.

Blood donation process

The blood donation involves four stages which are:

1. Donor registration
2. Medical history questionnaire and mini-physical examination
3. Collection of blood
4. Recovery stage

Donor Registration

This is the first step in the process of blood donation where, a blood bank official takes note of the basic donor information like name, age, sex, address and contact details. Identity of a donor is confirmed by crosschecking with a valid identification document provided by the donor. The donor is then explained the blood donation process in a language he/she understands followed by signing of consent form by the donor.[16]

Questionnaire and physical examination

In the next step of blood donation, the donor is asked to fill-up a donor questionnaire form. This form contains questions pertaining to the health of the donor and his/her blood donation history. These questions help the blood bank official to determine the donor eligibility for blood donation and ensure the safety and integrity of blood being donated.[16]

General physical examination of the donor is carried out by the blood bank officer, which includes recording of height, weight, temperature, pulse, blood pressure along with checking for pallor, icterus, cyanosis, edema etc. A blood sample is then taken from the donor to determine his/her blood haemoglobin levels. The donor is declared "fit for blood donation" when the medical officer is satisfied that the donor is free from any form of illnesses and meets the donor selection criteria. These steps not only protect the blood recipient from any complications but also protect the blood donor.[16]

Criteria for blood donation -Adapted from WHO recommendations:[1,39]

- Age: 18 to 65 years

- Weight: above 50 Kg
- Normal vital signs (Pulse rate, blood pressure and respiratory rate)
- Minimum hemoglobin level (pre-donation): ≥12.5 g/dl
- Frequency of donations: Minimum of 3 months or 12 weeks interval between two consecutive blood donations
- No evidence or history of infections like hepatitis, malaria, HIV/AIDS, tuberculosis, typhoid and sexually transmitted diseases
- No evidence or history of diseases involving/affecting heart, lung, kidney, liver, chronic conditions like diabetes, cancer/malignancy, epilepsy and abnormal bleeding tendencies
- No history of medication like antibiotics, aspirin, alcohol, steroids and vaccination within the past 72 hours.
- No history of dog bite or rabies vaccine within past 1 year.
- No history of any surgery/ blood transfusions in the past 6 months.
- For women donors: No evidence of pregnancy, abortion in the past 3 months, having child less than 1 year old, breast feeding and there should be minimum 3 days gap after menstruation.

Collection of blood

The donor is taken to a bleeding room, where he/she is asked to lie down on a bed. The skin at the puncture site is prepared using antibacterial scrub. This process minimizes the risk of bacterial contamination of blood. Whole blood is collected into

sterilized bag sets containing anticoagulant and attached satellite bags to facilitate component separation. Phlebotomy is performed and typically 350ml of blood is collected for whole blood while 450ml is taken for component preparation.[16]

Another method of blood collection is the Apheresis technique, wherein a single component is be taken from the donor and the other components are returned back. The most common use of this technique is in collection of platelets known as "Single donor platelets". However, this method can also be used for collection of other components like plasma, red blood cells and leukocytes.[40]

Recovery stage

The last step in blood donation involves a recovery period of approximately 10-15 minutes. During this stage, the donor is monitored for any physical reaction or complication due to blood donation. The donor is meanwhile provided with refreshments like fruit juice to compensate for the fluid loss which rehydrates the body. This period allows the blood donor to understand the process of donation and clear his/her doubts or misconceptions regarding blood donation, thus positively influencing donor behaviour regarding future donations.[16]

Advice Post-donation to the blood donors

Post-donation advice to the blood donors is an extremely important exercise as it prevents any untoward incidents and experiences. It is the responsibility of the blood transfusion service to make the donor aware of what to expect post-donation so as to avoid any apprehensions during future donations.

- The donor is advised to drink plenty of fluids during the next 48 hours to replenish the volume lost.[41]

- The donor is also asked to avoid lifting heavy weights or carry out strenuous physical activities over the next couple of days to prevent bruising of the venepuncture site.[41]

- Care of the venepuncture site should be taken. It is important for the donor to understand that slight bruising near the puncture site is a normal phenomenon, which resolves in a week and hence, there is no cause for alarm. If there is increased bruising or swelling at the puncture site, it is advisable to consult a doctor.[41]

- If there is bleeding from puncture site, the donor is advised to apply gentle pressure and keep the arm raised for 3-5 minutes. Bandage should be applied over the site which can be removed after 5 hours.[41]

- In case the donor feels unwell or dizzy, he/she should immediately take a seat or lie down, with feet raised, until the feeling passes. During such times the donor is advised to keep calm and take slow and deep breaths. Any tight or restrictive garments should be loosened immediately. If the situation doesn't improve, contact the blood transfusion service or a doctor immediately.[41]

Types of Donors

There are mainly three types of blood donors, namely:

- Voluntary (non-remunerated) donors- These are the donors who donate blood willingly, without any pressure or monetary benefit. Such type of donation is encouraged by a blood transfusion service as these donors belong to low risk category and willingly participate in blood donation regularly. These donors also respond to appeals during emergencies.[42]

- Replacement or relative donors- These donors are family members or relatives of the patient in need of blood, who donate their blood as replacement for the actual blood needed for the patient.[42]

- Professional or commercial paid donors- These donors derive direct or indirect monetary benefit for the blood they donate. This type of donation is not encouraged and is prohibited by law in many countries including India.[42]

Special donations:

- Directed donation: This type of donation is made specifically for use by a particular patient. These donations are generally made by family members, relatives or friends of the patient.[20,42]

- Autologous donation: It is the blood donated for the donor's own use. Such blood can be used if the donor is about to undergo a surgery. This method eliminates the risk of transfusion transmitted infections.[20,42]

Hemoglobin level in blood donors

A minimum Hb level of 12.5 g/dl or a haematocrit level of 38% in the blood donor is required before he/she can donate blood. A blood donor donates approximately 450 ml of blood per donation and the Hb level of a donor decreases by 1-1.5 g/dl after donation of single unit of whole blood.[39] Thus, it is important to know the pre-donation Hb levels of the donor, so as to prevent harm to the donor.

One gram of Hb contains about 3.4 mg of iron. So, in a normal individual, 100 ml of blood contains approximately 50 mg of iron, meaning with loss of every 2 ml of blood, 1mg of iron is lost. Hence, in 450 ml of blood taken per donation, a donor loses 220-240 mg of iron.[3,5] A healthy donor, generally recovers in a short

period of time and hemoglobin level comes back to normal as the iron stores in his/her body are mobilized.

Hemoglobin screening methods

As the Hb level decreases by 1-1.5 g/dl per donation, every blood donor has to be screened for pre-existing anemia or who may become anemic post donation. This will also avoid unnecessary deferral of blood if the donor turns out to be anemic. Several methods are available for Hb estimation these days each having its own advantages and disadvantages. Some of them are:

1. Copper sulphate method
2. Microhematocrit method
3. HemoCue method
4. Automated hematology analyzer

Copper Sulphate ($CuSO_4$) Method:

Principle of this method is based on the specific gravity of blood. Specific gravity of 1.053 corresponds to a Hb level of 12.5g/dl. So, when a drop of blood is allowed to drop in a copper sulphate solution of specific gravity 1.053, it becomes encased in sac of copper proteinate, preventing dispersion of fluid for 15 seconds. If the Hb level is 12.5 g/dl or more the drop will sink within 15 seconds and the donor is then accepted. If the drop sinks to the middle and floats or starts to rise, it could indicate anemia in the donor. In such cases, repeat testing using other methods is performed before donor deferral.[39,40]

Although this method is quick, cheap and easy to perform, it has its disadvantages. Some disadvantages are, subjective end point, high rate of false negative results and may give false value in donors with elevated protein levels.[39]

Microhematocrit method:

In this method the haematocrit in the blood is estimated. Blood is obtained from a finger-prick and collected in a capillary tube coated with an anticoagulant, which is then centrifuged and the percentage of packed red cell is estimated. This method is generally used as an ancillary test for donors who are deferred on copper sulphate method. Although this method is inexpensive and easy to perform, it takes about 5-7 minutes to give its results.[39,40]

HemoCue method:

This method estimates the concentration of Hb using a spectrophotometer. The system consists of microcuvettes containing a reagent in dry form and 10µl of capillary blood is used. In this method, sodium deoxycholate hemolyses the RBCs to release the Hb. Sodium nitrite then converts this Hemoglobin to methemoglobin which along with sodium azide forms azidemethemoglobin. Hb concentration is then estimated by measuring absorbance at two wavelengths (570 nm and 880 nm).This method is cost-effective, quick, portable and gives accurate results.[43]

Automated Hematology analyzers:

These are highly sophisticated machines which can not only determine Hb but also various other parameters like RBC count, MCV, MCH, MCHC, RDW, WBC count, Differential count, Platelet count, Reticulocyte count etc. Hb determination can be done by either Cyanmethemoglobin or Oxyhemoglobin or Sodium lauryl sulphate

method. No matter which method is used, the Hb estimation is done by measuring the concentration of the end product using absorbance at 540 nm.[39]

These analyzers are accurate, have high precision and enable high sample throughput. However, not only they require regular maintenance, calibration and trained personnel, they are also expensive and non-portable. Hence, they are not suitable for donor screening especially in remote locations (Figure 1).

Figure 1: Automated Hematology Analyzer (Sysmex XN-1000)

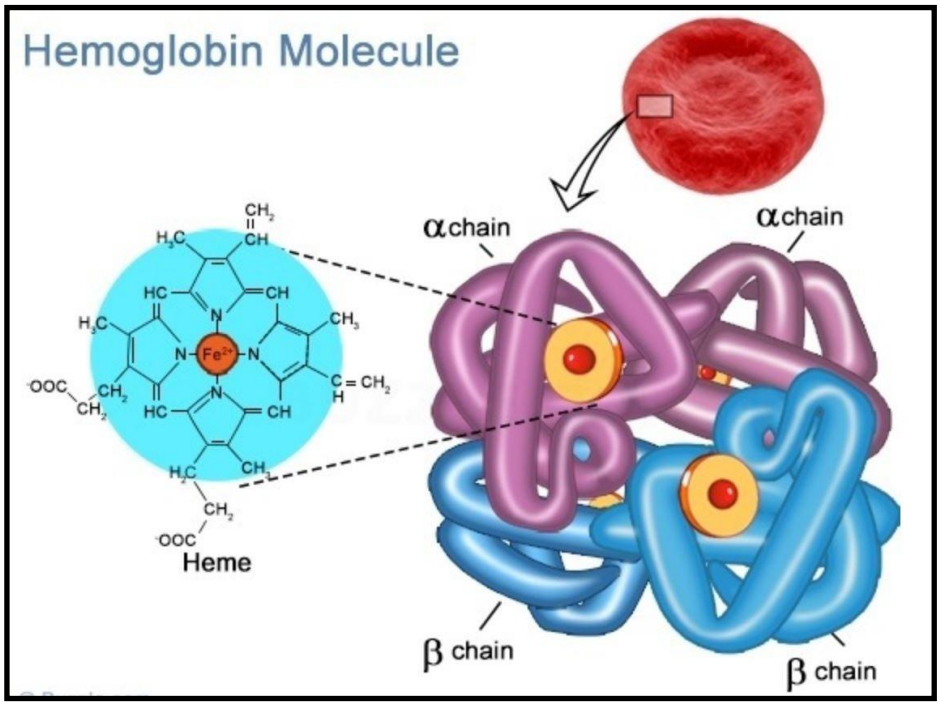
Hemoglobin structure and function

It was Dr. Max Perutz who demonstrated the 3-dimensional molecular structure of Hemoglobin using X-ray crystallography in 1960,[44] for which he received the Nobel Prize in 1962. Hemoglobin is globular protein which is made up of 4 subunits. Each of these subunits contains a polypeptide chain called globin and a heme group.[45]

Each molecule of adult Hb contains a total of 4 globin polypeptide chains, i.e. 2 alpha and 2 beta chains. There are minute differences between these 2 sets of polypeptide chains. Normally, α chain contains 141 amino acids while the β chain contains 146 amino acids.[45] The 4 globin chains are arranged together as helical structures.[46] Each of these polypeptide chains contain a heme molecule in the heme pocket present in these chains (Figure 2).

Heme is a derivative of porphyrin ring. This porphyrin ring is formed by the fusion of 4 pyrrole rings. An ion of iron (Fe^{2+}) is present at the centre of these 4 pyrrole rings and is bound to the four nitrogen atoms.[45] This heme group is responsible for the reversible binding of oxygen and carbon dioxide molecules. Each heme group binds with 1 molecule of oxygen. So, a single molecule of Hb can carry 4 molecules of oxygen.

Figure 2: Structure of Hemoglobin



Hb plays an important role in ensuring oxygen supply to all the cells in the body. The primary function of Hb is oxygen transport from the lungs to the peripheral tissues. Hb also helps in transport of carbon dioxide released from the peripheral tissues to the lungs.[46] The steps in oxygen and carbon dioxide transport are:

- Oxygen pickup: In the lungs, the inhaled oxygen diffuses through the alveolar membrane and capillaries and binds to the Hb in the RBCs. Since, each Hb has 4 heme groups, it can carry 4 oxygen molecules.
- Oxygen delivery: When Hb reaches the peripheral tissues, due to the low pH encountered, there is a decrease in affinity for oxygen and oxygen is released which is taken up by the tissues. This phenomenon is also known as Bohr effect.
- Carbon dioxide pickup: Carbon dioxide molecules bind to the deoxyhemoglobin as they have higher affinity for CO_2 than oxygen. This phenomenon is called as Haldane effect.
- Carbon dioxide delivery: Dissociation of carbon dioxide occurs in the lungs in the presence of oxygen and the Hb is now free for oxygen pick-up again.

Anemia in blood donors

In the past decade or so, major gains have been made with respect to adequacy of blood. This has been possible due to implantation of better collection technology, making donations more convenient and active community outreach programmes to build a donor base. Unfortunately, this success has come at a big price, i.e. anemia in regular blood donors.[47]

Functionally, anemia is defined as a reduction in the capacity of blood to ferry oxygen to tissues, hence leading to tissue hypoxia, but in a clinical setting, anemia refers to a reduction in the concentration of Hb or RBCs. However, anemia is not a disease but merely the manifestation of an underlying disorder.[48]

There are many disorders which can cause anemia and these disorders are classified according to the underlying mechanism. For example, hemoglobinopathies, malaria, hypersplenism are all classified under disease caused increased red cell destruction, while nutritional deficiencies, chronic diseases, renal failure lead to decreased red cell production and hence anemia.[49] The most common cause of anemia, in a developing country like India, is Iron deficiency anemia, in which there is a nutritional deficiency of iron. Understandably, the most common cause of anemia in regular blood donors too is iron deficiency anemia, as iron stores are depleted with every blood donation.

Iron deficiency anemia is the most common form of nutritional deficiency in the world. The causes of iron deficiency anemia are dietary iron deficiency, increased blood loss, hemodialysis and malabsorption syndromes. Hence, iron deficiency anemia is especially common in predominantly vegetarian populations and countries with high incidence of intestinal hookworm infestations.

Iron deficiency comprises of 3 stages.[48,50] First is the stage of iron depletion, wherein there is no anemia and erythrocyte morphology remains normal. In this stage the iron stores in the body are mobilized and exhausted, which is indicated by decrease in serum ferritin levels. RDW is frequently elevated at this stage and is often the first hematologic indication of iron deficiency. The second stage is that of iron deficient erythropoiesis, where there is insufficient iron to form heme. Anemia and

hypochromia are still not detected but the RBCs can be slightly microcytic. The final stage is that of iron deficiency anemia, wherein the classic picture of microcytic hypochromic anemia is seen in the peripheral blood smear. This stage represents the advanced stage of the disease. The time taken for this stage to develop can shorten drastically if there is significant blood loss. Hence, regular blood donors are at increased risk of developing iron deficiency anemia.

This is one of the reasons why, the donor Hb level is part of the blood donor eligibility criteria list. So, donors with low Hb are not allowed to donate blood to prevent the development of iron deficiency anemia. These donors are deferred temporarily by the blood centre, up until the time when donor Hb levels come back to the normal.[51] This makes anemia in blood donor as one of the most common causes of donor deferral.[52–54]

Anemia and iron deficiency in blood donors- Worldwide

In 1981, Simon *et al*.[6] examined 516 female and 505 male blood donors of Albuquerque, New Mexico. They observed a trend in ferritin values and iron stores with frequency of donation. As the frequency of blood donation increased, there was a reduction in the plasma ferritin levels. They also showed that there was no change in ferritin levels with number of lifetime donations.

A Danish study by Milman *et al*.[55] conducted in Copenhagen during 1982-1984, studied the impact of blood donation on the iron status of blood donors. They noted significantly lower serum ferritin levels in blood donors compared to non-donors. However, no significant difference in Hb levels was noted between the two groups. Iron deficiency anemia was noted in 0.26% of donors and 0.10% of non-donors.

Cancado *et al.*[7] studied the frequency of iron deficiency in the blood donors of Sao Paulo, Brazil, by evaluating their serum iron, total iron binding capacity, transferrin saturation index, serum ferritin and RBC indices. It was noted that 11% out of the total 300 donors screened were found to have iron deficiency. They also noted that the frequency of iron deficiency was higher in multi-time blood donors compared to first-time donors and this difference was statistically significant.

In a study conducted amongst the Nigerian Blood donors by Jeremiah *et al.*[56] an assessment of all hematological parameters was done. It was noted that 18.8% of total 112 donors were anemic. They observed significant reductions in HCT, RBC count, Hb and MCV in the regular blood donors. They also noted that 12.5% of the donors were leukopenic. They also reported significant negative correlation between HCT and absolute lymphocyte, HCT and RDW, WBC and percent lymphocytes and MCV and platelet count.

An Iranian study, conducted by Shahshahani *et al.*[57] on blood donors from Yazd, Iran showed reduction of iron stores with increase in number of donations. They observed that 7.5% of men and 33.3% of women had Iron deficiency among 199 male and 137 female donors, respectively. Iron deficiency was noted in 41.2% of regular blood donors while Iron deficiency anemia was seen in 26.5% of the regular blood donors.

Norashikin *et al.*[58] evaluated the serum ferritin levels among the first time and regular male blood donors in Malaysia. They observed that amongst a total of 211 blood donors, the serum ferritin levels were significantly lower in the regular donors compared to the first-time donors. They showed a significant correlation between

frequency of donation and serum ferritin level, and between number of donations and haemoglobin levels.

Another Malaysian study by Nadarajan *et al.*[59] studied the hemoglobin and iron status of the blood donors in Malaysia. They observed that 21.3% of the total 187 donors had a Hb level of less than 12.5 g/dl. 15.7% of repeat male donors and 22.7% of repeat female donors were found to be iron deficient, while among the first-time donors, 1.6% of males and 18.2% of females were iron deficient. They also observed a hemoglobinopathy prevalence rate of 6.4% amongst the blood donors.

Alvarez-Ossorio *et al.*[60] screened the Hb and serum ferritin levels of blood donors in Germany. They observed that, out of the 632 regular donors, low Hb levels were noted in 74 cases with majority of them being females. Among the 171 first-time donors, only 5 were anemic and incidentally, all of which were females. They also noted iron deficiency, with serum ferritin levels <15 µg/L, in 26% and 5% of the regular and first-time blood donors, respectively.

Scenario in India

Mahida *et al.*[3] studied the iron status among the regular blood donors in Surat, Gujarat. It was observed that 9.5% of male and 26.7% of female regular blood donors were anemic. They noted there was a gradual decrease in Hb, MCV and MCH, as the number of past blood donation increased. They also showed inverse relationship of serum iron, ferritin and transferrin saturation with number of past blood donations.

A study conducted by Kumari *et al.*[61] in the western part of Rajasthan, to observe the prevalence and pattern of anemia in blood donors. They reported a 1.7% rate of prevalence of anemia in blood donors, with anemia being more prevalent in the female donors. They also noted Iron deficiency anemia to be the most common cause

of anemia in blood donors. Microcytic hypochromic anemia was found to be the commonest morphological type in their study.

Tailor *et al*.[62] studied the changes in hematological parameters and iron status of voluntary blood donors in Gujarat. They noted a decrease in the mean Hb levels of repeat donors compared to first-time donors. They also observed a reduction in MCV and MCH parameters in repeat donors. Serum ferritin was greatly reduced in the repeat donors compared to first time donors.

A study conducted in Chandigarh, by Mittal *et al*.[4] evaluated the iron stores among the blood donors. Although none of the donors in their study were anemic, they observed a significant reduction in Serum ferritin after 3 donations/ year in the repeat blood donors. The ferritin levels of female donors were also found to be lower compared to males.

A study conducted in a rural tertiary care centre of Himachal Pradesh[63] revealed that amongst the total 992 blood donors, 2.3% of the donors were anemic. The prevalence of anemia in female donors was found to be higher at 26.31% compared to males at 1.84%. They also noted a donor deferral rate of 25.5% due to anemia in the blood donors.

Many more studies have been done to assess the donor deferral rate and prevalence of anemia in blood donors. The donor deferral rate ranged from 3% to15% in most of these studies conducted in various parts of India.[53,64–66]

MATERIALS AND METHODS

Source of data

A cross-sectional study was carried out on the blood donors, fulfilling the inclusion criteria, donating at the blood bank at BLDE (DU) Shri B.M. Patil Medical College, Hospital and Research Centre, Vijayapur and at the voluntary blood donation camps organized by it.

Study period: 1st November, 2016 to 30th June, 2018.

Inclusion criteria:

All voluntary, healthy blood donors in the age group of 18-60 years who have donated blood ≥3 times in the previous year with the last donation at least 12 weeks back.

Exclusion criteria:

Donors with history of blood transfusions or iron therapy in the last one year

Methods of collection of data

- After taking informed consent, under aseptic precautions, venous blood sample was collected from the blood donors before blood donation.
- Two ml of blood was taken in an EDTA vacutainer and immediately analyzed for complete blood count, including Hb, RBC count, HCT, MCV, MCH, MCHC, RDW, WBC count, Differential count and Platelet count using an automated 5-part differential hematology analyzer (SYSMEX XN-1000).
- Donors who did not fulfil the donor selection criteria were deferred from donation.

Sample size

By using the formula:

$$n = \frac{z^2 p(1-p)}{d^2}$$

Where, z= z statistic at 5% level of significance

d= margin of error

p= anticipated prevalence rate

A sample size of 132 subjects allowed the study to determine the hematological profile of multiple blood donors with a confidence interval of ±5% with finite population correction.

Statistical analysis

All characteristics were summarized descriptively. For continuous variables, the summary statistics of mean, standard deviation (SD) were used. For categorical data, the number and percentage were used in the data summaries. Chi-square (χ^2)/ Freeman-Halton Fisher exact test was employed to determine the significance of differences between groups for categorical data.

The difference of the means of analysis variables between two independent groups was tested by unpaired t test. The difference of the means of analysis variables between two time points in same group was tested by paired t test. The t test (also called Student's T Test) compares two averages (means) and tells if they are different from each other. The difference of the means of analysis variables between more than two independent groups was tested by ANOVA and F test of testing of equality of Variance. Bivariate correlation analysis using Pearson's correlation coefficient (r) was

used to test the strength and direction of relationships between the interval levels of variables.

If the p-value was < 0.05, then the results were considered to be statistically significant otherwise it was considered as not statistically significant. Data were analyzed using SPSS software v.23.0. and Microsoft office.

RESULTS

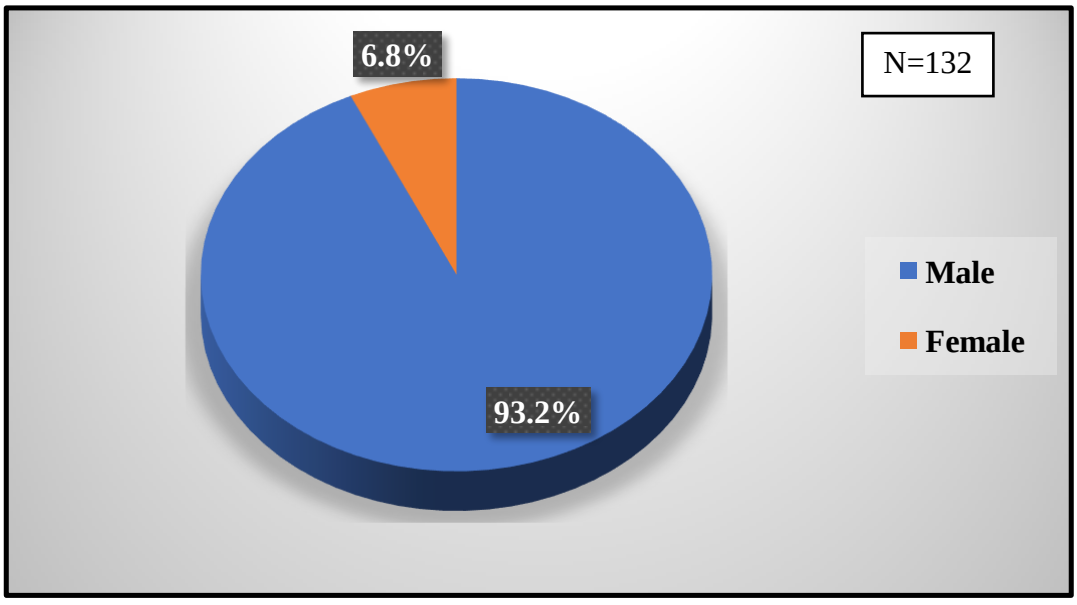
DISTRIBUTION OF DONORS ACCORDING TO GENDER

A total of 132 blood donors participated in the study out of which 123 donors (93.2%) were male and 9 donors (6.8%) were females. (Table 1, Figure 3). The male to female ratio was 13.6:1.

Table 1: Gender-wise distribution of donors

Gender	N	Percentage (%)
Male	123	93.2
Female	9	6.8
TOTAL	132	100

Figure 3: Gender-wise distribution of donors



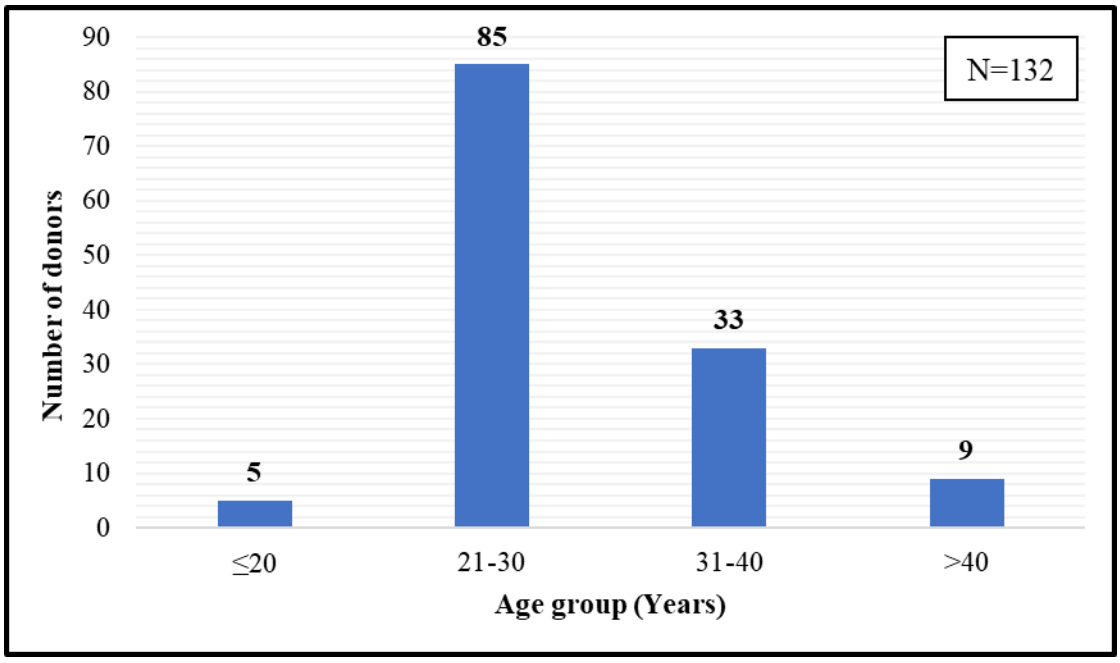
DISTRIBUTION OF DONORS ACCORDING TO AGE

Out of the 132 donors who participated in the study, most of the donors belonged to the age group of 21-30 years while least number of donors were less than 21 years of age. (Table 2, Figure 4)

Table 2: Age-wise distribution of donors

Age (Years)	N	Percentage (%)
≤20	5	3.8
21-30	85	64.4
31-40	33	25
>40	9	6.8
Total	132	100

Figure 4: Age-wise distribution of donors



Among the 132 donors, the minimum age of donors was 20 years, while the maximum age was 58 years. The mean age of the donors was calculated to be 29.04 years. (Table 3)

Table 3: Mean age of Donors

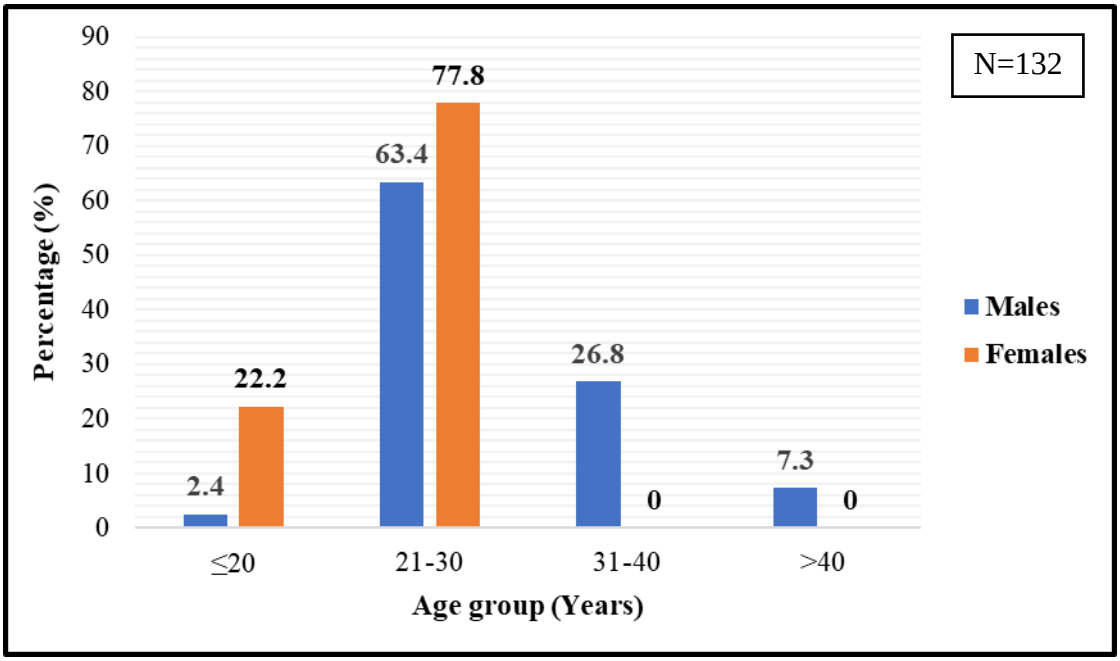
	Minimum	Maximum	Mean	SD
Age (Years)	20	58	29.04	7.07

Out of the 132 donors, most of the male and female donors belonged to the age group of 21-30 years. While there were no female donors beyond the age of 31, least number of male donors were less than 21 years of age. (Table 4, Figure 5)

Table 4: Distribution of Donors by Age and Gender

Age (Years)	Male		Female	
	N	%	N	%
≤20	3	2.4	2	22.2
21-30	78	63.4	7	77.8
31-40	33	26.8	0	0.0
>40	9	7.3	0	0.0
Total	123	100	9	100

Figure 5: Distribution of donors by age and gender



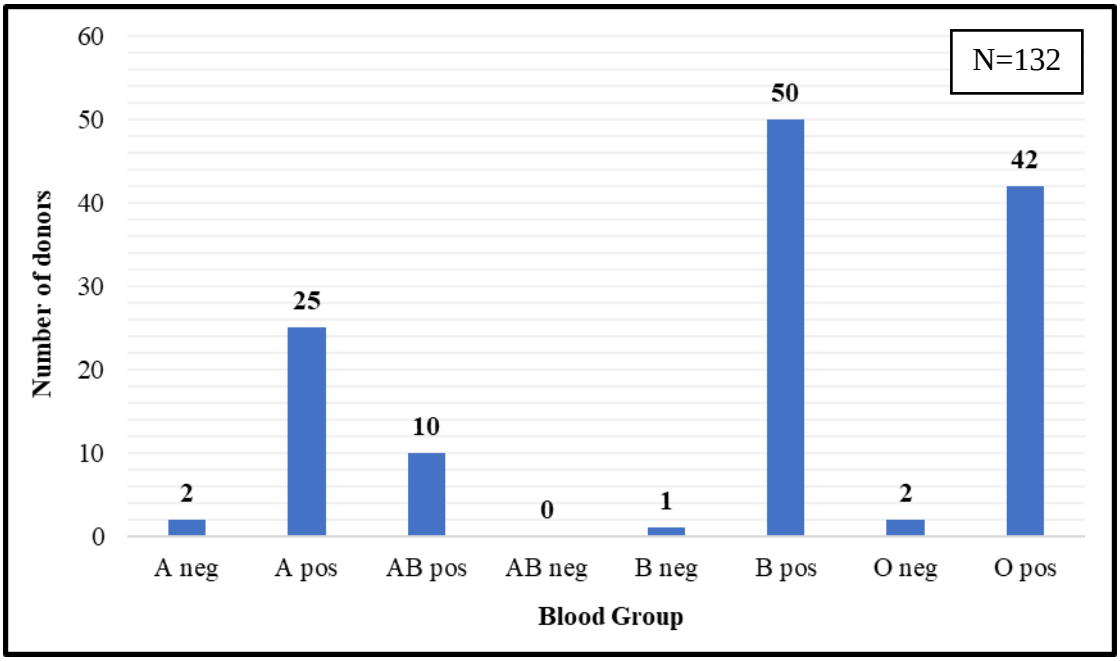
DISTRIBUTION OF DONORS ACCORDING TO BLOOD GROUP

Amongst the 132 donors, the commonest blood group was B positive (37.9% of the donors) while none of the donors were AB negative, making it the rarest blood group of this study. (Table 5, Figure 6)

Table 5: Blood Group-wise distribution of donors

Blood Group	N	Percentage (%)
A negative	2	1.5
A positive	25	18.9
AB positive	10	7.6
AB negative	0	0.0
B negative	1	0.8
B positive	50	37.9
O negative	2	1.5
O positive	42	31.8
Total	132	100

Figure 6: Blood Group-wise distribution of donors



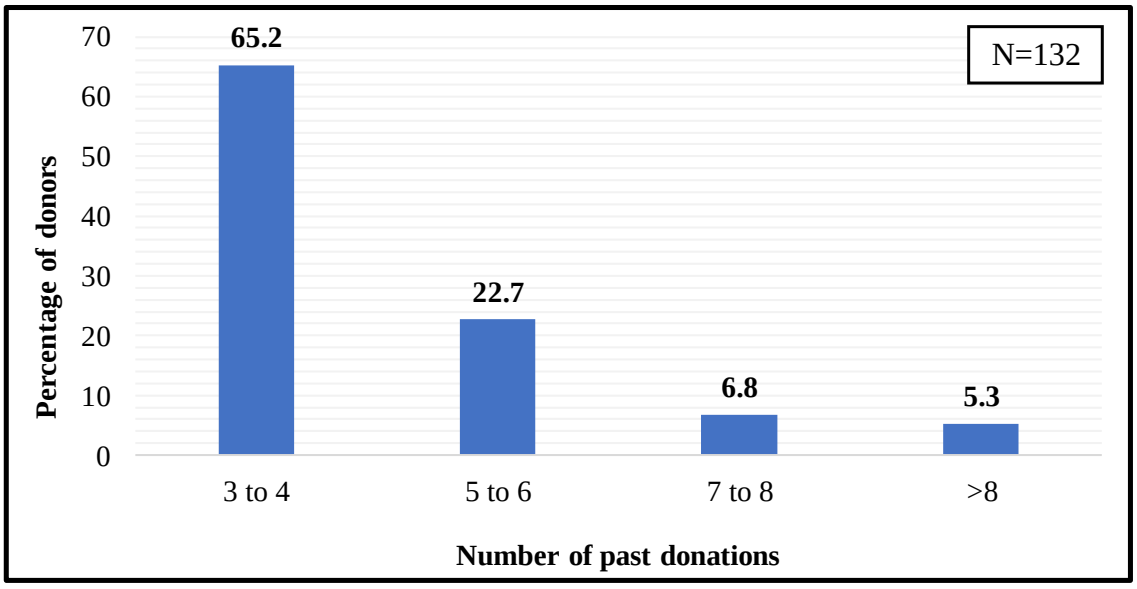
DISTRIBUTION OF DONORS ACCORDING TO NUMBER OF DONATIONS

Out of the 132 donors, most of the donors had donated at least 3-4 times, while few donors had donated blood more than 8 times. (Table 6, Figure 7)

Table 6: Distribution of donors according to number of past donations

Number of donations	N	Percentage (%)
3-4	86	65.2
5-6	30	22.7
7-8	9	6.8
>8	7	5.3
Total	132	100

Figure 7: Distribution of donors according to number of past donations



The minimum number of past donations by the donors was 3, while the maximum number of past donations by one of the donors was found to be 35. The mean number of donations per donor was 4.71. (Table 7)

Table 7: Mean number of donations

Number of donations	Minimum	Maximum	Mean	SD
	3	35	4.71	±3.47

RED CELL PARAMETERS OF BLOOD DONORS

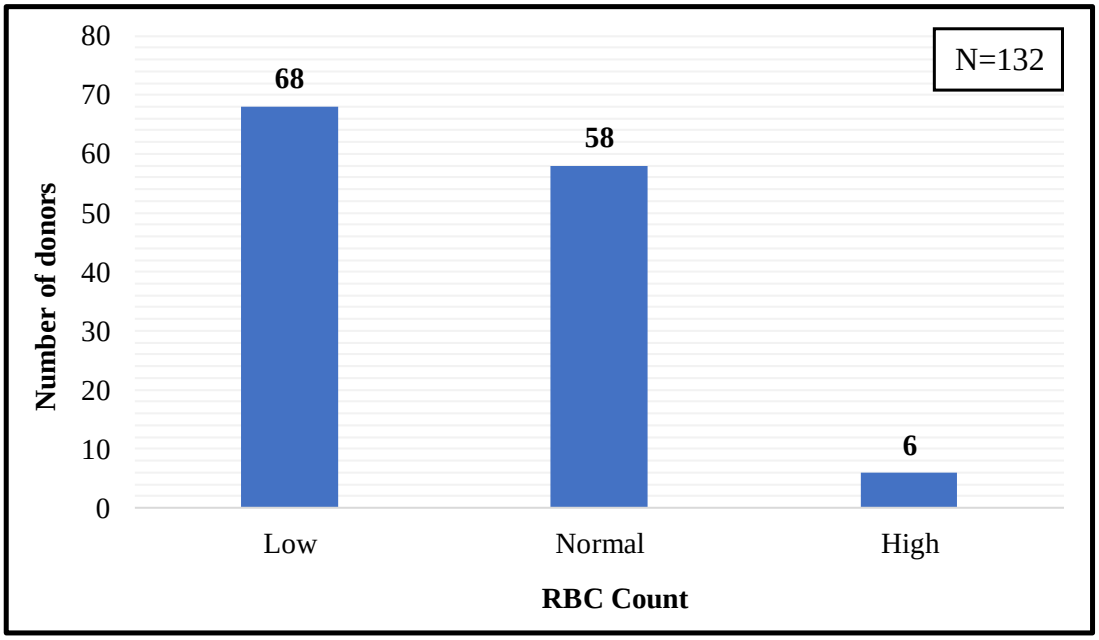
RBC Count

Out of the 132 donors, a total of 68 donors had low RBC count, accounting for 51.5% of all the blood donors. (Table 8, Figure 8). The mean RBC count of the donors was noted to be 4.4 million/mm^3 with a SD of 0.63 million/mm^3.

Table 8: RBC count among the blood donors

RBC Count (millions/mm^3)	N	Percentage (%)
Low (<4.5)	68	51.5
Normal (4.5-5.5)	58	43.9
High (>5.5)	6	4.5
Total	132	100

Figure 8: RBC Count among the blood donors



Hemoglobin

Out of the 132 donors, 52 donors were found to be anemic, comprising 39.4% of the total blood donors. (Table 9, Figure 9). The mean Hb of the donors was noted to be 12.9 g/dl.

Table 9: Number of Anemic donors

Hemoglobin (g/dl)	N	Percentage (%)
Anemia (<12.5)	52	39.4
Normal (>12.5)	80	60.6
Total	132	100

Figure 9: Number of Anemic donors

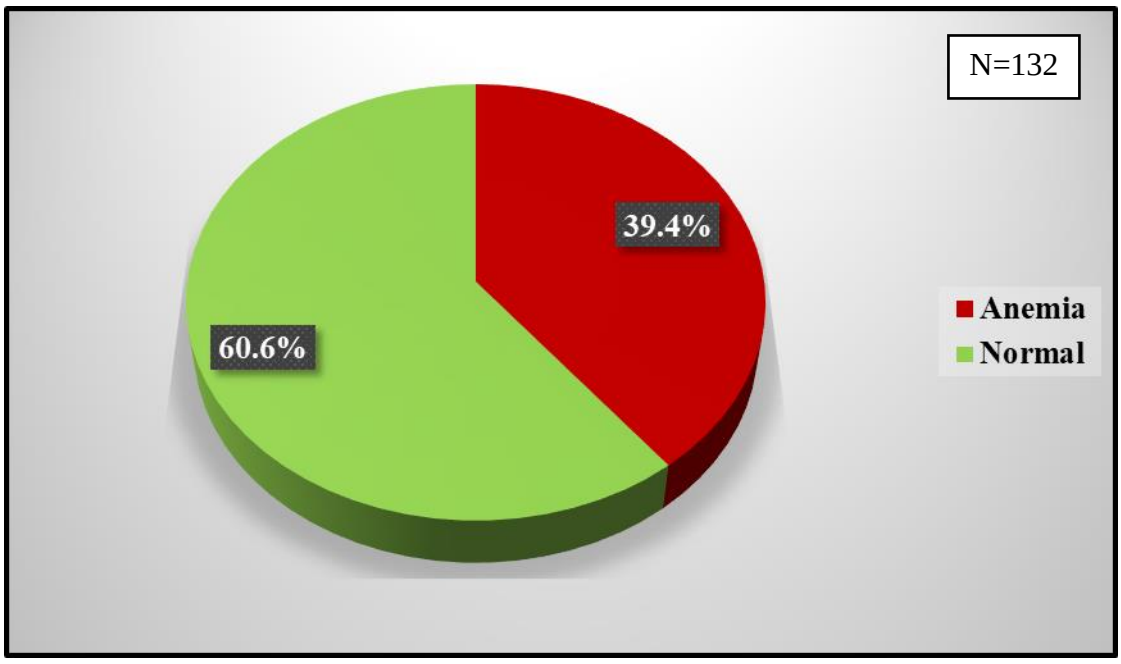
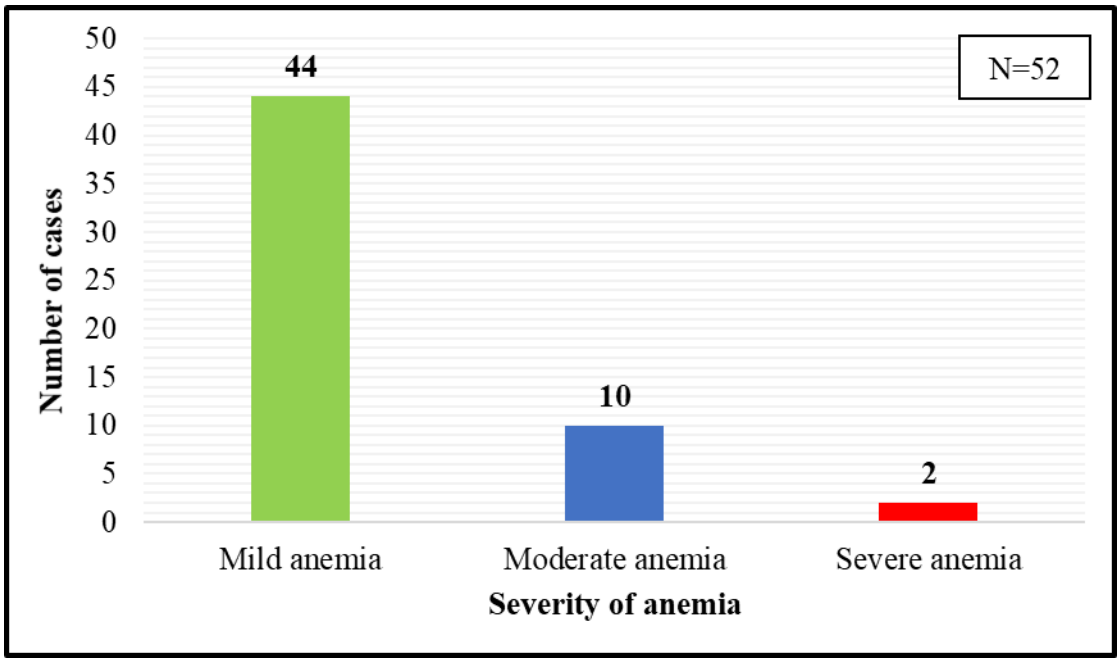


Table 10 and Figure 10 show the severity of anemia in the blood donors. A total of 33.3% of the blood donors were mildly anemic, 7.6% showed moderate anemia, while 1.5% of the donors were severely anemic.

Table 10: Severity of anemia among blood donors

Number of donors	Total Anemic (<12.5 g/dl)		Mild anemia (11-12.4 g/dl)		Moderate anemia (8-10.9 g/dl)		Severe anemia (<7.9 g/dl)	
	N	**%**	**N**	**%**	**N**	**%**	**N**	**%**
	52	42.4	44	33.3	10	7.6	2	1.5

Figure 10: Severity of anemia among blood donors



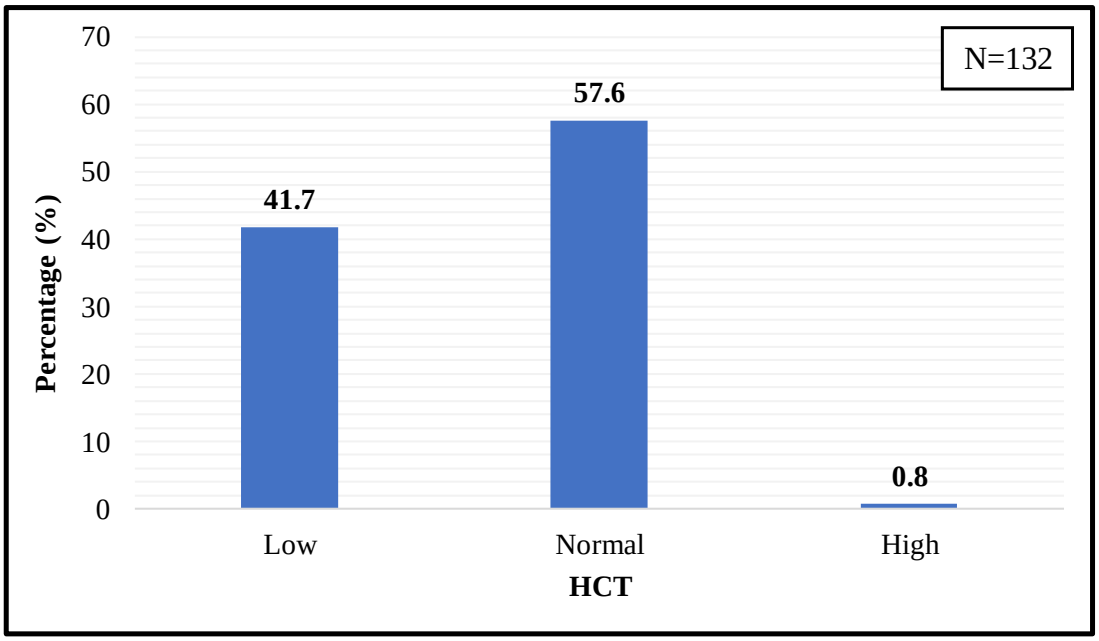
Hematocrit

Out of the 132 donors, 55 donors were found to have low HCT, accounting for 41.7% of the total donors, while only 1 donor had HCT on the higher side. (Table 11, Figure 11). The mean HCT was noted to be 40.67% with a SD of 4.23%.

Table 11: HCT level in blood donors

HCT (%)	N	Percentage (%)
Low (<40)	55	41.7
Normal (40-50)	76	57.6
High (>50)	1	0.8
Total	132	100

Figure 11: HCT level in blood donors



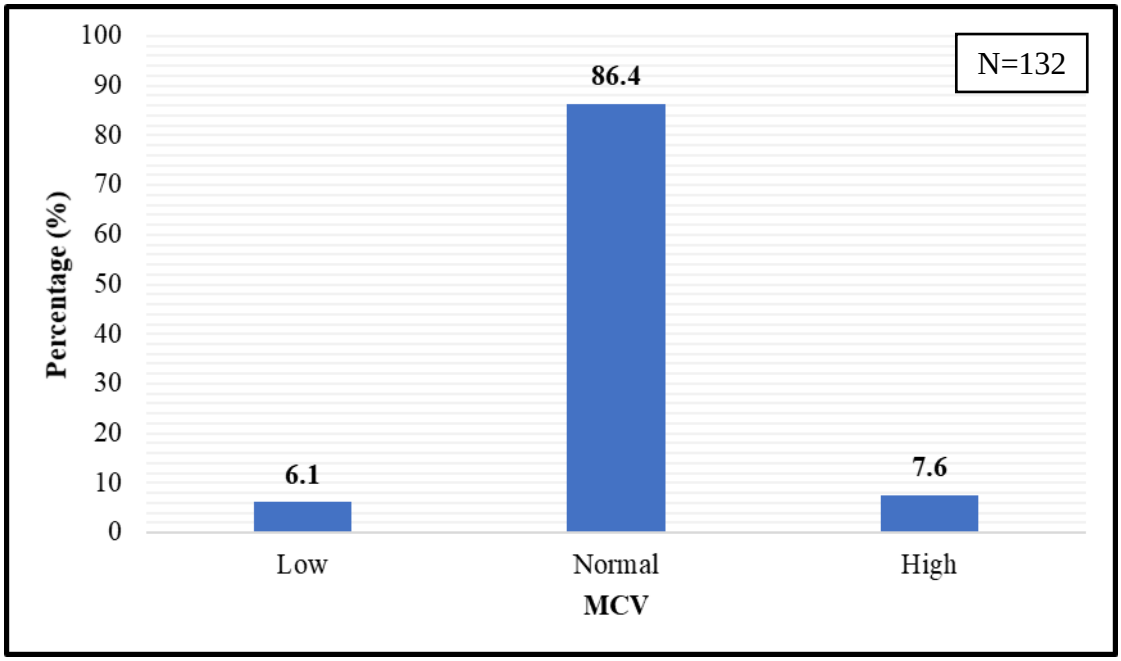
Mean Corpuscular Volume

A total of 6.1% of the total 132 blood donors had low MCV, while 7.6% of them had MCV on the higher side. (Table 12, Figure 12). The mean MCV was noted to be 91.56fl with a SD of 7.57fl.

Table 12: MCV level in blood donors

MCV (fl)	N	Percentage (%)
Low (<82)	8	6.1
Normal (82-101)	114	86.4
High (>101)	10	7.6
Total	132	100

Figure 12: MCV level in blood donors



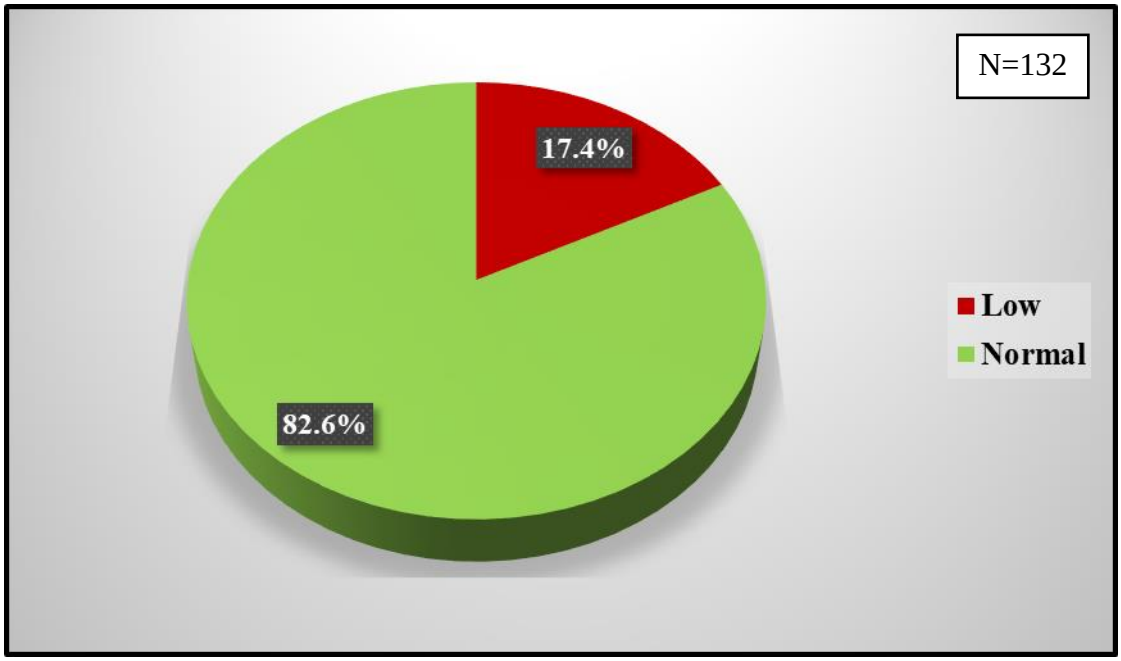
Mean Corpuscular Hemoglobin

Out of the 132 blood donors screened, a total of 23 donors had low MCH levels while the rest had normal MCH values. (Table 13, Figure 13). The mean MCH was 29.42 pg with a SD of 5.94 pg.

Table 13: MCH level in blood donors

MCH (pg)	N	Percentage (%)
Low (<27)	23	17.4
Normal (≥27)	109	82.6
Total	132	100

Figure 13: MCH level in blood donors



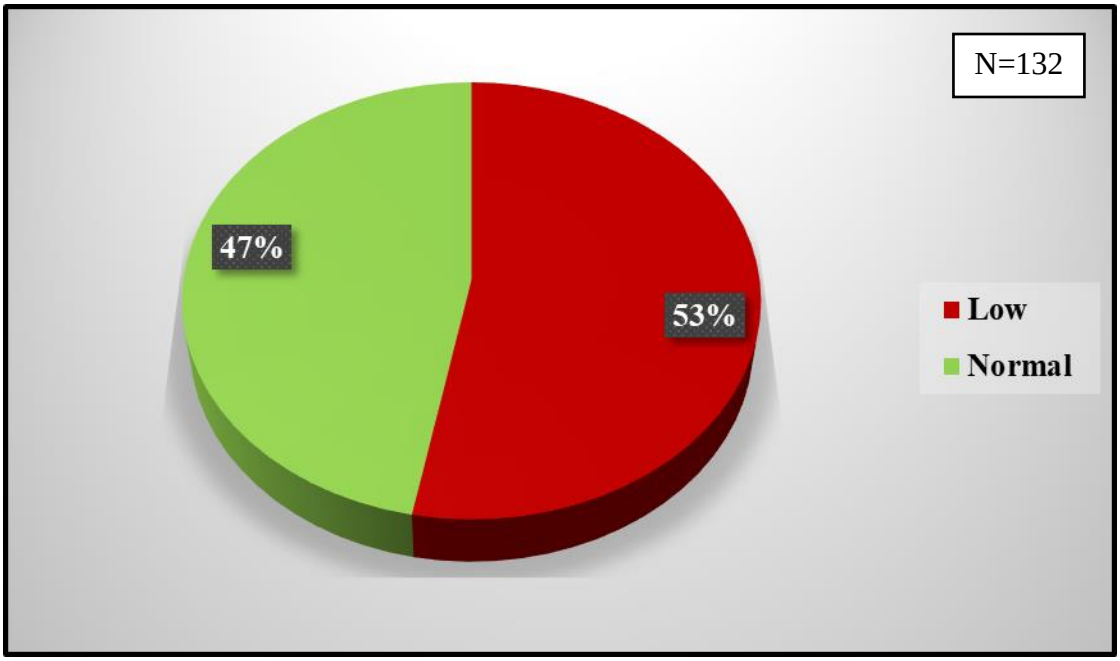
Mean Corpuscular Hemoglobin Concentration

Fifty-three percent of the total 132 blood donors screened, showed decreased MCHC values. (Table 14, Figure 14). The mean MCHC was 31.51 g/dl with a SD of 1.43 g/dl.

Table 14: MCHC values in blood donors

MCHC (g/dl)	N	Percentage (%)
Low (<31.5)	70	53
Normal (≥31.5)	62	47
Total	132	100

Figure 14: MCHC values in blood donors



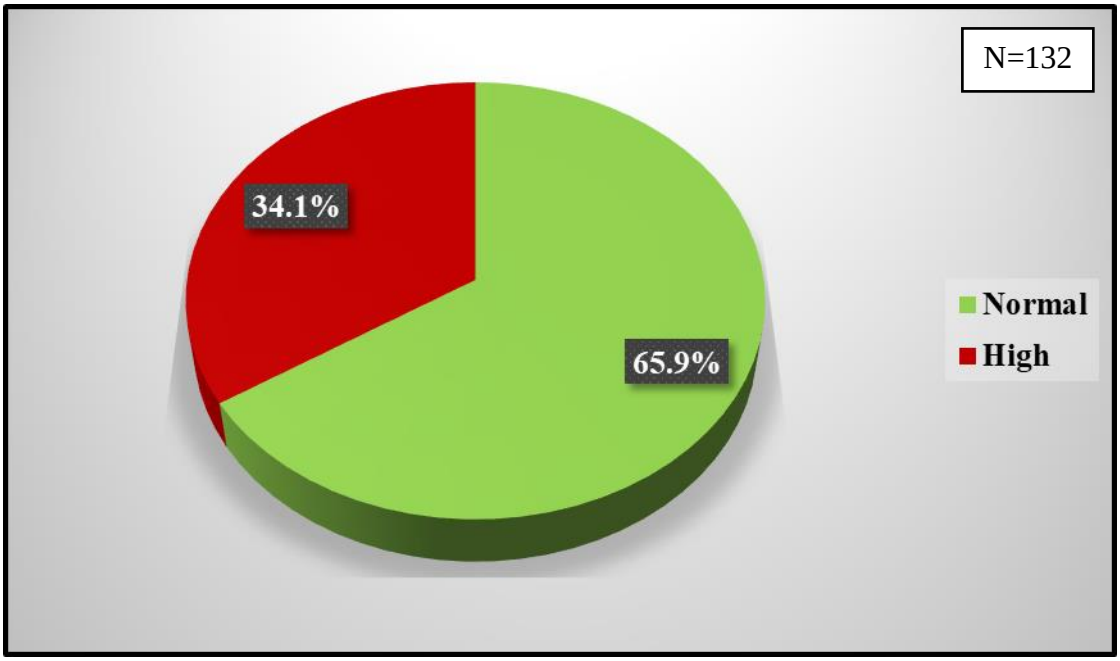
Red cell Distribution Width

Out of the 132 blood donors screened, 45 donors had increased RDW, accounting for 34.1% of all the donors. (Table 15, Figure 15). The mean RDW was 13.98 with a SD of 1.55.

Table 15: RDW of the blood donors

RDW	N	Percentage (%)
Normal (≤14)	87	65.9
High (>14)	45	34.1
Total	132	100

Figure 15: RDW of the blood donors



A significant number of non-anemic donors showed variation in some of the red cell parameters like RBC count, HCT, MCHC and RDW. Table 16 shows the number of anemic and non-anemic donors showing variation in these parameters. A total of 32.5% of the donors with normal Hb showed low RBC count. Low HCT was noted in 11.3% of the non-anemic donors. MCHC levels were decreased in 35% of the non-anemic donors. High RDW levels were noted in 28.8% of the donors with normal Hb levels.

Table 16: Variation of Red cell parameters in non-anemic donors

Donors / Parameters	Anemic		Non-Anemic	
	N	**%**	**N**	**%**
Low RBC count	42	80.8	26	32.5
Low HCT	46	88.5	9	11.3
Low MCHC	42	80.8	28	35
High RDW	22	42.3	23	28.8

WBC PARAMETERS OF BLOOD DONORS

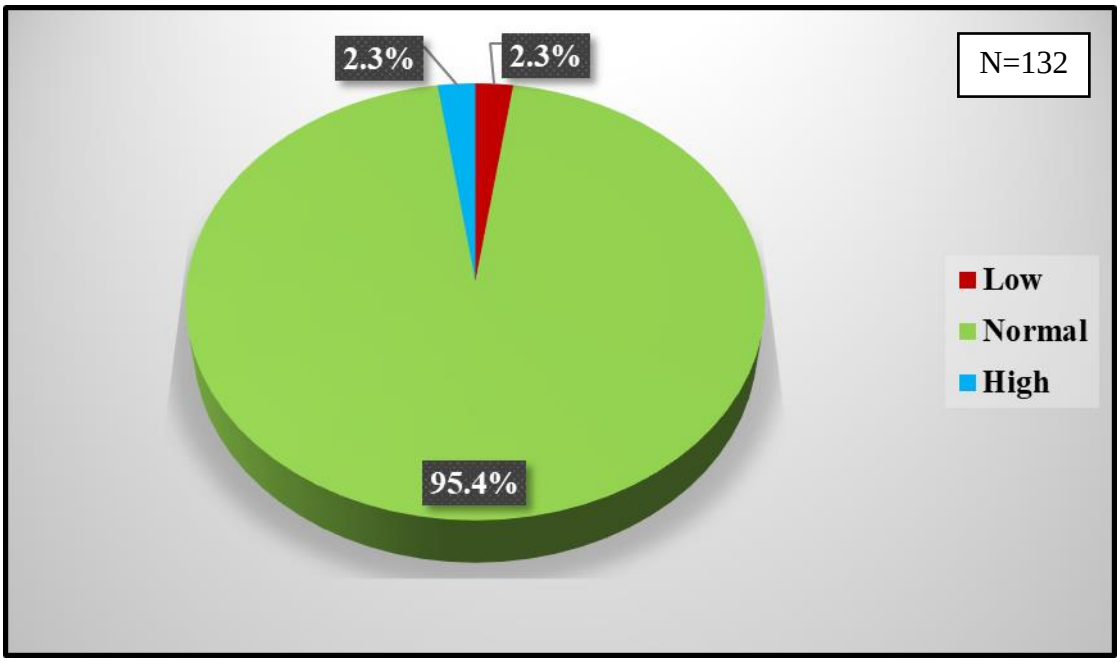
WBC Count

Out of the 123 blood donors, 3 of the donors had leukopenia, 3 had leucocytosis, while others had normal WBC count. (Table 17, Figure 16). The mean WBC count was 7074.92 cells/mm^3 with a SD of 1701.39 cells/mm^3.

Table 17: WBC count among the blood donors

WBC (cells/mm^3)	N	Percentage (%)
Low (<4000)	3	2.3
Normal (4000-11000)	126	95.5
High (>11000)	3	2.3
Total	132	100

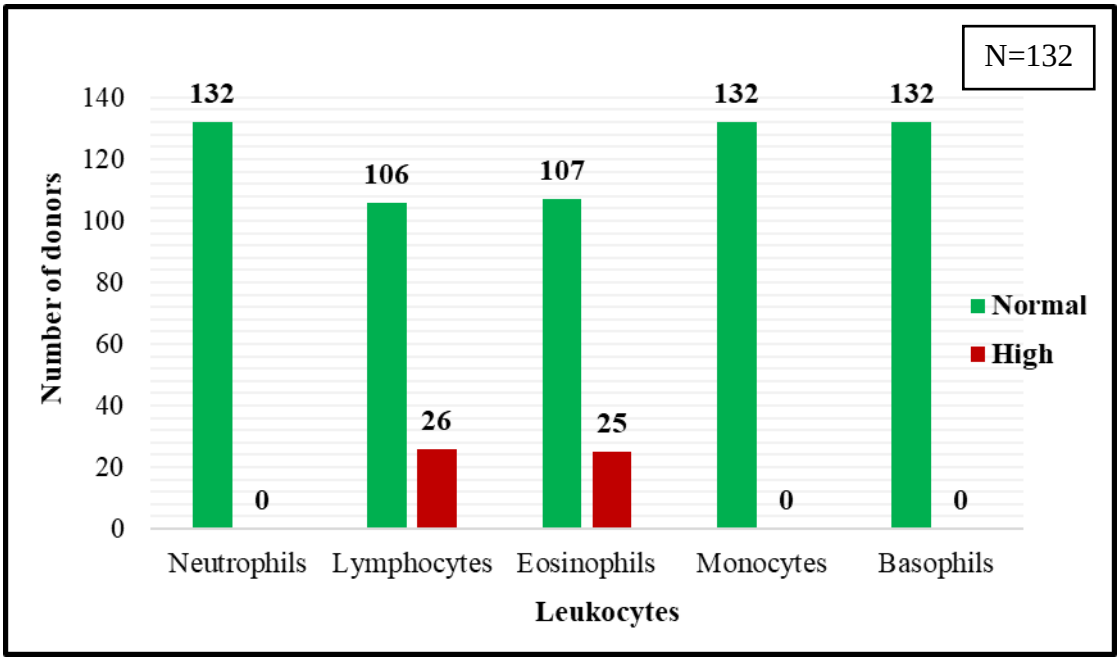
Figure 16: WBC count among the blood donors



Differential leukocyte count

In the 132 donors screened, neutrophils, monocytes and basophils differential count were noted to be normal in all the donors. Lymphocytosis was noted in 26 donors while eosinophilia was noted in 25 donors. (Figure 17).

Figure 17: Differential count of blood donors



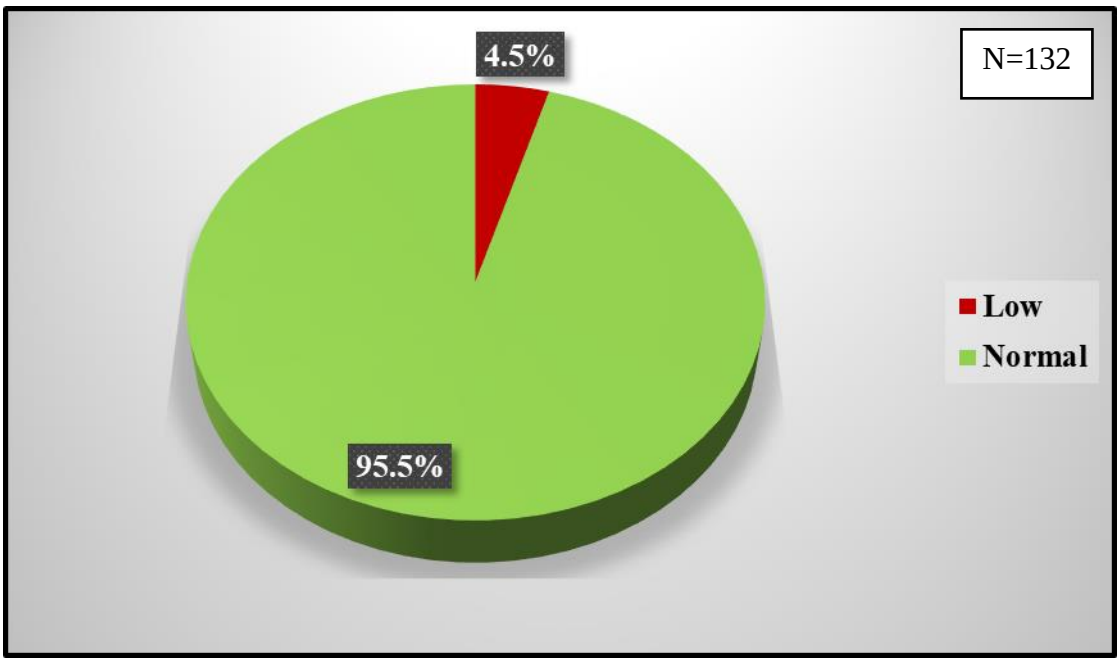
PLATELET COUNT OF BLOOD DONORS

Out of the 132 blood donors, 6 of the donors showed thrombocytopenia, while the rest had normal platelet count. (Table 18, Figure 18). The mean platelet count was noted to be 2.63 lakh/mm^3 with a SD of 0.72 lakh/mm^3.

Table 18: Platelet count among the blood donors

Platelet count (lakh/mm^3)	N	Percentage (%)
Low (<1.5)	6	4.5
Normal (1.5-4)	126	95.5
Total	132	100

Figure 18: Platelet count among the blood donors



HEMATOLOGICAL CHANGES IN ANEMIC DONORS

The mean of all the parameters between anemic and non-anemic donors have been compared in Table 19. It was noted that there was reduction in RBC count, Hb, HCT, MCH and MCHC levels, while there was an increase in RDW in anemic donors compared to non-anemic donors. Within these parameters, significant changes were observed in levels of Hb, HCT, MCH, MCHC and RDW.

Table 19: Comparison of mean parameters in anemic and non-anemic donors

Parameters	Anemic (N=52)		Non-Anemic (N=80)		p value
	Mean	SD	Mean	SD	
WBC count	7081.35	1650.54	7070.75	1743.95	0.972
RBC count	4.05	0.66	4.68	0.47	0.22
Hb	11.32	1.07	13.94	2.15	**<0.001***
HCT	37.03	3.46	43.03	2.75	**<0.001***
MCV	91.00	8.86	91.92	6.64	0.494
MCH	28.16	3.62	30.23	6.95	**0.049***
MCHC	30.64	1.36	32.07	1.17	**<0.001***
Platelet count	2.71	0.72	2.58	0.73	0.295
RDW	14.42	2.09	13.69	0.96	**0.007***

Note: *significant when p value ≤ 0.05

CORRELATION OF PARAMETERS WITH NUMBER OF DONATIONS

Table 20 shows the change in the various parameters with the number of donations. A negative correlation was observed between RBC count, Hb, HCT, MCV, MCH, MCHC and platelet count with the number of donations. This meant that there was a reduction in these parameters as the number of past donations increased. Significant reduction was noted in RBC count, Hb and HCT.

Table 20: Pearson correlation coefficient of parameters with number of donations

Parameters	r value	p value
WBC count	0.009	0.923
RBC count	-0.197	**0.024***
Hb	-0.164	**0.049***
HCT	-0.248	**0.004***
MCV	-0.067	0.448
MCH	-0.048	0.587
MCHC	-0.031	0.721
Platelet count	-0.03	0.729
RDW	0.09	0.303

Note: ***significant when p value ≤ 0.05

DISCUSSION

Voluntary, non-remunerated blood donors form the core of any blood transfusion service as, they are the foundation of a safe and sustainable blood supply. These donors not only donate blood at regular intervals but also respond to calls during disasters and emergencies. Hence, one should consider these donors a valuable national asset.[10]

WHO, quite for some time now, has been pushing towards 100% voluntary unpaid blood donations. Most of the developed countries and even a few developing ones have achieved this goal, while other developing countries are on their way towards achieving it. Although this action has led to greater availability of safe blood in many countries, a pattern has emerged among these voluntary regular blood donors everywhere. Many studies have been published from all over the world and these authors have reported varying degree of anemia among their donor base. This anemia has been widely attributed to Iron deficiency, which occurs due to multiple blood donations as, with every donation a donor loses approximately 220-240 mg of iron.[3,5]

Hence, Hb estimation is an important criterion for blood donor selection and the minimum Hb cut-off recommended by WHO is 12.5 g/dl. Various screening tests are available for screening of Hb in the blood donors, however there is no consensus among the blood banks as to which method is best suitable for screening. In most of the laboratories, the automated hematology analyzer is taken as the gold standard for Hb estimation as the results are accurate and reliable. Nevertheless, this method cannot be employed everywhere like remote locations. For this reason, many blood banks including ours rely on copper sulphate method for screening of the donors during camps in remote areas.

There is gross gender inequality when it comes to blood donation. Many studies have noted that majority of the regular blood donors are generally males and regular female blood donors are rare. This could be due to cultural issues, lack of motivation and misconceptions about blood donation. Another reason is the high prevalence of anemia among females leading to donor deferral, which inversely affects donor attitude towards blood donation.

Table 21 compares the gender distribution of the blood donors from different parts of the world. In the present study, 93.2 % of the donors were males. In comparison, other studies like Jeremiah *et al.*[56], Mahida *et al.*[3] and Kumari *et al.*[61] have also reported similar gender distribution in donors.

Table 21: Gender distribution of blood donors compared to other studies

Studies	Male donors	Female donors
Present Study	**93.2%**	**6.8%**
Jeremiah *et al.*[56]	93.3%	6.7%
Mahida *et al.*[3]	88%	12%
Kumari *et al.*[61]	97.6%	2.4%
Singh *et al.*[63]	98%	2%

In the present study, the mean age of the donors was 29 years, which represents a young donor base. Most of the donors belonged to the age group of 21-30 years. This is obvious as, India has an expanding young adult population and more importantly this age-group can be motivated for regular blood donation. Similar findings were noted by Mahida *et al.*[3] and Mittal *et al.*[4]. However, Jeremiah *et al.*[56] noted most of their regular blood donors were above 40 years of age.

It was noted in the present study that most of the blood donors had B blood group (38.6%), followed by O blood group and 96.2% of the donors were positive for Rh antigen. In comparison, another study by Jeremiah *et al*.[56] found that where most of their donors were of O blood group. (Table 22)

Table 22: Comparison of Blood group distribution of donors

Blood group	**Present study**	Jeremiah *et al*.[56]
A	**20.4%**	7.2%
B	**38.6%**	8%
AB	**7.6%**	0%
O	**33.4%**	84.8%
RhD positive	**96.2%**	92%
RhD negative	**3.8%**	8%

The only possible complication of blood donation is development of anemia among the regular blood donors. This makes sense, because as every time a person donates blood, he/she loses 1-1.5 g/dl of Hb and approximately 220-240 mg of iron.[3] Table 23 shows the comparison of prevalence of anemia in blood donors in various studies from different parts of the world. In the present study, it was noted that 39.4% of the regular donors were anemic. These findings are consistent with other studies conducted by Kumari *et al*.[61], Nadarajan *et al*.[59], Jeremiah *et al*.[8] and Shahshahani *et al*.[57] However, other studies conducted by Milman *et al*.[55], Bahadur *et al*.[65] and Tailor *et al*.[62] noted low prevalence of anemia among regular blood donors. One possible reason for this could be due to the high prevalence of anemia in the general population in India.

Table 23: Prevalence of anemia among regular blood donors in various studies

Studies	Anemic donors	Non-Anemic donors
Present study	**39.4%**	**60.6%**
Kumari *et al.*[61]	23.1%	76.9%
Nadarajan *et al.*[59]	21.3%	78.7%
Bahadur *et al.*[65]	15.5%	84.5%
Shahshahani *et al.*[57]	26.4%	73.6%
Jeremiah *et al.*[56]	18.8%	81.2%
Milman *et al.*[55]	1.3%	98.7%
Tailor *et al.*[62]	19.6%	80.4%
Jeremiah *et al.*[8]	25.8%	74.2%
Mahida *et al.*[3]	11.6%	88.4%

In the present study, the mean Hb of the donors was noted to be 12.9 g/dl with 44 of the donors showing mild anemia, 10 showing moderate while 2 showing severe anemia. Table 24 shows the mean Hb of the blood donors in similar studies by Tailor *et al.*[62], Mahida *et al.*[3], Jeremiah *et al.*[56] and Nadarajan *et al.*[59]

Table 24: Comparison of mean Hb among regular blood donors

Studies	Mean Hb (g/dl)	SD
Present study	**12.9**	**±2.21**
Tailor *et al.*[62]	13.1	±1.32
Nadarajan *et al.*[59]	13.9	±1.42
Mahida *et al.*[3]	13	±1.6
Jeremiah *et al.*[56]	12.4	±2

The mean RBC count was found to be 4.4 million/mm^3 in the present study, which is below the normal. Low RBC can be caused by iron deficiency anemia, vitamin B12 or folate deficiency, internal bleeding and even in kidney diseases. Regular blood donors are likely to suffer from iron deficiency anemia. In fact, 51.5% of the donors in our study had RBC count below normal limit (Figure 8), while 39.4% donors were actually anemic (Figure 9). Similar findings were noted by Tailor *et al.*[62] and Jeremiah *et al.*[56] as shown in Table 25. Nadarajan *et al.*[59] found the mean RBC count to be 5.2 million/mm^3 among the blood donors in their study.

Table 25: Comparison of mean RBC count among regular blood donors

Studies	Mean RBC count (millions/mm^3)	SD
Present study	**4.4**	**±0.63**
Tailor *et al.*[62]	4.8	±0.56
Nadarajan *et al.*[59]	5.2	±0.6
Jeremiah *et al.*[56]	4.9	±1.1

Table 26 shows the mean HCT of the blood donors. In the present study, the mean haematocrit level was 40.6% which is on the lower side. This can be explained by the low level of RBC count among the blood donors. Approximately 41.7% of our donors had low haematocrit (Figure 11), compared to 39.4% of actually anemic donors (Figure 9). Similar findings have been noted by Nadarajan *et al.*[59] Jeremiah *et al.*[56] noted the haematocrit to be as low as 30.5%.

Table 26: Comparison of mean HCT among regular blood donors

Studies	Mean HCT (%)	SD
Present study	**40.6**	**±4.2**
Nadarajan *et al.*[59]	44	±4
Jeremiah *et al.*[56]	30.5	±5.5

Table 27 shows the mean MCV level among regular blood donors. In the present study, the mean MCV was noted to be 91.56 fl which was consistent with the findings of other studies conducted by Baart *et al.*[67], Tailor *et al.*[62], Nadarajan *et al.*[59] and Mahida *et al.*[3] However, Jeremiah *et al.*[56] noted the mean MCV to be as low as 70.1 fl in their study.

Table 27: Comparison of mean MCV among regular blood donors

Studies	Mean MCV (fl)	SD
Present study	**91.56**	**±7.57**
Tailor *et al.*[62]	85.17	±7.15
Nadarajan *et al.*[59]	85.2	±6.2
Mahida *et al.*[3]	79.5	±6.8
Baart *et al.*[67]	88	±5
Jeremiah *et al.*[56]	70.1	±11.3

In the present study, the mean MCH value was found to be 29.42 pg, which is on the lower side. Similar observations were made by Tailor *et al.*[62] Nadarajan *et al.*[59], Mahida *et al.*[3] and Baart *et al.*[67] as shown in Table 28. Jeremiah *et al.*[56] reported mean MCH to be 22.1 pg in their study.

Table 28: Comparison of mean MCH among regular blood donors

Studies	Mean MCH (pg)	SD
Present study	**29.42**	**±5.94**
Tailor *et al.*[62]	28.05	±3.04
Nadarajan *et al.*[59]	27	±2.3
Mahida *et al.*[3]	27.5	±3
Baart *et al.*[67]	29.3	±2.5
Jeremiah *et al.*[56]	22.1	±4.4

The mean MCHC was noted to be 31.5 g/dl in the present study, which was on the lower side of normal. In fact, 53% of the donors in the present study had low MCHC levels (Figure 14), while only 39.4% donors were anemic (Figure 9). This could be due to iron deficiency in the blood donors. This finding is consistent with the observations of Nadarajan *et al*.[59] and Jeremiah *et al*.[56], as depicted in Table 29.

Table 29: Comparison of mean MCHC among regular blood donors

Studies	Mean MCHC (g/dl)	SD
Present study	**31.5**	**±1.4**
Nadarajan *et al*.[59]	31.4	±3
Jeremiah *et al*.[56]	30.1	±4

Table 30 shows the mean RDW among blood donors across various studies. The mean RDW in the present study was noted to be 13.9, similar to Mahida *et al*.[3] While other studies conducted by Nadarajan *et al*.[59] and Jeremiah *et al*.[56] have noted higher RDW levels in regular donors. This is due to the increased anisopoikilocytosis of RBCs. In the present study, 65.9% of the donors had increased RDW (Figure 15), while only 39.4% were anemic (Figure 9). This shows that RDW can act as a marker for iron deficiency in the non-anemic donors.

Table 30: Comparison of mean RDW among regular blood donors

Studies	Mean RDW	SD
Present study	**13.98**	**±1.5**
Nadarajan *et al*.[59]	15.5	±1.8
Mahida *et al*.[3]	14.1	±2.3
Jeremiah *et al*.[56]	17.7	±3.6

Table 31 shows the mean WBC count among regular donors compared with other studies. In the present study, the mean WBC count was 7074.9 cells/mm^3. Similar findings were noted by Nubila *et al*.[68] However, Jeremiah *et al*.[56] noted subclinical leukopenia among the regular blood donors, with a mean of 3600 cells/mm^3. In the present study only 3 donors showed leukopenia (Table 17), while Jeremiah *et al*.[56] noted leukopenia among 14 donors. This variation could be due to the high number of professional donors among the Nigerian population.

Table 31: Comparison of mean WBC count among regular blood donors

Studies	Mean WBC count (cells/mm^3)	SD
Present study	**7074.9**	**±1701.4**
Nubila *et al*.[68]	5880	±330
Jeremiah *et al*.[56]	3600	±1800

In the present study, a total of 25 donors showed eosinophilia, an unexpected finding. Mild eosinophilia can be caused by allergies, asthma, atopic dermatitis and eosinophilic esophagitis, while moderate to severe eosinophilia can be dur to sinusitis, aspergillosis, eosinophilic pneumonia, parasites, autoimmune disorders and rarely idiopathic hyper-eosinophilic syndrome.[69] Few studies have reported eosinophilia among their blood donors and they have attributed this to parasitic infestations.[70,71] In the present study, the eosinophilia noted in the donors could be attributed to helminthic infestations which is prevalent in the Indian subcontinent. Hence, all these blood donors need to be screened further, to confirm the cause of the eosinophilia and managed accordingly.

The mean platelet count in the present study was noted to be 2.63 lakh/mm^3 as shown in Table 32. Similar findings were noted by Jeremiah *et al.*[56] and Abbas *et al.*[72] However, Nair *et al.*[73] noted mild thrombocytopenia with mean platelet count at 1.38 lakh/mm^3 in their study population.

Table 32: Comparison of mean Platelet count among blood donors

Studies	Mean Platelet count (lakh/mm^3)	SD
Present study	**2.63**	**±0.72**
Jeremiah *et al.*[56]	2.49	±0.96
Abbas *et al.*[72]	2.15	±0.68
Nair *et al.*[73]	1.38	±0.56

Table 33 shows the hematological changes noted in the anemic blood donors of the present study compared to another study by Jeremiah *et al*.[56] In the present study, significant changes were noted in mean levels of Hb, HCT, MCH, MCHC and RDW among the anemic blood donors in comparison to non-anemic donors. These findings are consistent with Jeremiah *et al*.[56], who noted significant changes in RBC count, Hb, HCT, MCH, RDW and WBC count.

Table 33: Hematological changes noted in anemic donors compared to non-anemic donors in different studies

Parameters	**Present study**		Jeremiah *et al*.[56]	
	Mean ± SD	**p value**	Mean ± SD	p value
WBC count	**7081.3 ± 1650.5**	**0.972**	3.2 ± 1.6	0.047*
RBC count	**4.05 ± 0.66**	**0.22**	4.1 ± 1.2	0.001*
Hb	**11.32 ± 1.07**	**<0.001***	10.5 ± 1.9	0.001*
HCT	**37.03 ± 3.46**	**<0.001***	30.0 ± 4.5	0.001*
MCV	**91.00 ± 8.86**	**0.494**	74.9 ± 9.2	0.163
MCH	**28.16 ± 3.62**	**0.049***	20.7 ± 4.2	0.016*
MCHC	**30.64 ± 1.36**	**<0.001***	26.8 ± 4.8	0.342
Platelet count	**2.71 ± 0.72**	**0.295**	2.55 ± 1.12	0.386
RDW	**14.42 ± 2.09**	**0.007***	19.8 ± 4.4	0.001*

Note: *****significant when p value ≤ 0.05

Significant hematological changes have been noted in the anemic donors, as expected, and this has been observed by other studies as well. However, in the present study, it was noted that some of the RBC parameters showed significant change in non-anemic donors as well, as shown in Table 16. A significant number of non-

anemic donors showed reduction in RBC count, HCT and MCHC levels. Many of these donors also had high RDW levels. So, it is possible that these parameters could act as a marker of iron deficiency or as a marker of eminent anemia in an apparently non-anemic blood donor. However, these findings would have to validated, in larger study population with a simultaneous comparison with iron studies, among the non-anemic donors.

It is evident from the above discussion that anemia has become a notorious problem among the regular blood donors worldwide. Although the prevalence varies from region to region, most of the countries are facing this menace and this problem is only getting bigger with each day. On one hand, the WHO has been pushing for voluntary blood donors and is encouraging transfusion centres to have a donor base, while on the other hand, the increasing trend of fast food consumption, which provides empty calories, has only compounded the problem of anemia in the population.

Role of Iron Supplementation

There is no doubt from the above observations that regular blood donation can lead to significant hematological changes including anemia and significant loss of iron in the blood donors. Moreover, their levels are bound to worsen with every donation unless appropriate measures are taken to address the issue head-on. These could be preventive measures or interventional.

Preventive measures include proper counselling of the blood donors. They need to be made aware of the possible adverse effects of multiple blood donations and the risk of developing anemia or iron deficiency in the long run. Hence, they should be encouraged to maintain a healthy nutritious diet which contains good quantity of

iron. They should also be encouraged to undergo regular health check-ups to rule out any subclinical illnesses. However, counselling should be done in a manner that does not discourage a potential young donor from donating blood.

Many studies have advised the use of iron supplements for the blood donors, post donation.[60,74–76] These studies have reported quicker recovery of iron levels, even in non-anemic blood donors. They have advised an iron supplementation of 100 mg daily for 20 days post donation for all donors. This practice will not only raise the iron levels among the iron deficient blood donors but also prevent anemia in the healthy blood donors.

SUMMARY

- The present study was a cross-sectional study, carried out from 1st November, 2016 to 30th June, 2018.
- A total of 132 regular blood donors who had donated blood at least 3 times in the previous year were selected for the study.
- Two ml of blood was collected in EDTA vacutainer before the blood donation.
- The samples were analyzed within 4-6 hours of collection.
- These blood samples were tested using an automated 5-part differential hematology analyzer (SYSMEX-XN1000) for hematological parameters like Hb, RBC count, HCT, MCV, MCH, MCHC, RDW, WBC count, Differential count and Platelet count.
- It was observed that 39.4% of the regular donors were anemic, with majority of them showing mild degree of anemia.
- A negative correlation was observed between the RBC count, Hb, HCT, MCV, MCH, MCHC and platelet count with number of previous blood donations, with significant reductions noted in RBC count, Hb and HCT.
- A significant reduction in Hb, HCT, MCH, MCHC was noted in anemic blood donors compared to non-anemic donors. There was also a significant increase in RDW of anemic donors.
- A proportion of non-anemic donors showed significant reduction in RBC count, HCT, MCHC and increase in RDW. Hence, these parameters could potentially act as markers of iron deficiency among the non-anemic,

apparently healthy blood donors, as undertaking iron studies is not feasible for every donor.

- An unexpected finding of eosinophilia was noted in 25 blood donors. This finding needs to evaluated further to pinpoint the cause and take appropriate steps in management of the donor.
- Due to the high prevalence of anemia in the blood donor population, it is recommended that the donors should be properly counselled regarding the long-term risks of regular blood donation.
- Donors should be encouraged to take iron rich nutritious diet. In addition, iron supplementation (100 mg daily) should be provided to the donors for 20 days post donation, free of cost, to treat and prevent anemia in the blood donors.

CONCLUSION

The present study shows that there is a high prevalence of anemia in the regular blood donors, which only worsens with every donation. In addition, significant reduction in RBC count, HCT and MCHC and increase in RDW was also noted in these donors as well as many apparently healthy non-anemic donors. Therefore, these parameters should also be evaluated in non-anemic donors, as these donors are at-risk of developing anemia and could very well have an underlying iron deficiency.

In a country like India, where anemia is highly prevalent among the general population and there is ever increasing demand for blood and its components, it becomes imperative that our blood donors are fit, healthy and free from iron deficiency. In this regard, free iron supplementation has to be given to all donors, especially, the regular blood donors.

It is high time that the health of the blood donor became as important as the health of the recipient.

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ANNEXURE-I

Institutional Ethical Committee
BIJAPUR-586 103.
OUTWARD
No. 611/2016
Date. 06/10/16
Shri B.M. Patil Medical College

B.L.D.E. UNIVERSITY'S
SHRI.B.M.PATIL MEDICAL COLLEGE, BIJAPUR-586 103
INSTITUTIONAL ETHICAL COMMITTEE

INSTITUTIONAL ETHICAL CLEARANCE CERTIFICATE

The Ethical Committee of this college met on 04/10/2016 *at* 3-00pm *to scrutinize the Synopsis of Postgraduate Students of this college from Ethical Clearance point of view. After scrutiny the following original/corrected & revised version synopsis of the Thesis has been accorded Ethical Clearance.*

Title "A study on changes in hematological parameters due to matile blood donations"

Name of P.G. student Ankur Kumar
Dept in Pathology

Name of Guide/Co-investigator Dr Prakash M. Patil
Associate Professor in Pathology

DR.TEJASWINI. VALLABHA
CHAIRMAN
INSTITUTIONAL ETHICAL COMMITTEE
BLDEU'S, SHRI.B.M.PATIL
MEDICAL COLLEGE, BIJAPUR.

Following documents were placed before E.C. for Scrutinization
1) Copy of Synopsis/Research project.
2) Copy of informed consent form
3) Any other relevant documents.

ANNEXURE-II

INFORMED CONSENT FORM FOR DISSERTATION/REASEARCH

I, the undersigned_____, S/O D/O W/O , aged _____ years, ordinarily resident of do hereby state/declare that Dr ANKUR KUMAR of BLDE Hospital has examined me thoroughly on _____ at (place) and it has been explained to me in my own language.

Further Dr ANKUR KUMAR informed me that he/she is conducting dissertation/research Titled "A STUDY ON CHANGES IN HEMATOLOGICAL PARAMETERS DUE TO MULTIPLE BLOOD DONATIONS" under the guidance of Dr PRAKASH M. PATIL requesting my participation in the study.

Further doctor has informed me that my participation in this study help in evaluation of the results of the study which is useful reference to treatment of other similar cases in near future.

The Doctor has also informed me that information given by me, observations made/ photographs/ video graphs taken upon me by the investigator will be kept secret and not assessed by the person other than me or my legal hirer except for academic purposes.

The Doctor did inform me that though my participation is purely voluntary, based on information given by me, I can ask any clarification during the course of treatment / study related to diagnosis, procedure of treatment, result of treatment or prognosis. At the same time, I have been informed that I can withdraw from my participation in this study at any time if I want or the investigator can terminate me

from the study at any time from the study but not the procedure of treatment and follow-up unless I request to be discharged.

After understanding the nature of dissertation or research, diagnosis made, mode of treatment,

I the undersigned Shri/ Smt _____ under my full conscious state of mind agree to participate in the said research/ dissertation.

Signature of donor:

Signature of doctor:

Witness: 1.

2.

Date:

Place

ANNEXURE-III

PROFORMA FOR THE STUDY

Sample no:

Name (Optional):

Age: Sex: M/F Blood Group:

Donated blood previously: YES/NO

a. If yes, no. of donations:
b. Last donated:

History of any illness or surgery in the last one year: YES/NO

c. If yes, Type of illness:
d. Duration of illness:
e. Treatment taken:

General physical examination:

Pallor : Icterus:

Cyanosis : Edema:

Vitals:

Investigations:

WBC Count (cells/mm^3)	
RBC Count (million/mm^3)	
Hb (g/dl)	
HCT (%)	
MCV (fl)	
MCH (pg)	
MCHC (g/dl)	
Platelet Count (lakh/mm^3)	
RDW	
Neutrophils (%)	
Lymphocytes (%)	
Monocytes (%)	
Eosinophils (%)	
Basophils (%)	

KEY TO MASTER CHART

WBC	White Blood Cell count (cells/mm^3)
RBC	Red Blood Cell count (million/mm^3)
Hb	Hemoglobin (g/dl)
HCT	Hematocrit (%)
MCV	Mean Corpuscular Volume (fl)
MCH	Mean Corpuscular Hemoglobin (pg)
MCHC	Mean Corpuscular Hemoglobin Concentration (g/dl)
PLT	Platelet Count (lakh/mm^3)
RDW	Red cell Distribution Width
N	Neutrophil (%)
L	Lymphocyte (%)
M	Monocyte (%)
E	Eosinophil (%)
B	Basophil (%)

MASTER CHART

Sample No.	Age	Gender	Blood group	No. of donations	WBC	RBC	Hb	HCT	MCV	MCH	MCHC	PLT	RDW	N	L	M	E	B
1	58	M	O pos	6	7300	4.64	13.9	44.3	95.5	30	31.4	0.85	12.3	71	22	4	3	0
2	21	M	A pos	4	7180	5.38	15.4	48.3	89.8	28.6	31.9	4.16	13.2	57	35	4	3	1
3	30	M	A pos	6	5490	2.68	7.9	25.1	93.7	29.5	31.5	2.89	16.4	58	31	6	5	0
4	26	M	O pos	5	6530	4.74	12.7	39.2	82.7	26.8	32.4	0.8	14.8	58	37	3	2	0
5	23	M	AB pos	3	6080	4.83	13.3	4305	90.1	27.5	30.6	3.39	14.9	47	43	6	4	0
6	23	M	B pos	5	12180	4.63	14.6	43.9	94.8	31.5	33.3	2.63	13.4	61	33	4	2	0
7	36	M	O pos	5	7420	4.5	12.5	40.1	89.1	27.8	31.2	3.03	12.9	51	42	3	3	1
8	34	M	B pos	3	9790	5.04	13.7	43.6	86.5	27.2	31.4	2.3	12.9	47	36	5	11	1
9	29	M	A pos	4	7650	4.1	11.6	38.2	93.2	28.3	30.4	3.48	12.8	68	25	5	2	0
10	48	M	B pos	6	4550	4.62	13.6	43.5	80.8	26.6	32.9	1.8	14.2	48	45	4	2	1
11	22	M	O pos	3	6000	3.66	13.2	52.3	92.4	30.4	32.9	3.21	14.7	71	22	4	3	0
12	25	M	A pos	3	7000	3.15	11.4	40.5	92.2	28	30.3	4.3	12.8	61	33	4	2	0
13	28	M	O pos	20	7740	2.2	7.9	25.2	76.7	23.6	30.7	3.8	18	77	15	6	2	0
14	25	M	A pos	6	5320	3.4	11.5	39.2	86.7	28.3	30.3	2.1	14.1	65	28	4	3	0
15	27	M	O pos	6	6450	3.15	9.3	31.9	101.3	29.5	29.2	1.68	13.3	55	36	5	3	1

16	25	M	B pos	6	6680	5.28	13.2	45	85	25	29.4	2.92	14.1	54	32	4	10	0
17	22	M	B pos	3	5840	5.14	15.4	47	91.4	30	32.8	2.74	13.3	64	28	4	3	1
18	20	F	A pos	3	6140	4.1	11.1	37.5	91.5	27.1	29.6	2.04	14.2	62	32	5	1	0
19	20	M	AB pos	4	8330	6.36	12.9	44.2	69.5	20.3	29.2	3.52	17.9	54	35	5	6	0
20	20	F	B pos	3	9680	3.06	9	30.3	99	29.4	29.7	4.3	18.3	57	38	4	1	0
21	38	M	O pos	3	5420	4.27	12.2	40.9	95.8	28.6	29.8	3.23	13.9	56	36	5	3	0
22	31	M	O pos	3	6290	4.8	14.3	45.5	94.8	29.8	31.4	2.72	14	41	46	5	7	1
23	31	M	B pos	3	4260	4.86	14.2	42.2	86.8	29.2	33.6	1.79	13.3	51	40	5	3	1
24	26	M	B pos	3	6860	4.62	13.6	43	93.1	29.4	31.6	2.48	12.6	60	34	4	2	0
25	24	M	B pos	3	6150	4.39	12.3	39	88.8	28	31.5	2.67	12.7	61	27	6	6	0
26	25	M	B pos	3	6610	4.36	12.3	39.5	90.6	28.2	31.1	2.41	14.2	35	53	6	6	0
27	27	M	B pos	3	8280	4.35	11.8	39.1	89.9	27.1	30.2	2.22	13.2	57	31	6	5	1
28	26	M	A neg	3	6320	4.73	13.1	42.7	90.3	27.7	30.7	1.14	13.2	58	34	5	2	1
29	33	M	O pos	8	8580	4.42	13.6	43	97.3	30.8	31.6	2.08	13.7	43	50	4	3	0
30	27	M	B pos	4	5340	4.18	11	36.6	87.6	26.3	30.1	1.87	13.3	39	49	5	7	0
31	27	M	A pos	3	8400	4.41	13.5	43	97.5	30.6	31.4	2.49	13.4	46	36	5	12	1
32	32	M	B pos	5	6980	4.35	12.3	39.8	91.5	28.3	30.9	2.36	15.3	58	26	6	9	1
33	29	M	B neg	6	10860	4.67	13.4	43.5	93.1	28.7	30.8	2.77	13.7	62	27	5	5	1
34	33	M	B pos	5	10380	4.52	13.2	41.3	91.4	29.2	32	2.71	13.4	44	44	5	6	1

35	32	M	O pos	9	8070	4.2	13.1	40.8	97.1	31.2	32.1	2.45	14.6	50	41	6	3	0
36	27	M	B pos	8	4720	4.89	12.7	41.4	84.7	26	30.7	1.18	13.9	59	29	5	6	1
37	24	M	A pos	7	5330	4.14	11.3	38.4	92.8	27.3	29.4	2.07	14.6	39	49	6	6	0
38	32	M	AB pos	7	7620	4.23	12.6	40.8	96.5	29.8	30.9	2.78	12.9	61	30	5	4	0
39	24	M	O pos	3	7390	4.58	13.7	44.4	96.9	29.9	30.9	3.36	14.7	57	56	4	3	0
40	31	M	O pos	3	6360	4.83	13.9	45.5	94.2	28.8	30.5	2.1	14	49	42	4	4	1
41	24	M	A pos	4	5530	3.83	11.8	37.8	98.7	30.8	31.2	2.35	12.5	55	36	6	2	1
42	37	M	O pos	5	6400	4.62	13.1	42.3	91.6	28.4	31	2.15	13.9	53	32	5	10	0
43	34	M	A pos	3	5390	4.57	12.7	40.5	88.6	87.8	31.4	1.97	13.1	49	41	6	4	0
44	44	M	O pos	7	6320	4.55	15.2	48.4	106.4	33.4	31.4	1.95	13.9	61	29	4	5	1
45	28	M	O pos	4	4970	4.87	13.7	44	90.3	28.1	31.1	2.37	13.6	52	33	5	9	1
46	22	M	B pos	4	8310	4.73	13.7	45.3	95.8	29	30.2	2.57	13.9	47	47	5	1	0
47	33	M	O pos	5	7570	3.76	12.5	40.1	106.6	33.2	31.2	3.76	12.4	50	36	5	8	1
48	29	M	O pos	4	6320	4.29	12.3	39.1	91.1	28.7	31.5	1.72	13.1	59	34	5	2	0
49	40	M	B pos	3	6370	4.84	13.4	42.6	88	27.7	31.5	2.71	13.4	49	42	5	4	0
50	26	M	A pos	3	5630	4.83	12.4	42.2	87.4	25.7	29.4	3.35	13.1	59	35	4	2	0
51	23	M	O pos	3	6380	4.02	12.4	41.3	102.7	30.8	30	2.1	12.6	66	27	6	1	0
52	28	M	O neg	5	6350	3.95	13.9	42.8	108.4	35.2	32.5	2.51	13.1	63	29	5	3	0
53	28	M	B pos	5	7940	4.84	13.1	41.2	85.1	27.1	31.8	3.18	13.6	58	30	5	7	0

54	24	M	O pos	10	7420	4.77	13.9	43.2	90.6	29.1	32.2	2.91	13	55	35	6	4	0
55	30	M	B pos	3	7000	4.33	13.3	40.3	93.1	30.7	33	2.29	12.9	70	24	2	4	0
56	36	M	AB pos	3	8000	4.94	12.9	40.9	82.8	26.1	31.5	2.24	14.8	77	20	2	1	0
57	32	M	B pos	5	6000	4.08	11.2	35.8	87.7	27.5	31.3	2.19	12.9	64	33	3	0	0
58	26	M	B pos	5	10000	5.03	14.3	44.4	88.3	28.4	32.2	2.5	14.6	46	42	6	6	0
59	26	M	O pos	3	5000	4.31	14	41.1	95.4	32.5	34.1	1.99	12.9	74	20	2	4	0
60	30	M	AB pos	3	11000	4.19	13	39.7	94.7	31	32.7	3.22	13.7	47	20	1	32	0
61	37	M	B pos	10	5000	4.35	13.2	42.1	96.8	30.3	31.4	2.23	14.3	68	28	0	4	0
62	20	M	O pos	3	3000	4.45	12.7	41.3	92.8	28.5	30.8	2.05	13.2	70	28	1	1	0
63	29	M	A pos	4	8000	4.45	14.8	42.7	96	33.3	34.7	3.5	14.1	60	39	1	0	0
64	33	M	O pos	6	6000	3.65	11.3	35.2	96.4	31	32.1	1.59	13.1	77	20	1	2	0
65	24	M	O pos	4	6000	4.15	12.5	39.7	95.7	30.1	31.5	2.47	13.5	50	40	3	7	0
66	43	M	B pos	4	4000	4.23	11.9	38.4	90.8	28.1	31	1.85	13.5	47	40	1	12	0
67	37	M	B pos	11	6500	4.33	12	38.1	88	37.7	31.5	2.52	13.5	78	20	0	2	0
68	33	M	B pos	7	6000	4.8	14.6	45.6	95	30.4	32	2.46	12.2	52	40	2	6	0
69	24	M	B pos	4	8000	3.8	11.2	36.8	96.8	29.5	30.4	3.3	14.5	64	36	2	8	0
70	28	M	O pos	4	4000	5.47	9.8	35.9	65.6	17.9	27.3	2.5	21.6	62	30	2	6	0
71	30	M	O pos	6	6000	5.44	16.6	49.2	90.4	30.5	33.7	1.81	13.2	78	20	0	2	0

72	24	M	B pos	4	5200	4.39	13.1	45.1	102.7	29.8	29	2.51	13	41	52	5	2	0
73	23	M	B pos	3	6050	4.92	13.4	43.9	89.2	27.2	30.5	3.29	14.2	46	42	5	7	0
74	51	M	A pos	5	8060	3.39	9.4	32.9	97.1	27.7	28.6	3.17	14.2	56	36	4	3	1
75	24	M	AB pos	4	6500	4.68	11	39.7	84.8	23.5	27.7	2.88	15.7	69	26	4	1	0
76	25	M	B pos	5	6000	4.07	10.8	35.6	87.5	26.5	30.3	2.67	12.6	61	29	4	5	1
77	29	M	O pos	4	9440	4.64	12.4	43.1	92.9	26.7	28.8	2.87	14.2	62	27	5	5	1
78	33	M	O pos	4	7810	4.09	12	38.5	94.1	29.3	31.2	2.47	13.4	51	40	5	3	1
79	25	M	B pos	3	5950	4.79	12.8	40.1	83.7	26.7	31.9	3.3	13.3	51	40	5	3	1
80	30	M	B pos	10	8100	3.91	11.3	37.3	95.4	28.9	30.3	2.51	13.9	65	30	4	0	1
81	54	M	B pos	4	10200	4.62	11.9	39.5	85.5	25.8	30.1	3.05	14.9	49	43	3	4	1
82	24	M	B pos	4	9030	3.66	10.8	35.3	96.4	29.5	30.6	3.66	16.8	51	39	5	4	1
83	33	M	B pos	6	5500	3.59	12	37.2	103.6	33.4	32.3	2.03	14	51	41	4	4	0
84	26	M	A neg	5	6710	4.27	12.2	38.5	90.2	28.6	31.7	2.09	12.2	61	33	5	0	1
85	26	M	O pos	8	8380	3.86	12.2	38.7	100.3	31.6	31.5	2.81	13	71	19	5	4	1
86	51	M	B pos	3	7080	3.74	10.9	34	90.9	29.1	32.1	2.97	13.8	60	30	4	6	0
87	33	M	B pos	3	5530	4.95	13.9	41.7	84.2	28.1	33.3	0.82	13	56	33	5	5	1
88	41	M	O pos	5	7450	5.98	13.2	47.2	86.3	28.8	33.3	3.92	13.8	65	26	6	2	1
89	37	M	O pos	35	7330	3.86	11.4	34.8	90.2	29.5	32.8	2.6	14	68	24	4	4	0
90	40	M	O pos	4	8370	4.13	31.1	41.4	100.2	31.7	31.6	3.33	13.7	52	37	5	6	0

91	39	M	B pos	3	10010	5.16	16.1	47.3	91.7	31.2	34	2.94	14.5	53	40	4	2	1
92	32	M	AB pos	8	7330	5.58	14.7	46	82.4	26.3	32	3.14	14.3	64	30	4	2	0
93	20	M	B pos	4	6160	5.19	14	43.3	83.4	27	32.3	3.4	12.7	47	37	5	10	1
94	21	M	O pos	3	6840	3.85	12.4	36.3	94.3	32.2	34.2	1.7	13.6	66	27	4	3	0
95	29	M	B pos	8	8890	5.99	11.3	41	68.4	18.9	27.6	2.99	20.1	62	28	4	6	0
96	21	M	B pos	6	6570	4.38	12.6	39.6	90.4	28.8	31.8	2.19	12.7	51	36	4	9	0
97	23	M	A pos	4	4010	4.24	12.9	37.8	89.2	30.4	34.1	1.56	12.9	51	37	6	5	1
98	24	M	O pos	4	9750	5.49	11.1	36.4	66.3	20.2	30.5	2.61	16.7	57	33	6	4	0
99	21	M	A pos	3	8330	3.61	13	40.2	111.4	36	32.3	3.12	14.9	57	28	2	12	1
100	22	M	O pos	3	8120	4.19	12.4	39.5	94.3	29.6	31.4	3.91	14.5	64	25	3	8	0
101	28	M	A pos	3	4980	4.86	13.7	41.9	86.2	28.2	32.7	3.13	13.1	50	42	4	4	0
102	21	F	B pos	3	10150	3.99	10.9	35.4	88.7	27.3	30.8	3.34	13.5	68	26	3	3	0
103	23	M	B pos	4	7930	5.55	15.5	48.6	87.6	27.9	31.9	2.87	13.5	61	31	4	3	1
104	26	M	O pos	3	6190	4.81	15.3	45.4	94.4	31.8	33.7	3.17	12	61	30	4	5	0
105	23	M	O pos	5	6420	3.86	11.5	35.6	92.2	29.8	32.3	1.66	12.5	50	31	3	16	0
106	26	F	O pos	3	10170	3.38	10.4	34	100.6	30.8	30.6	3.13	13.7	66	30	3	1	0
107	28	M	B pos	3	6560	4.96	14.4	43.4	87.5	29	33.2	3.21	12.5	59	37	2	2	0
108	31	M	B pos	3	6210	4.66	14.8	44.3	95.1	31.8	33.4	2.4	13.2	42	44	4	10	0
109	25	M	A pos	6	5070	4.1	11.9	39	95.1	29	30.5	2.18	12.7	48	45	4	3	0

110	28	M	A pos	4	6740	4.82	13.3	39.9	82.8	27.6	33.3	2.18	13.8	56	39	4	1	0
111	32	M	O pos	3	6630	5.33	13.8	44.5	83.5	25.9	31	2.56	16.2	53	40	5	2	0
112	29	M	B pos	3	5050	3.99	12.3	37.8	94.7	30.8	32.5	1.77	12.8	62	29	5	4	0
113	25	M	A pos	4	6860	4.13	12.5	39	94.4	30.3	32.1	2.66	13.2	64	27	5	4	0
114	26	M	AB pos	3	5190	4.7	15.3	45.7	97.2	32.6	33.5	2.66	13.8	51	42	5	2	0
115	27	M	O pos	3	5210	4.69	13.3	41.3	88.1	28.4	32.2	2	14.7	52	42	4	2	0
116	28	M	A pos	3	7570	4.28	13.3	41.1	96	31.1	32.4	3.87	15.5	67	26	5	1	1
117	29	M	O pos	3	8120	4.55	13.9	43.9	96.5	30.5	31.7	2.2	12.6	62	30	5	3	0
118	24	M	A pos	4	6310	4.98	15.8	46.9	94.2	31.7	33.7	2.88	12.6	47	38	5	10	0
119	27	M	B pos	3	9550	4.31	13.1	40.9	94.9	30.4	32	3.42	13	47	34	5	14	0
120	23	F	B pos	3	10120	4.52	11	35.7	79	24.3	30.8	4.01	20.6	58	37	3	2	0
121	25	F	A pos	6	7170	3.15	11.7	35.6	113	37.1	32.9	2.98	14.6	65	30	3	2	0
122	27	M	O pos	3	7710	4.52	13.8	41.8	92.5	30.5	33	2.61	14.3	74	20	3	3	0
123	23	M	B pos	3	6680	4.22	12.6	39	92.4	29.9	32.3	2.02	13.4	52	25	5	18	0
124	41	M	O pos	3	7370	4.35	14.1	42.6	97.9	32.4	33.1	2.44	15	64	26	5	5	0
125	25	F	A pos	3	5910	3.75	11.7	36.8	98.1	31.2	31.8	3.73	14.6	66	31	2	1	0
126	36	M	B pos	3	7920	5	15	44.7	89.4	30	33.6	0.69	13.3	55	35	6	4	0
127	35	M	A pos	3	7610	4.67	13.7	42.6	91.2	29.3	32.2	3.75	12.9	52	38	5	5	0
128	28	M	B pos	3	6310	5.05	13.7	43.2	85.5	27.1	31.7	2.61	13.6	37	55	3	5	0

129	25	M	AB pos	5	6650	4540	11.7	38.6	85	25.8	30.3	2.88	13.7	56	36	6	2	0
130	32	M	AB pos	3	11820	4.36	14	41.7	95.6	32.1	33.6	2.42	15.9	57	24	4	15	0
131	23	F	A pos	4	9840	4.8	10.6	36.8	76.7	22.1	28.8	3.4	16.2	55	40	3	2	0
132	24	F	O neg	3	9200	3.68	12.7	37.7	102.4	34.5	33.7	2.5	14.4	63	32	4	1	0