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Comparative study of lipid profile and hematologic parameters among regular and first-time blood donors at National Blood Bank Service of Ethiopia.

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A Thesis Submitted to Department of Medical Biochemistry, School of Medicine, College of Health Sciences , Addis Ababa University, in Partial Fulfillment of the Requirements for the Degree of Masters of Science in Medical Biochemistry.

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This is to certify that the dissertation prepared By Abbul Hasano, entitled: **Comparative study of lipid profile and haematologic parameters among regular and first-time blood donors at National Blood Bank Service of Ethiopia, 2020**, and submitted for the partial fulfillment of the requirements of the degree "Master of Science in Biochemistry" in the Department of Medical Biochemistry complies with regulations of the university and meets the accepted standards with respect to originality and quality.

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DECLARATION

I declare that this research paper entitled: **Comparative study of lipid profile and haematologic parameters among regular and first-time blood donors at National Blood Bank Service of Ethiopia, 2020,** is my original work and has not been presented for any degree in any other university and that all sources of materials used for the research have duly been acknowledged.

Abbul Hasano Kebalo

Signature _____

Date _____

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ABBREVIATIONS AND ACRONYMS

BMI	Body mass index
CBC	Complete blood count
CVD	Cardiovascular disease
FTD	First-time donors
HDL-c	High-density lipoprotein cholesterol
IDL-c	Intermediate-density lipoprotein cholesterol
LDL-c	Low-density lipoprotein cholesterol
NBBS	National Blood Bank Service
NLR	Neutrophil to lymphocyte ratio
PLR	Platelet to lymphocyte ratio
RBC	Red blood cell
RBD	Regular blood donors
TC	Total cholesterol
TG	Triglyceride
VLDL-c	Very-low density lipoprotein cholesterol
WBC	White blood cell
WHR	Waist to hip ratio

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ABSTRACT

Background: There is a shortage of blood in the blood banks due to limited donation. By and large, it is widely believed that blood donation has multiple health benefits. However, there are limited studies that support health benefits; As a result, assessing the lipid profile and hematologic parameters of the regular blood donors is an indispensable to evaluate an individual's risk for chronic inflammation.

Objective: We strived to compare lipid and haematological profiles of the regular and first-time blood donors in the National Blood Bank Service of Ethiopia.

Materials and Methods: This comparative cross-sectional study was involved 104 samples (52 each of regular and first-time donors). Anthropometric parameters of donors measured and Blood was drawn from each participants for hematologic and lipid profile determination. Data were analyzed using SPSS version 21, Chi-square (χ^2) was used to compare the relationship between categorical variables and independent t-test was used to compare the mean of the two groups. A p-value < 0.05 was considered statistically significant.

Results: The mean total cholesterol, LDL-c, LDL-c/HDL-c and TC/HDL-c ratios were significantly lower in the regular blood donors than the first-time donors. Even though the level of HDL-c was higher in regular blood donors compared to first-time blood donors, it was not statistically significant. The mean of some hematologic parameters like a platelet, RDW, lymphocyte, and MCH were significantly lower in regular blood donors than first-time blood donors. BMI and WHR in regular donor were less than the first time donors, albeit statistically insignificant.

Conclusions: Interestingly, blood donation has a significant health benefit by lowering TC, TG and LDL-c that have a potential risk for developing chronic inflammation.

Key word: Blood donation, lipid profile, hematologic parameters, National Blood Bank Service of Ethiopia, and chronic inflammation.

1. INTRODUCTION

1.1. Background

Blood is an important element of human life and there is no compensation for it (Nubila, 2014). Blood donation is the life-saving process for people who have lost large volumes of blood from serious accidents, obstetric and gynecological hemorrhages and during various surgical procedures. The blood taken from donors could be either whole blood or blood products (WHO, 2013).

WHO gives a global rate of 117.5 million blood donations annually, of which 60% are voluntary donors and the rest are for family or paid donors (WHO, 2017). The blood donation is the only source of blood, but the recruitment of voluntary and unpaid donors has been a challenge all over the world (Alharbi et al., 2018).

Although blood is very essential to save every life, acute shortage of blood and blood products encounter even in big cities with high population fail to supply 50% of their demand. Every year, millions of people depend on the generosity of another person to donate blood. Nevertheless, blood donation rates vary substantially and the demands for blood and blood products are rising worldwide. To fulfill the demand, more people must step forward to give blood voluntarily and regularly (WHO, 2013).

Blood transfusions in Sub-Saharan Africa were emphasized area of concern, yet there have been very few studies of its history. Record found that transfusions were first reported in Africa in the early 1920s and organized transfusion practices were established before the Second World War and then blood transfusion grew rapidly after 1945. However, there is a great shortage of blood and blood product in Sub-Saharan Africa (Schneider, 2013). The Ethiopia

National blood bank service selects blood donors in a way that ensures the safety of the donor and that of the prospective recipient of the donated blood and blood products. The service strives to build and maintain a pool of safe, voluntary non-remunerated blood donors and takes all necessary steps to ensure that the products derived from donated blood are effective for the recipient, with a no risk of infection that could be transmitted through their transfusion. Routine evaluation of lipid profile and haematological parameters can be done for the diagnosis of various chronic inflammation as its deviation from the normal value indicates that there is a pathological condition (Bharadwaj, 2005).

Lipoproteins are lipid and protein composites that play a great role in transport of lipids and lipophilic molecules in the plasma. Lipoproteins are classified based on their density ranging from high-density lipoproteins (HDL-c) to low-density lipoproteins (LDL-c), intermediate-density lipoproteins (IDL-c), very low-density lipoproteins (VLDL-c), and chylomicrons (CM) (Jairam, 2012).

Blood donation makes blood less viscous and lowers LDL-c and TC due to loss of fibrinogen, albumin and lipid loss during donation. However, the value of LDL-c and TC is higher and HDL lower in chronic inflammation; Therefore, evaluating lipid profile is very important to assess risk factors for chronic illnesses like diabetes mellitus, hypertension, heart disease, etc. (Uche et al., 2013). Blood donation is also reduce enzyme responsible for oxidation of LDL-c, which is linked with the development of atherosclerosis (Salonen et al., 1998).

Dyslipidemia is also shown in a patient suffering from rheumatoid arthritis indicated an unfavorable lipid profile that is a fundamental risk factor of cardiovascular abnormalities or another chronic disease. It is suggested that there is a relationship between inflammation and dyslipidemia (Park et al., 2002).

Haematological parameters determine the amount of haemoglobin and the counts of red blood cells (RBCs), white blood cells (WBCs), and platelets. The tests for haemoglobin and RBCs are essential ways to identify anemia, a condition often caused by insufficient iron in the patient. The hematocrit, also known as packed cell volume or erythrocyte volume fraction, is the volume percentage of RBCs in blood and functions as a major determinant of blood viscosity, blood pressure, venous return, cardiac output, and platelet adhesiveness (Brooks et al., 2014). Testing for white blood cells can reveal some different conditions; fewer or higher white blood cells than normal values can be an indication of infections, failure of production, hematologic pre-malignant and clonal disorders. Generally, a bacterial infection may be suspected among patients with increased total WBC counts than normal. Hemostatic disorders may be accompanied by low or high platelet counts (Lugos et al., 2019). Therefore, haematologic parameters analysis is good indicator to assess the significance of blood donation.

1.2. Statement of the problem

Blood donation is considered as altruistic behavior and it has a benefit for both donors and recipients. Blood services are facing a shortage of blood all over the world. The demand for blood is rising alarmingly and current blood donation is insufficient to meet the demand. (WHO, 2017).

Eighty (80) and two million units of blood are donated each year globally and in Sub-Saharan Africa, respectively, even though the demand in the later is below the required (Schneider, 2013). Adequate and safe blood supply has remained a challenge in developing countries (WHO, 2017). In Ethiopia, there has been inadequacy and in equitability in access to blood. According to a report from the Ethiopian Ministry of Health, Ethiopia collected 223,000 units of blood in 2019/ 20, meeting only 22% of its require as per the standard of the World Health Organization. The goals of the National Blood Bank Service is to improve service and

utilization of blood; ensure the existence of adequate stock of blood in health facilities; and monitor the establishment and proper functioning of hospital blood transfusion (Beyene, 2020 and Arage et al., 2017).

While donating whole blood or blood products, the volume of the blood decreases and there is an exchange of fluid between intracellular and extra-cellular compartments. The exchange makes blood less viscous or dilutes more as well as mechanical removal of protein like fibrinogen, albumin and enzyme responsible for low-density lipoprotein oxidation. All listed above have the potential to make blood more viscous and turbid which is a risk factor for cardiovascular disease (Finland, 1997 and Kessler et al., 2013).

Evaluation of lipid profile (HDL-c, LDL-c, TC, and TG) among blood donors is crucial to assess, whether blood donation has beneficial effects on lipid profile and its association with cardiovascular disease and other chronic inflammation (Mziwira et al., 2005).

Hematologic profile determines the amount of haemoglobin (Hb), WBCs, RBCs and platelets (Tailor et al., 2017). Studies indicated that there were significant changes in haematological profiles among regular blood donors as compared to non-donors individuals (Tailor et al., 2017 and Nubila, 2014).

Some studies reported blood donation decrease risk of cardiovascular and other chronic diseases (vanden Hurk, 2017 and Uche et al., 2013). However, limited studies conducted worldwide and as far as our knowledge, there were no study in Ethiopia that compare the lipid profile and hematologic parameters among regular and first-time blood donors.

Therefore, this study primarily focused on comparing the lipid profile and hematologic parameters among regular blood donors and first-time blood donors at the National Blood Bank Service of Ethiopia.

1.3. Significance of the study

Blood is the essence of life and the most precious donation. Regular donors are the basis of the safe and sustainable distribution system of blood products. The goal of WHO toward a blood donation is to raise awareness of individuals to save lives and improve the health of others by donating blood (WHO, 2012). According to studies, some of the benefits of blood donation includes reduce obesity; reduce risk cardiovascular disease and other chronic inflammation (Clement, 2017; Salonen, 1998 and Nubila, 2014).

This study was planned to assess the level of serum lipid profiles and hematologic parameters from blood donors to establish baseline information; to analyze the effects of frequent blood donation on donors lipid profiles and hematologic parameters; to know health benefits of blood donation by providing evidence based explanation and encourage donors and health professionals to reduce the acute shortage of the blood in the national blood bank service.

1. 4. The hypothesis of the study

The null hypothesis (H_o) assumes that regular blood donation does not affect the lipid profile, haematological and anthropometric parameters of regular blood donors.

The alternative hypothesis (H_a) supposes that regular blood donors affect the lipid profile, haematological and anthropometric parameters of regular blood donors.

2. LITERATURE REVIEW

2.1. Overview of blood donation

Transfusion of blood and blood products serves as the life-saving process. It can help patients suffering from life-threatening conditions to live longer and help those who went through complex surgical procedures. Besides, it has played an essential role in maternal and prenatal care (WHO, 2013).

WHO recommends that every country should set policy, ensure safety, access to quality and timely available blood and blood products to meet the demands of blood transfusion (WHO, 2012). Around 117.4 million people donated blood annually. Of these, 42% collected from high-income countries that only accounts for 16% of world populations. The collected blood is transfused more for under five-years-old children and older age group (Myers and Collins, 2019).

The safety and adequacy of blood donation were important issues in Africa; however, only fewer studies were done in Sub-Sahara Africa (Schneider, 2013). In Ethiopia, there has been gross insufficiency access to blood. Blood banks have to provide adequate and safe blood to the community (Arage et al., 2017).

2.2. Health benefits of blood donation

There are a few studies on the beneficial effects of blood donation and its relationship to ischemic heart disease compared to non-donors. Blood donors seemed to have more favorable lipoprotein concentrations, such as lower levels of LDL-c, TG and Apo B and elevated HDL-c cholesterol concentration when compared to non-donors (van Jaarsveld, 2002). The role of blood loss and consequent decrease in body iron on the risk of acute myocardial infarction and coronary disease were studied by comparing voluntary blood donors to non-blood donors. For

every 300-500 mL whole blood donated, there is around 225-250 mg of heme iron removed. The preliminary findings showed that individuals who had donated blood regularly had greatly reduced the risk of acute myocardial infarction compared with non-blood donors (van den Hurk et al., 2017).

A study done on hypertensive patients showed that after four-times of blood donations, the systolic and diastolic blood pressure decreased from a mean of 155.9 to 143.7 mmHg and from 91.4 to 84.5 mmHg, respectively. After at least four blood donations, donors with Stage II hypertensive baseline values (160 mmHg SBP and/or 100 mmHg DBP) were found to have the most marked reduction in BP with 17.1 mmHg (Kamhieh-Milz et al., 2016).

2.3. Over-view of the function and metabolism of lipoprotein

Majority of lipids found in the human body were from ingesting food and the liver and tissue synthesize the others endogenously. After ingested food is digested in the stomach and small intestine by the help of a locally secreted enzyme, then it gets absorbed through the blood vessels of the small intestine (Rodgers, 2011).

Lipoprotein metabolism is shown in Figure 1.

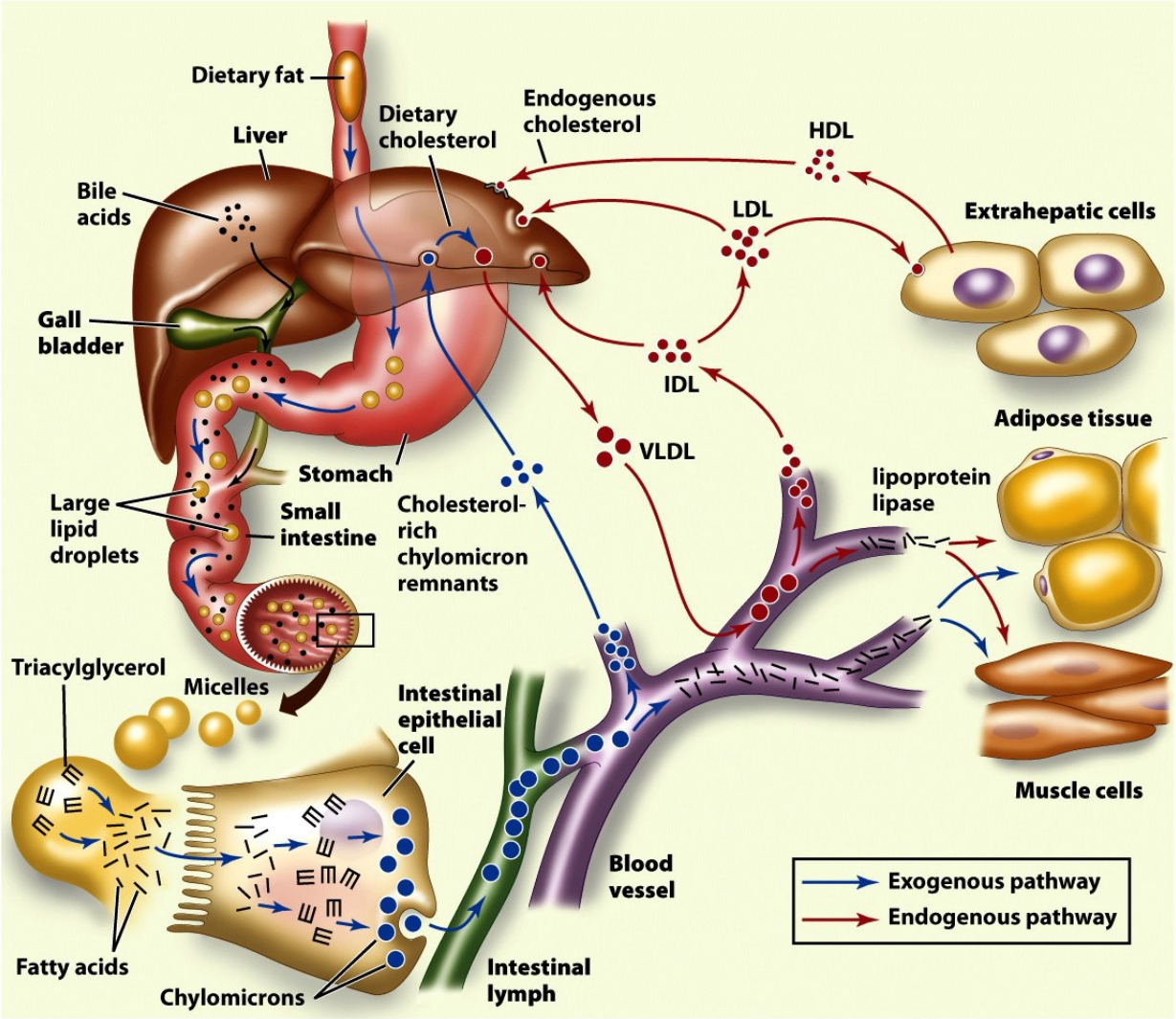

Figure 1. Schematic representation of lipoprotein metabolism, chylomicrons, transport from the intestine through lymphatic vessels into blood/periphery VLDL-c, IDL-c, and LDL-c made by the liver to transport endogenous lipids to periphery and HDL-c transport cholesterol from the periphery back to the liver. The more the lipid content the lower the density of the lipoprotein particle (Jairam et al., 2012).

Plasma levels of LDL-c are mainly determined by the production of Apo B, the Apo lipoprotein of LDL-c, by the conversion of VLDL-c to LDL-c, and by LDL-receptor mediated clearance. The LDL-c molecules are often called bad cholesterol because they can transport their content of many fat molecules into artery walls, attract macrophages, and thus drive atherosclerosis, whereas HDL-c carries cholesterol from tissues back to liver (Song et al., 2010). While LDL

is the major source of atherosclerotic lipid storage, HDL-c is not atherosclerogenic. LDL-c consists of various classes with different size and density that includes large buoyant, intermediate, and small. It had been reported that LDL-c has greater atherogenicity than other types of lipoprotein. Circulating LD-c undergoes multiple modifications in blood plasma such as desialylation, glycation, and oxidation, which is basic for cardiovascular risk factors (Ivanova et al., 2017). LDL-c trapped and accumulate as foam After, it enters to the intima of the whole blood, it gets modified to play a role in the production of pro-inflammatory cytokines (Jaakkola et al., 1993 and Tabash et al., 2019).

Endothelial injury or dysfunction is characterized by enhanced endothelial permeability and LDL-c deposition in the sub-endothelial space followed by leukocyte adhesion and transmigration across the endothelium (Alouffi et al., 2018). Uptake of oxidatively modified LDL-c into macrophages can be so extensive that cell morphology converts to that of a foam cell. Oxidized LDL-c directly delivers various lipid oxides and hydro peroxides to target cells. These compounds variably act as cytotoxins, monocyte, chemoattractant, and stimulators of cholesterol ester accumulation by macrophages. The accumulation of LDL-c in the vessel wall contributes to the formation of fatty strikes (Bharadwaj, 2005; Stocker and Keaney Jr, 2004).

HDL-c is a type of lipoprotein that mainly contain Apo lipoprotein A-I (ApoA-I) as a major structural and functional protein component. The atheroprotective activity of HDL-c is often explained by the unique ability of these lipoproteins to remove cholesterol from peripheral tissues including the arterial wall and transport it to the liver (Calabresi et al., 2003). HDL–c associated enzyme protects LDL-c from oxidizing because oxidized LDL-c facilitate for the formation of platelet activation factor and production of free radicals that are potential to produce pro-inflammatory cytokine. HDL-c also inhibit platelet-activating factor (PAF) synthesis in the endothelial cell by inhibiting PAF acetyltransferase (van Jaarsveld, 2002 and Uche et al., 2013). Low HDL-c in a patient with metabolic syndrome cause increase plasma

TG, which is a potential cause of oxidized LDL-c that is a risk for cardiovascular disease (Calabresi et al., 2003).

2.4. Composition of lipoprotein

Classes of lipoproteins based on their density are shown in Figure 2 below.

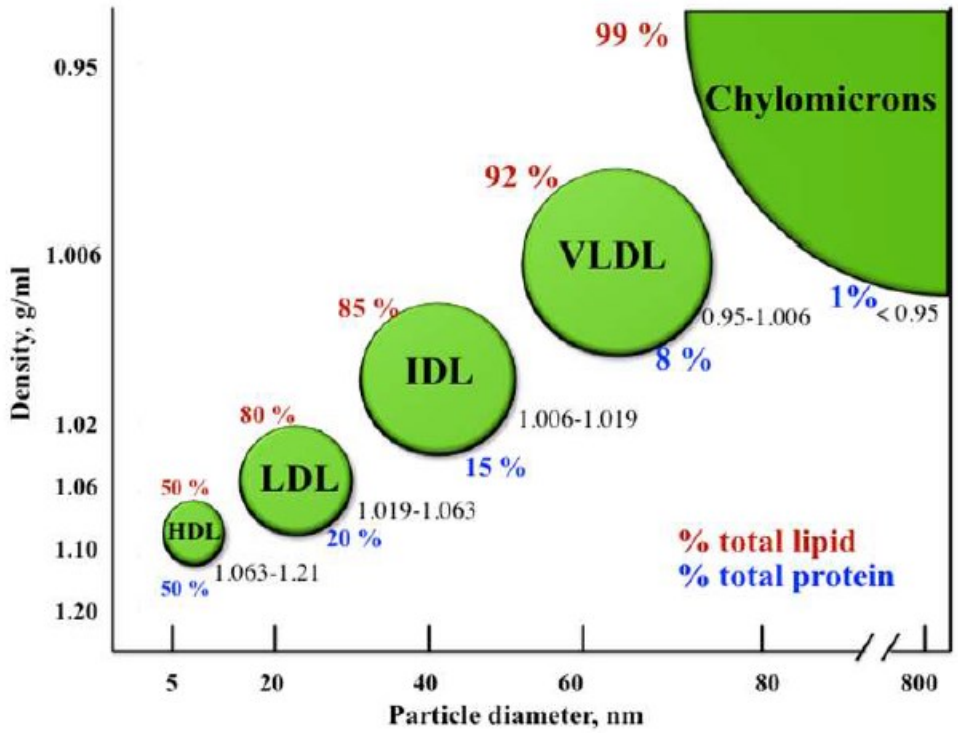

Figure 2. Compositions of lipoprotein classes. The classification of the major types of lipoproteins is based on their densities obtained by flotation ultracentrifugation analysis. The density range for each class is shown and also the contents of lipid (red) and protein (blue) (Jairam et al., 2012).

2.5. Lipid profile in blood donors

An elevated level of oxidized LDL-c is found in ischemic heart disease patient; however, it is not found in-patient with dilated cardiomyopathy. This suggests that LDL-c is responsible for

spasm of the blood vessel, which is easily blocked by plaque. Blood donation has various beneficial effects such as increasing HDL-c and Apo A; a higher oxidative potential of LDL-c; a lower level of LDL-c peroxidation resulting in an LDL-c particle with higher oxidative potential (Jaakkola et al., 1993; Ivanova et al., 2017 and van Jaarsveld, 2002).

A case-control study conducted in Palestine to assess the lipid profile and haematological parameters among 40 regular blood donors, 40 first time donors, and 40 non-donors reported that the mean total cholesterol, TG, LDL-c, LDL-c/HDL-c ratio and TC/HDL-c ratio was found lower in the regular blood donors than the first time donors and non-donors. In addition, HDL-c was increased in regular blood donors than first-time donors and non-donors. The above-mentioned outcome asserts a protective effect against atherosclerosis (Tabash et al., 2019).

Another study done on the evaluation of the lipid profile of regular blood donors in Cameroon reported that the mean total cholesterol (173.54 ± 46.28 mg/dl), low-density lipoprotein (96.68 ± 39.37mg/dl), and triglycerides (79.28 ± 42.95mg/dl) were comparatively lower in the regular blood donors than the irregular donors (174.61 ± 50.57, 98.16 ± 42.71, 79.82 ± 50.07), respectively, even though it was not statistically significant ($P > 0.05$). The mean LDL-c/HDL-c ratio was also lower in the regular donors than in irregular donors. Blood donors as a whole may be said to have a reduced risk of developing coronary heart disease as reflected by the low prevalence of risky LDL-c/HDL-c ratio and this benefit may be enhanced with regular blood donation as reflected by the lower TC, LDL-c, and TG levels in regular blood donors (Clement et al., 2017).

A cross-sectional study was also done on the level of hyperlipidemia among blood donors. A significant correlation was found among hyperlipidemic level in a single time donors and regular donors. Markers of increase cardiovascular risk appear to be lower in regular blood

donors compared with single time donors as reflected by significantly lower TC and LDL-c (Fatima et al., 2019).

The study conducted in Argentina reported that HDL-c of regular blood donors (at least two times in twelve months) have higher HDL-c and lower systolic blood pressure than irregular or non-donors, which indicated that blood donation has potential to decrease cardiovascular risk factor and other chronic inflammation (Kessler et al., 2013).

From 20 hyper-cholesterolemia individual selected in India to look whether regular blood donation treats hypercholesterolemia or not, there was a significant decrease in LDL-c and TC, but unchanged HDL-c among 17 individuals (Kumar, 1994).

A study done among 82 individuals, (52 were regular and 30 non-donors). The risk for cardiovascular complication appeared lower in regular blood donors compared with non-donors as reflected by significantly lower TC and LDL-c levels in regular donors compared to non-donors. Although HDL-c appears to be significantly higher in the non-donors population, the LDL-c/HDL-c ratio – which is a better predictor of increased cardiovascular risk – is lower in regular blood donors (Uche et al., 2013).

2.6. Hematologic parameters of blood donors

Haematological parameters that include RBC and WBC counts, haemoglobin and haematocrit determinations, platelet count, and RBC indices are the backbones of any laboratory evaluation. While WBC response for inflammation, whereas RBCs and platelets have a role in transporting oxygen between lungs and tissues and clotting, respectively. The abnormal value may be associated with various pathological conditions (Shapiro and Greenfield, 1987 and Mohammed, 2016).

A cross-sectional study done on some hematologic parameters of blood donors at Nigeria National Blood Bank reported that among 102 participants, more than 95% of their hematologic level fell in the normal range (Lugos et al., 2019).

In a comparative study done on various hematologic parameters among 393 voluntary blood donors (111 firs-time and 282 regulars) in India. Compared to the first time donors, the level of Hb, MCV, MCH, RBC and serum ferritin were significantly decreased in regular blood donors (Tailor et al., 2017).

Another study conducted on CBC in Sudan Hospitals Blood Bank showed that 70% with normal range, 26% increased, and 4% decreased in RBCs count. On WBCs count, 82% and 18% were in normal range and decreased, respectively. In addition, when 90% of the PLTs count is in the normal range, 8% and 2% were increased and decreased in PLTs count, respectively (Elnour et al., 2016).

Case-control study title in Effect of Repeated Whole Blood Donations on Hemoglobin in Belgium reported that the value of Hematocrit in repeated blood donors was decreased 11% than non-donors groups (Meurrens et al., 2016).

Another study showed that as the frequency of blood donation increases, hematocrit value of first (41.9±0.66%), second (40.1±0.47%), third (39.1±0.54%) and fourth (33.3±0.56%) time decreased compared with control (43.9±0.55%) (Okpokam et al., 2016).

A red cell distribution width (RDW) is an independent predictor of cardiovascular mortality in the general population and those with various high risks (Nada, 2015). Increased RDW causes impairment on RBCs deformability and negatively affects blood flow through microcirculation because of the complete standstill movement of RBCs (Wang et al., 2013).

3. OBJECTIVES

3.1. General objective

- To compare lipid profile and haematologic parameters among regular and first time blood donors at National Blood Bank Service of Ethiopia, 2020.

3.2. Specific objectives

- To compare anthropometric parameters of regular blood donors with first-time donors.
- To compare the lipid profile (TC, LDL-c, HDL-c, and TG) of regular blood donors and first-time donors.
- To compare haematological parameter (CBC) of regular blood donors and first-time donors.
- To assess the effects of regular blood donation on lipid profile and hematologic parameters.
- To insight the health benefits of blood donation

4. METHODS AND MATERIALS

4.2. Study area

This study was conducted in the Department of Medical Biochemistry, School of Medicine, CHS, AAU, in collaboration with the National Blood Bank Services of Ethiopia, both located in Addis Ababa, Ethiopia. National Blood Bank Services of Ethiopia was established in 1969 by Ethiopian Red Cross society but has been under the Federal Ministry of Health, since 2004. The head quarter of National Blood Bank Service is located in Addis Ababa and has the responsibility to provide blood to regional blood banks, supervise and monitor their activities. The regional blood banks provide blood and supervise about 8-12 hospitals in 100 Km radius.

4.3. Study design and period

The comparative cross-sectional study design was conducted to compare the lipid profile and haematological parameters of study participants at the National Blood Bank Service of Ethiopia, from January to December 2020.

4.4. Source and study population

4.4.1. Source population

The source population for this study was all blood donors who have voluntarily donated the blood at National Blood Bank Service of Ethiopia.

4.4.2. Study population

All RBD and FTD who attending National Blood Bank Service of Ethiopia during the study period and who were included in the study.

4.5. Eligibility criteria

4.5.1. Inclusion criteria

- Blood donors between aged 18–65 years.
- Regular and First time blood donors.

4.5.2. Exclusion criteria

- Smoking and chronic alcohol consumption.
- Hypertension and diabetes mellitus.
- Pregnant women.
- Donors weight less than 45 Kg.
- Patient on lipid-lowering medication.
- Anaemic patients.

4.6. Sample size determination

The sample size was determined based on the specific mean ± standard deviation for each sub-groups since there were no reported studies in Ethiopia. The mean ± SD of HDL-c for regular blood donors was 0.86 ± 0.24 (mmol/L) and for non-donors was 1.0 ± 0.26 (mmol/L), which was taken from the study done in Nigeria entitle "lipid profile among blood donors"(Uche et al., 2013). The sample size is determined by using the G power software Statistical Power Analyses for Windows (3.1.2.9 version) with the following assumptions: Significance level = 95%, power = 80%, type of test = two independent mean T-test, Z α/2=the critical value at 95% (α= 0.05) and 1:1 ratio. Computed sample size based on the above assumption was 104, which comprised of 52 samples from each category.

4.7. Sampling technique

Convenient sampling methods were used, for both regular and the first- time donors groups until the required sample size was attained.

4.7. 1. Methods of sample and data collection

A structured questionnaire translated to the local language, Amharic, was used for the sociodemographic data collection. Anthropometric measurements including weight, height, waist-circumference and hip-circumference were measured with the subjects wearing light clothing. Body mass index (Kg/m^2) was calculated by dividing the weight in Kg to height in m^2 and waist-to-hip ratio (WHR) were calculated.

4.7. 2. Blood collection and processing

After we obtained informed consent from each participants, the questionnaire was filled by face-to-face interview and some anthropometric indicators were assessed and measured side by side. In addition, about 6mL blood sample was drawn by qualified health care professionals and divided in to two (3ml for lipid profile and 3ml for CBC). Three-milliliter blood was added in SST and allowed to stand for 30 minutes at room temperature for complete clotting and clot retraction. Then, Serum was separated by centrifugation at 3500 rpm for 15 minutes by Megafuge r 1.0 Heraeus centrifuge (Germany, 2017). The serum was used to determine the levels of TC, HDL-c and TG by a Cobas 6000 Chemistry Analyzer (Germany, 2020). LDL-c cholesterol was calculated using the Fried-Wald formula (LDL-c=TC-(TG/5+HDL)) (Friedewald et al., 1972). Other 3 mL for CBC was added in to EDTA tube to determine hematologic parameters by Beckman hematology Analyzer (Germany, 2018).

4.8. Test principles of the laboratory analyses

4.8.2. Estimation of serum lipid profiles

Determination of total cholesterol

Principles of the Method: Total cholesterol was measured enzymatically in serum in a series of a couple of reactions that hydrolyzed cholesteryl esters and oxidized the 3-OH group of cholesterol. Cholesterol ester was hydrolyzed to free cholesterol-by-cholesterol ester hydrolase. The free cholesterol produce was oxidized by cholesterol Oxidase to cholest-4-en-3-one with the simultaneous production of hydrogen peroxide (H2O2), which oxidatively couples with 4-aminoantipyrine and phenol in the presence of peroxidase to yield Quinoneimine dye with maximum absorption between 500-550 nm (Röschlau et al., 1975).

The reaction sequence is as follows

$$\text{Cholesteryl ester} + H_2O \xrightarrow{\text{Cholesteryl ester hydrolase}} \text{cholesterol} + \text{fatty acid}$$

$$\text{Cholesterol} + O_2 \xrightarrow{\text{cholesteryl Ester Hydrolase}} \text{Cholest -4-en-3-one} + H_2O_2$$

$$2H_2O_2 + \text{4-aminophenazone} + \text{phenol} \xrightarrow{\text{peroxide}} \text{quinoneimine} + 4H_2O$$

The test comes in the form of a commercial kit in which the serum sample was incubated with enzymes and reagents from the kit and the change in absorption at 500 nm was measured spectrophotometrically. This change in absorption is proportional to the concentration of total cholesterol in serum sample and can be calculated by comparison with absorption changes that occur with standard solutions containing known cholesterol concentrations (Röschlau et al., 1975).

Procedure: Ten microliters (10 µL) serum was added into the sample cups and put on the sample disk, which rotated to bring the desire sample cup into position next to the sample probe for specimen sampling. One thousand microliters (1000 µL) reaction reagent (4-Aminophenazone, phenol, peroxidase, cholesterol esterase, cholesterol oxidize) was pipetted into reagent bottles level for TC and put on reagent disk and then on the screen menu of the machine, TC will enter as a parameter to be tested. The sample probe is pipetted sample from the sample disk and transfer to the reaction disk, which contains cuvettes. On the other side of the machine, the reagent probe was pipette reagents from the reagent disk and transfer it into reaction disk which is a large rotatable disk holding reusable cuvettes with a stirring paddle to stir or mix thoroughly the sample and the reagents. The cuvette was immersed into a reaction water bath and incubate at 37^0C for 5 minutes. Next, the reaction disk was rotated the cells to all reaction stations including the photometer light path. Finally, the light was passed through the cuvettes and absorbance of the sample was measured at 500nm (Röschlau et al., 1975).

Determination of triglyceride

Principles of the Method: The method was based on the enzymatic hydrolysis of triglycerides to glycerol and frees fatty acids by lipoprotein lipase (LPL). Glycerol was converted to glycerol-3-phosphate and adenosine-5-phosphate (ADP) by glycerol kinase and ATP. Glycerol-3 phosphate was oxidized by glycerol phosphate Oxidase to form dihydroxyacetone phosphate and H2O2. In the presence of peroxidase and H2O2, 4-aminoantipyrine couples with phenol to form a colored product (quinoneimine) that can be measured spectrophotometric ally at a wavelength of 500nm.

The reaction sequence is as follows:

$$\text{Triglycerides} + 3H_2O \xrightarrow{\text{Lipase}} \text{glycerol} + \text{fatty acid}$$

$$\text{Glycerol} + \text{ATP} \xrightarrow{\text{glycerol kinase}} \text{glycerol-3-phosphate ADP}$$

$$\text{Glycerol-3-phosphate} + O_2 \xrightarrow{\text{glycerol phosphate oxidase}} \text{dihydroxyacetone} + \text{phosphate} + H_2O_2$$

$$H_2O_2 + \text{4-aminoantipyrine} \xrightarrow{\text{peroxidase}} \text{quinoneimine} + 2H_2O$$

The triglyceride test comes in the form of a commercial kit containing the reagents, reactants and enzymes needed. Serum samples were incubated with the kit reagents and enzymes for 5 minutes at 37 ^{0}C and absorbance was measured at 500 nm against the reagent blank and known concentrations of standard triglyceride concentrations. The change in absorbance is proportional to the concentration of triglyceride in the serum sample.

Procedure: Ten microliters (10 µL) serum was added into the sample cups and put on the sample disk, which rotates to bring the desire sample cup into position next to the sample probe for specimen sampling. One thousand microliters (1000 µL) buffer and 1000µL substrate were pipetted into reagent bottles level for TG and put on the reagent disk. Then, on the screen menu of the machine, TG will enter as a parameter tope test. The sample probe pipette sample from the sample disk and transfer to the reaction disk, which contains cuvettes. On the other side of the machine, the reagent probe pipette reagents from the reagent disk and transfer it into rotatable reaction disk holding reusable cuvettes with a stirring paddle to stir or mix thoroughly the sample and the reagents. The cuvette was immersed into a reaction water bath and incubates at 37^{0}C for 5 minutes. Next, the reaction disk was rotated the cells to all reaction stations

including the photometer light path. Finally, the light was passed through the cuvettes and absorbance of the sample measure at 500 nm (Röschlau et al., 1975).

Determination of high-density lipoprotein cholesterol

Principles of the method: The basic principle of the method was as follows. The Apo-B containing lipoproteins in the specimen react with antibodies to Apo-B that renders them nonreactive with the enzymatic cholesterol reagent under conditions of the assay. The enzymes used were also pegylated, and this allows them to react only with HDL-c and not with antibody-bound LDL-c, VLDL-c chylomicrons. The Apo-Containing 39 lipoproteins were thus effectively excluded from the assay and only HDL-c was detected under the assay conditions (Jacobs *et al.*, 1990). The HDL-c test was a two reagent homogenous system for the selective measurement of serum or plasma HDL-c in the presence of other lipoprotein particles. The assay was comprised of two distinct phases. In phase one, it was likely that in the presence of slightly alkaline buffer and magnesium sulfate and dextran sulfate selectively form water-soluble complexes with LDL-c, LDL-c and chylomicrons, which were resistant to polyethylene glycol (PEG) modified enzymes. In phase two, the cholesterol concentration of HDL-c cholesterol was determined enzymatically by 22-cholesterol esterase and cholesterol oxidase coupled with PEG to the amino groups (approximately 40%).

$$\text{HDL-cholesterol esters} + H_2O \xrightarrow[\text{Esterase}]{\text{PEG}} \text{cholesterol} + \text{free fatty acids}$$

Cholesterol esters are broken down quantitatively into free cholesterol and fatty acids by cholesterol esterase.

$$\text{Cholesterol} + O_2 \xrightarrow[\text{Oxidase}]{\text{PEG cholesterol}} \text{cholestenone} + H_2O_2$$

In the presence of oxygen, cholesterol is oxidized by cholesterol oxidase to be converted into cholestenone and hydrogen peroxide.

$2H_2O_2$ + 4-amino – antipyrine + HSDA* + H^+ +H_2O $\xrightarrow{\text{Peroxidase}}$ purple-blue pigment + $5H_2O$

*Where: HSDA= N-(2-hydroxy-3-sulfopropyl)-3, 5-dimethoxyaniline.*In the presence of peroxidase, the hydrogen peroxide generated reacts with 4- aminoantipyrine and HSDA to form a purple-blue dye. The color intensity of this dye is proportional to the cholesterol concentration and can be measured by spectrophotometric ally (Jacobs Jr et al., 1990).

Procedure: Ten microliters (10 µL) serum samples were added into the sample cups and put on the sample disk, which rotates to bring the desire sample cup into position next to the sample probe for specimen sampling. One thousand microliters (1000 µL) each of buffer and substrate were pipetted into reagent bottles levelled for HDL-c and put on the reagent disk. Then on the screen menu of the machine. HDL-c was entered as a parameter to be tested. The sample probe pipetted sample from the sample disk and transferred to the reaction disk, which contains cuvettes. On the other side of the machine, the reagent probe pipetted reagents from the reagent disk and transferred it into rotatable reaction disk holding reusable cuvettes with a stirring paddle to stir or mix thoroughly 23 the sample and the reagents. The cuvettes were immersed in the reaction water bath and incubated at 37^0C for 5 minutes. Next, the reaction disk was rotated the cells to all reaction stations including the photometer light path. Finally, the absorbance of the sample was measured at 500 nm (Jacobs Jr et al., 1990).

Determination of low-density lipoprotein cholesterol

Most of the circulating cholesterol was found in three major lipoprotein fractions: VLDL, LDL, and HDL. LDL-c was calculated from measured values of total cholesterol, triglycerides and HDL-c according to the Fried Ewald equation: LDL-c = TC - [HDL-c + TG/5)]. Where [TG]/5 was an estimate of VLDL-c. All values are expressed in mg/dL. The equation is derived from

another equation: [Total Cholesterol] = [VLDL-c] + [LDL-c] + [HDL-c], but TG is easier to estimate than VLDL-c and [TG/5] is a good estimate of VLD-cL(Jacobs Jr et al., 1990, Röschlau et al., 1975).

4.8.1. Haematologic parameters

Determination of haematological parameters (CBC)

Principally Beckman analyzer is based on the electronic resistance (impedance) detection method for counting and sizing recognition of the leukocytes, erythrocyte, and platelet using three hydraulic systems for WBC, RBC, platelets, and haemoglobin and displays the results on the liquid crystal displayer (LCD) and printed out the results in thermal paper. The analyses were performed using automated haematology analyzers Bekman (Germany, 2018) using EDTA anticoagulated fresh venous blood sample. Red blood cells (RBCs), haematocrit (HCT), haemoglobin (HGB), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), absolute neutrophils count (ANC), and absolute lymphocytes count (ALC) were determined in an automated haematological counter and the reference value of haematological parameters (Bekman, 2018).

Principle and procedure of Sysmex KX-21N

Measurement of blood cells (RBCs, WBCs, & platelets) and haematological concentration were measured obtained by aspiration of a small volume of well-mixed EDTA blood by sample probe and mixed with isotonic diluents in a nebulizer. Diluted mixture aspiration was delivered to RBCs aperture both for providing information about RBCs and platelet based on the cell size. Particles of 2 to 20 fL counted as platelet and above 36 fL were counted as a reamed cell. Some portion of aspiration mixture induced into WBCs both in which hemolytic reagent (Stromatolyzer) was added automatically to measure haemoglobin concentration in build calorimeter, based on cyanomethemogolobin method. Blood cell was counted, size information

was also generated in triplicate pulses according to electronic conductivity, and translated into a digital number using in-build calculator programmed and designed for that RBCs, WBCs count. Three parameters can be directly measured and displayed on (LCD). Other values of red cell indices, platelet, and leukocyte differential and absolute count were calculated from given information and automated constructed histograms. The results were printed out according to the setting mode.

Table 1: Normal range of lipid profile.

S.No.	Lipid profile	Normal range (mg/dL)
1	TC	150-200
2	TG	100-150
3	LDL-c	< 130
4	HDL-c	> 35
5	LDL-c/HDL-c	< 4
6	TC/HDL-c	< 5

* It is based on a report by Rusdian, 2009.

4.8.3. Anthropometrical measurement procedure

The weight and height of the study participants was measured using a standard balance with height-measuring device attached to it. Body mass index (BMI) was calculated from the body weight (kg) and height (meter) as follows: BMI=Weight (in kg) / (Height in m^2) (Tambe et al., 2010). Waist and hip circumference of the participant was also measured. Waist circumference was measured over light clothing at the level halfway between the iliac crest and the costal margin in the mid-axillary line after exhaling, with the subject in standing position with the body weight evenly distributed across the feet. Hip circumference was measured over light

clothing at the level of greater trochanters with the subject in standing position and both feet together. Waist to hip ratio was calculated by dividing waist circumference by hip circumference (Tambe et al., 2010).

Table 2: Description of anthropometric parameters.

S. No	Profile (Kg/m^2)	Range	Description
1	BMI	< 18..5	Underweight
		18.5 – 24.5	Normal
		24.5 – 29.5	Overweight
		29.5– 34.5	Obese
		> 34.5	Morbidity obese
2	WHR	< 0.9 for male	Normal
		< 0.8 for female	
		0.9-0.99 for male	Moderate risk
		0.8-0.89 for female	
		> 1 for male	High risk
		> 0.9 for female	

* It is based on a report by Tambe, 2010.

4. 9. Variables

4.9.1. Dependent variables

- Serum lipid profile (TC, LDL-c, HDL-c and TG)
- Haematological parameters (CBC)

4.9. 2. Independent variable

- Socio-demographic factors
- Anthropometric indicators

4.10. Operational definition

Lipid Profile: is a panel of blood tests that serves as an initial broad medical screening tool for abnormalities in lipids, such as cholesterol, HDL-c, LDL-c, and triglycerides.

Haematologic parameters: is complete blood count widely used clinical indicators of disease include WBC, RBC, Platelet, haemoglobin, WBC differential and RBC indices.

Regular blood donors: individuals who had donated blood every three months, at least for the last two years.

First-time blood donors: an individual who has donated blood for the first time with no history of blood donation previously.

4.11. Data quality control and management

Data collection tools were prepared to meet the highest quality and monitored at the time of sampling. Professional laboratory technologists handled all the experimental procedures and all instruments were operated according to the manufacturer's instructions. High-quality standards used as a control and calibration used to calibrate the instruments and no analysis were done if the control is out of normal range.

4.12. Data processing and analysis

Data obtained from questionnaire and laboratory analysis were checked for completeness and refined. It was coded and entered to Epi-Data statistical software version 3.1 and exported to SPSS software version 21 package and different variables were tested and analyzed. Simple descriptive statistics were used to present the socio-demographic characteristics of the study subjects. While the Chi-square ($\chi 2$) test was used to compare categorical variables, the continuous variable was present as mean ± SD and compared using the Student t-tests for

groups. A p-value of less than 0.05 at 95% confidence level was considered to be statistically significant.

4.13. Ethical considerations

Before the study, ethical clearance letter (with a reference number SOM/DRERC/BCHM/088/2012) was obtained from the Research and Ethics Review Committee of the Department of Medical Biochemistry, School of Medicine, College of Health Sciences, Addis Ababa University. Collaboration letter for data collection was also obtained from the National Blood Bank Service. The research proposal was presented and thoroughly discussed at National Blood Bank Service of Ethiopia as well. The objective of the study was briefly clarified and explained for each participant before enrolling any of the eligible study participants. Samples and data were collected after informed consent had been obtained from the study participants. Confidentiality, anonymity, neutrality, accountability and academic honesty were maintained throughout the study.

4.14. Dissemination of results

The finding of this study will be submitted and presented in open defense to the Department of Medical Biochemistry, School of Medicine, College of Health Sciences, Addis Ababa University. The findings will also be disseminated to National Blood Bank Service of Ethiopia.

The effort will also be made to publish in peer-reviewed journal and present in different national and international conferences and seminars.

5. RESULTS

5.1. Socio-demographic characteristics of the blood donors

Table 3 illustrates the socio-demographic characteristics of the study populations. The study included 104 study participants: 52 of each were regular and first-time blood donors. Among the regular blood donors, 84.6% and 15.4% were donated four and three times in a year, respectively.

Among regular blood donors, 65.3 % were in 18-38 while 34.6% were in 39-58 years: from first-time donors, 73.1% and 26.9% were 18-38 and 39-58 years, respectively ($P > 0.05$). Eighty-four percent (84.6%) of regular blood donors and 71.15% of first-time blood donors were found males ($P > 0.05$).

Among regular blood donors, 57.69% were Orthodox, 30.77% Protestant, and 11.5% Muslim. Likewise, from first-time blood donors, 51.9% were Orthodox, 25% Muslim and 19.23% protestant ($P > 0.05$).

More than 98.5% and 50% were urban dwellers and married, respectively, of regular blood donors. Among regular blood donors, 1.9% has no formal education and 44.2% graduated from college or university level. From first-time blood donors, 3.8% have no formal education and 51.9% graduated from college or university level ($p>0.05$). Our sociodemographic data reflected an ideal matching between the study participants.

Table 3. Socio-demographic characteristics of the study participants at National Blood Bank Service, Ethiopia, from June to July 2020 (n=104).

Variables	Category	RBD (%)	FTB (%)	P-value
Gender	Male	84.6	71.2	0.098
	Female	15.4	28.8	
Age group	18-38	65.3	73.1	0.095
	39-58	34.6	26.9	
Marital status	Married	50	55.8	0.84
	Unmarried	48.1	42.3	
	Divorced	1.9	1.9	
Religion	Orthodox	57.7	51.9	0.11
	Muslim	11.5	25	
	Protestant	30.8	19.2	
	Others	0	3.8	
Residence	Urban	100	96.2	0.15
	Rural	0	3.8	
Level of education	Illiterate	1.9	3.8	0.75
	Primary school	7.7	1.9	
	Secondary school	25	25	
	Undergraduate	21.2	17.3	
	Graduate	44.2	51.9	

5.2. Anthropometric parameters of study participants

This study showed that the mean ± SD of BMI of regular blood donors were 23.33 ± 2.77 kg/m^2 while that of the first time blood donors were 23.86 ± 2.84 kg/m2. Regarding the waist-to-hip ratio, the mean ± SD found 0.85 ± 0.03 and 0.86 ± 0.04 for regular blood and first-time blood donors, respectively ($P > 0.05$). The result indicated that the physical status of donors has no effects on the level of lipid profile and haematological parameters

Table 4. Anthropometric parameters of study participant at National Blood Bank Service, Ethiopia, from June to July 2020 (n=104).

S.No	Variable	Mean ± SD		P-value
		RBD	FBD	
1	Height (m)	1.73 ± 0.07	1.70 ± 0.08	0.11
2	Weight (kg)	70.4 ± 10.1	69.5 ± 11.2	0.68
3	BMI (kg/m^2)	23.3 ± 2.78	23.9 ± 2.84	0.34
4	WC (cm)	84.4 ± 4.82	83.9 ± 5.74	0.67
5	HC (cm)	98 ± 4.0	97.8 ± 3.9	0.85
6	WHR	0.86 ± 0.03	0.87 ± 0.04	0.74

5.3. Lipid profile of the study participants

The lipid profile data showed that the regular blood donors had lower mean TC (144.3±28 mg/dL), TG (159.3±88.2 mg/dL), LDL-c (75.9±25.9 mg/dL) and higher HDL-c (39.8±8.8 mg/dL) than the first time blood donors with values of 158.1±38.94 mg/dL, 163.9±82.7mg/dL, 93.1±31.5mg/dL and 36.8±7.7 mg/dL, respectively (Table 5). LDL-c and TC in regular blood donors were significantly lower than in the first time blood donors. Succinctly, the ratio of LDL-c/HDL-c and TC/HDL-c found lower in regular blood donors when compared to the first-time donors ($P < 0.05$). Except for a few participants, the mean value of lipid profile among the groups falls within the normal biological range indicating there was no prior history of abnormal lipid profiles that affect the outcome of the present result.

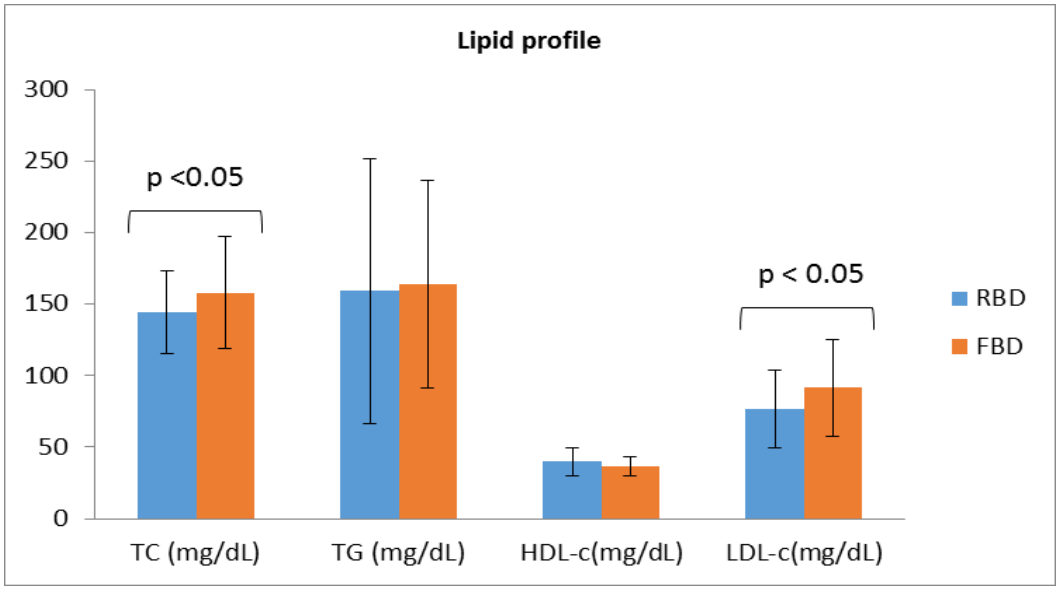

Figure 3: Levels of lipid profiles in regular and first-time blood donors at National Blood Bank Service, Ethiopia, from June to July 2020(n=104).

5.4. Hematologic parameters

Table 6 below illustrates the haematologic parameters of the 104 study participants classified as regular and first-time donors. The result showed that values of WBC, Neu%, and Lymph% (all in mean ± SD) were found in regular blood donors 5.59±1.5 *10^3/µL; 58.08 ± 10.5; 29.9 ± 9.1 compared to the first time donors 5.85 ±1.7 *10^3/µL; 52.2 ± 9.7; 35.7 ± 8.6. The neutrophils of regular blood donors were significantly higher than the first-time blood donors. However, the lymphocyte of regular blood donors was significantly lower than first-time blood donors. Mean corpuscular haemoglobin (MCH), Mean corpuscular haemoglobin concentration (MCHC) and RDW-SD found significantly lower in regular blood donors compared to first-time donors. However, the majority of RBC indices were found similar.

Concerning platelet indices, the values (mean ± SD) of regular blood donor versus first-time blood donors are as follows: PLT (208.3 ± 42.8 and 229.5 ± 59.5*10^3/µL) and mean platelet volume (MPV) (9.25 ± 1.08 and 9.28 ± 1.09 fL), respectively (Table 6). The platelets of regular

blood donors were significantly lower when compared to first-time blood donors. Ratio of Neutrophil to lymphocyte (NLR) and platelet to lymphocyte ratio (PLR) found lower in first time compared to regular blood donors.

Generally, the mean values of all hematologic parameters were in the normal range in both regular and first-time blood donors.

Table 5: Levels of haematological parameters in regular and first-time blood donors at National Blood Bank Service, Ethiopia, from June to July 2020 (n=104).

S.No	Variable	Mean ± SD		P-value
		Regular	First-time	
1	WBC $*10^3/\mu L$	5.6 ± 1.5	5.8 ± 1.7	0.43
2	Neu (%)	58.2 ± 10.5	52.2 ± 9.7	0.004*
3	Lymph (%)	29.9 ± 9.1	35.7 ± 8.6	0.001*
4	Plt $*10^3/\mu L$	208.3 ± 42.8	229.5 ± 59.5	0.04*
5	Mpv (FL)	9.2 ± 1.1	9.3 ± 1.1	0.89
6	RBC ($10^3/\mu L$)	4.76 ± 0.6	4.71 ± 0.5	0.65
7	HCT (%)	42 ± 4.3	42.3 ± 4.3	0.4
8	HGB (g/dL)	14.7 ± 1.7	15 ± 1.7	0.75
9	MCH (pg)	30.8 ± 2.1	31.7 ± 1.7	0.016*
10	MCHC (g/dL)	35 ± 1.0	35.5 ± 1.1	0.017*
11	RDW (%)	13.7 ± 1.1	14.2 ± 2.2	0.133
12	RDW-SD	42.2 ± 2.8	43.8 ± 3.7	0.01*
13	Neu/lymph	2.4 ± 1.0	1.7 ± 0.8	0.003*
14	Platelet/lymph	115.5 ± 24.3	113.3 ± 31.6	0.48

The * indicates $p < 0.05$.

6. DISCUSSION

This study assessed the lipid and haematologic profiles of the regular blood donors (those who donate every three months in a year) and first-time donors (no recorded history of blood donation) voluntarily come to the National Blood Bank Services of Ethiopia. The present study showed that the socio-demographic status of the blood donors, viz., age, religion, marital status and education level were well matched. This indicated that sociodemographic characteristics did not affect the results as a confiding, which is in congruence with previous reports (Uche et al., 2013 and Tabash et al., 2019). However, the number of male donors were found higher in regular blood donors (RBD) and first-time blood of donors (FTD), compared to females, albeit statistically insignificant. Rizvi's report showed a similar trend (Rizvi et al., 2020). This might be due to the very low-haemoglobin levels or/and the susceptibility of females to various anemia.

In addition, BMI and WHR were found similar between the regular and first-time blood donors. While our report's on BMI value is in agreement with two researchers in Iran and Palestine (Tabash et al., 2019), other groups claimed that BMI was lower (Getta et al., 2018) and higher (Adias et al., 2012) in regular blood donors when compared to the first-time donors. The later is due to the high interest of obese people who understood the health benefits of blood donation (Kamhieh-Milz et al., 2016).

Two decades back regular blood donors have been identified as a reduced risk of cardiovascular disease when compared with non-donors (Salonen et al., 1998). The present study compared the lipid profile of regular blood donors with the first time donors.

Our study vividly proved that TC, LDL-c, and the ratios of TC/HDL-c and LDL-c/HDL-c found significantly lower in regular blood donors (RBD) than the first-time donors (FTD). TG

and HDL-c were lower and higher, respectively, in RBDs compared to FTDs, although statistically insignificant.

Most of the previous studies here under were found in agreement with our outcomes: lower TC, LDL-c, LDL-c/HDL-c and TC/HDL-c (Tabash et al., 2019 and Uche et al., 2013); lower LDL-c (Rusdiah et al., 2018); lower TG and LDL-c (Kumar, 1994); lower TC and LDL-c (Fatima et al., 2019); healthier HDL-c and TC/HDL-c (Bharadwaj, 2005); higher HDL-c (van Jaarsveld, 2002; Salonen et al., 1998 and Kessler et al., 2013) levels in RBDs compared to FBDs. However, others reported there was no change in the values of TC, TG and LDL-c (van Jaarsveld, 2002) of both regular donors and higher HDL-c in non-regular donors (Rusdiah et al., 2018).

From our result, lower LDL-c and higher HDL-c have a health benefit for the regular blood donor. HDL-c is the type of lipoprotein, which mainly contains Apo lipoprotein A-I as a major protein component. The atheroprotective activity of HDL-c is often explained by the unique ability of these lipoproteins to remove cholesterol from peripheral tissues, including the arterial wall, and transport it to the liver (Calabresi et al., 2003). Therefore, the higher in the concentration of HDL-c in regular blood donors reduced the level of cholesterol by scavenging from peripheral tissues and transport to the liver for synthesis of other important biomolecules. In addition, the lower LDL-c in regular blood donors also advantageous over first-time donors. LDL-c is responsible for spasm of the blood vessels that easily blocked by plaques (Schmitz and Orso, 2015) and oxidized LDL-c delivers lipid oxides and hydroperoxides to target cells variable acts as cytotoxins, monocytes, chemoattractants and stimulators of cholesterol ester accumulation by macrophages, which leads to fatty strikes (Stocker and Keaney Jr, 2004). From our study, blood donation lowers LDL-c that increases its oxidative (not peroxidation) potential, in which the chance of formation of oxides and hydroperoxides, plaques, and fatty strikes (all the major contributors of atherosclerosis and other complications) are significantly

reduced. As a result, we can deduce that regular blood donors has benefited than the first time and non-donors.

Higher TC/HDL-c and LDL-c/HDL-c ratios are predictors of high cardiovascular risk and coronary artery disease (Panagiotakos and Toutouzas, 2003 and Awano, 2010). The present study indicated that these ratios were found significantly lower in regular blood donors compared to the first-time donors, which is in agreement with the previous study done in Cameroon (Clement et al., 2017). This confirmed that regular blood donors or blood donation, in general, have reduced the chance of developing cardiovascular risk and coronary heart disease (Kessler et al., 2013).

This study was also designed to compare the haematological parameters (RBC, WBC, PC, and packed cell volume) of regular blood donors with first-time blood donors. The analysis of our data showed that the levels of WBC, lymphocyte, platelet, MPV, HCT, HGB, MCH, RDW, and RDW-SD were lower in RBD compared to the FTD, albeit statistical significance was merely observed in lymphocyte, platelet, MCH, and RDW-SD ($P < 0.05$). However, the levels of RBC ($p > 0.05$) and Neutrophil ($p < 0.01$) were higher in RBD compared to FTD. None of the hematologic parameters of both groups deviated from the normal biological ranges, which is highly pronounced in regular blood donors. Elnour's group data is incongruous with ours (Elnour et al., 2016). This proved how safe is the procedure and adequate interval between consecutive blood donations. The later allows erythropoiesis to compensate for any minor decline in any of these haematological profiles.

The HCT, also known as packed cell volume or erythrocyte volume fraction, is the volume percentage of RBCs in blood and functions as a major determinant of blood viscosity, blood pressure, venous return, cardiac output, and platelet adhesiveness (Brooks et al., 2014). Although statistically insignificant, our result showed lower HCT level in regular blood donors

that is in agreement with previous reports (Tabash et al., 2019). As the frequency of donation increases (for example, first to fourth times), the haematocrit value significantly decreases (Okpokam et al., 2016).

In our study, the mean value of platelets counts was significantly lower in regular blood donors than first-time donors ($p< 0.05$). It coincided with the following researches (Okpokam et al., 2016 and Fayed et al., 2018).

RDW is a quantitative measure of the red blood cell volume (RBCV) heterogeneity. Increased RDW causes impairment on RBCs deformability and negatively affects blood flow through microcirculation because of the complete standstill movement of RBCs (Wang et al., 2013). Our analysis showed a significant decline ($p<0.05$) in the level of RDW in regular blood donors compared to the first time donors. This asserted regular blood donation may attribute to lower the risk of chronic inflammation.

NLR and PLR can be an important measure of systemic inflammation as it is cost-effective, readily available, and could be calculated easily (Turcato et al., 2019). The previous study showed that NLR of chronic disease like diabetes and hypertension were higher than a cut-off value (Imtiaz et al., 2012). In our study both NLR and PLR were lower in FTD compared to RBD. This indicated that NLR and PLR are not potential markers in regular blood donors.

7. CONCLUSION

Our study proved that the TC, TG, LDL-c and the ratios TC/HDL-c and LDL-c/HDL-c were found lower in regular blood donors. Interestingly, HDL-c was also found increase in regular blood donors compared to the first-time blood donors. Body-mass index (BMI) and waist-hip ratio (WHR) and some haematological profiles like, platelet, heamatocrit, white blood cell, lymphocyte and RDW were also found lower in regular blood donors. Combining all the above-mentioned parameters, the regular blood donors have gotten less chance toward developing the risk of cardiovascular complications and other chronic inflammation. As a result, we can deduce that blood donation has multiple health benefits to our best knowledge, this is the first-ever research in Ethiopia.

8. RECOMMENDATION

Based on the current research findings the following recommendations are forwarded.

- Regular blood donation reduces the level of lipid profile like LDL-c, TC, TG and the ratios of TC/HDL-c and LDL-c/HDL-c, which is essential to reduce the risk of developing chronic inflammation. Therefore, we recommend people to come forward to donate blood regularly.
- Regular blood donors should be monitored for their marked changes of lipid profile, haematological parameters and other risk factors and further studies should be conducted to understand the mechanisms how blood donation reduces lipid profile and hematologic parameters.
- Large observational studies are required to establish a possible role of regular blood donation in lipid and haematological alteration and its effect on the rest of biochemical values by using appropriate sample size.
- National Blood Bank Service of Ethiopia should do the routine investigation of lipid profile and CBC for voluntary blood donors.

9. STRENGTH AND LIMITATION

This study has the following major strengths:

- It tried to compare lipid profile and haematological parameters of regular and first-time blood donors groups by considering the socio-demographic, anthropometric parameters and frequency of blood donation that can affect the level lipid profile and hematologic parameters of the participants.
- Even though the data collection period coincided with the outbreak of the pandemic disease, we were dedicated to collect the sample and did the laboratory analysis by following the recommended preventive measures to protecting ourselves from covid-19.

Despite the strengths, the study had also the subsequent drawbacks.

- The sample size was small and a caution should be taken to generalize to the general population.
- No control and follow-up were made on other factors that may affect lipid profile and hematologic parameters.
- Moreover, this study did not include other biochemical molecules that are associated with chronic inflammation.

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11. ANNEXES

11.1. Annex 1. Information sheet (English Version)

Research Title**: Comparative study of lipid profiles and haematological parameters among regular and first-time blood donors at National Blood Bank Service of Ethiopia.**

Sponsoring organization: **Department of Medical Biochemistry, School of Graduate Studies, College of Health Sciences, Addis Ababa University**

Principal Investigator: **Abbul Hasano (BSc)**

Advisors**: Solomon Tebeje Gizaw (Ph.D.) and NatesanGnanasekran (Ph.D.)**

Introduction

Dear participants, you are kindly requested to take part voluntarily in this research project as a study participants. Please read the information provided in this sheet carefully and kindly respond freely and voluntarily to the data collectors.

The objective of the research

This information sheet is prepared by the investigator in consultation with the advisors at AAU and collaborators at National Blood Bank Services for a project titled "Comparative study of lipid profiles and haematological parameters among RBD and FTD at National Blood Bank Service of Ethiopia."

Procedure

If you are willing to take part in the study, the principal investigator will give you verbal and/or written information about the study and you will be given the consent form to sign. Also, the health professional will ask you some questions about your general health and perform a complete medical examination and assess whether you qualify to participate in the study. When confirmed, we will interview face-to-face and about 6 mL of blood samples will be collected for complete blood count (CBC) and lipid profile analysis.

Discomforts, risks, and benefits from participation

The degree of discomfort you may encounter while giving the blood is no more than when does in your routine examination. Licensed health care professionals will withdraw the blood. Please informed that you will not be provided with any direct incentives for your participation in the research.

Confidentiality

All pieces of information about the participants will be kept confidential. Logbooks and information sheets used in the laboratory will be coded. You have full right to withdraw from participating in this study at any time before and after consent even without explaining the reason.

Contact information: If you have, any questions contact the principal investigator through Phone: 0931971514; email: hasanoabbul@gmail.com.

11.2. Annex 2: Informed consent (English version)

Department of Medical Biochemistry, School of Graduate Studies, College of Health Sciences, Addis Ababa University.

Consent form for the participation of the study participants in the research project

Name of the study participant ……

Code number…..……

I have been informed about the research project that it aims to compare serum lipid profile and haematological parameters among regular and first-time blood donors. The objectives of the research project have clearly been explained to me and I have been told that the results obtained from me will help me as well as the community for better awareness about blood donation. I had been also informed about the confidentiality of this research project. Moreover, I have also been well informed of my right to keep hold of information, decline to cooperate and make myself withdraw from the study. Therefore, with a full understanding of the importance of the study, I agreed voluntarily to provide the requested samples and my benefit will be only from the free laboratory investigation results.

I_____ hereby give my consent for providing the requested information and blood sample.

Signature of participants ----------

Date_____

11.3. Annex 3: Questionnaire (English version)

Dear Respondents, you are kindly requested to give correct information accordingly. Thank you for your time and participation.

Questionnaire code number_____

No	Question	Categories
1	Sex	1. male 2. female
2	Age	----------
3	Marital status	1. married 2. unmarried 3. widow 4. divorced
4	Residence	1. rural 2. urban
5	Level of education	1. no formal education 2. primary 3. secondary 4. college or university
6	Religion	1. orthodox 2. Muslim 3. protestant 4. others
7	Weight	
8	Height	
9	Body mass index (kg/m2)	
10	Waist- circumference	
11	Hip–circumference	
12	Waist to hip ratio	
14	Have you ever donated blood before	1. yes 2. no
15	If your answer is yes how many times	----------
16	If question number "14" is yes, in what interval	1. Every three month 2. 2-3 per year 3. Specify, if any

11.4. Annex 4: Information sheet (Amharic version)

የተሳታፊዎች የፍቃደኝነት መተማመኛና መረጃ መስጫ ቅፅ፡፡

ጥናቱን ስፖንሰር ያደረገዉ ተቋም ፡- አዲስ አበባ ዩኒቨርሲቲ ጤና ሣይንስ ኮሌጅ ነው፡፡

መረጃ መስጫ ቅፅ

በአዲስ አበባ ዩኒቨርሲቲ ጤናሳይንስኮሌጅ የ ሕክምና ባዮኬሚስትሪ ት/ክፍል ሁለተኛዲግሪ ተማሪ የመመረቂያ ጥናት ጽሁፍ ላይ እንዲሳተፉ ተጋብዘዋል፡፡እባክዎ በዚህ ጥናት ለመሳተፍ ከመስማማትዎ በፉት ከዚህ ቀጥሎ የሚገኘውን በጥሞናያንብቡና ግልጽ ያልሆነ ነገር ካለ ይጠይቁ፡፡

የጥናቱ ርዕስ Comparative study of lipid profiles and hematological parameters in regular and first-time blood donors at National Blood Bank Service, Ethiopia ሲሆን አላማውም መደበኛ የደም ለጋሾች በደማቸው ውስጥ ያለውን የቅባት መጠንና የደምህዋሶች ለመጀመሪያ ጊዜ ደም ከሚለግሱ ጋር ለማነጻጸር ነው፡፡ የጥናቱ ውጤት ለደምለጋሾች ና ለሌሎች ማህበረሰብ የሚጠቅም ነው፡፡እናም እርስዎ በዚህጥናት ለማሳተፍ አስፈላጊ ሆነው ተመርጠዋል፡፡እርስዎ በዚህ ጥናት ላይ የሚያዳርጉት ተሳትፎ ሙሉ በሙሉ በበጎ ፍቃዳኝነት ላይ የተመሰረተ ነው፡ ፡በጥናቱ ለመሳተፍ ፈቃደኛ ከሆኑ

ለናሙና ይሆን ዘንድ 6ሚሊሊትር ያህል ደም በጤናባለሙያዎች የሚሰጡ ይሆናል፡፡በዚህ ጥናት ውስጥ ለመሳተፍ ከወሰኑ በኋላ ማቋረጥ ይችላሉ፡፡በጥናቱ ለመሳተፍ የሚስማሙ ከሆነ የስምምነት ቅጹ ላይ በጽሁፍ ወይም በጣት ፊርማዎን እንዲያስቀምጡ በትህትና እንጠይቀዎታለን፡፡ግልጽ ያለሆነና እንዲብራራለዎት የሚፈልጉት ነገር ካለ ሞባይል ቁ፡ **0931971514** አቡል ሃሳኖ ብለው በመደወል መጠየቅ ይችላሉ፡፡

11.5. Annex 5: Informed consent (Amharic version)

የተሳታፊዎች ስምምነት ማረጋገጫ ቅጽ

የሚስጥር ቁጥር----------

የተሳታፊ ስም----------

በመደበኛ እና ለመጀመሪያ ጊዜ ደም በሚለግሱ መካከል ያለዉን የቅባትና የደም-ህዋሶች መጠን ያለዉን ለማወዳደር በሚለዉ ጥናት ለመሳተፍ ፈቃደኛ ሆኜ አላማውና ጥቅሙ በሚገባ ተገልጾልኛል።ስለዚ መረጃና የስምምነት ቃሌን የምሰጠው በአጠቃላይ የ ጥናቱን አላማና ጥቅም በመረዳትና በፍጹም ፍቃዳኝነት ነው::በመጠይቁ ላይ የምሰጠው የእኔ መረጃ እንደማይባክንና በምስጢር እንደሚያዝም ተነግሮኛል::በተጨማሪም ጥናቱ ውስጥ ለላመሳተፍ ከፈለኩኝ መብቴ የተጠበቀ እንደሆነና በማንኛውም ጊዜ ከጥናቱ በራሴ ውሳኔ መውጣት ጭምር መብቴ መሆኑንና ከጥናቱ በመዉጣቴ ምንም አይነት ችግር እንደማይደርስብኝ በሚገባ ተገልጾልኛል::በተጨማሪም የምሰጠው የድምጽ ና ናሙና ለCholesterol.Triglycerides,HDL-C፣HDL-C፣ LDL-C እና complete blood count ምርመራዎች ብቻ እንደሚውል ተነግሮኝ ተስማምቻለሁ:: ማንኛውንም ያልገባኝን ነገር የመጠየቅ እድል ተሰጥቶኝ በሚገባኝ ቋንቋ መልስ አግኝቻለሁ::በተጨማሪም የሁሉም የሊብራቶሪ ምርመራ ውጤቶች በጊዜው ማወቅ ከፈለኩ ማግኘት እንደምችል ተነግሮኛል:: በአጠቃላይ እኔ ከላይ በመተማመኛ ቅፅ የተጠቀሱትን ሁሉ በሚገባ ና በተረጋጋ መንፈስ አንብቤአለሁ:: ስለዚህ በዚህ ጥናት ለመሳተፍ ፍቃደኛ መሆኔን በፊርማዬ አረጋግጣለሁ:: እኔ_____

ፊርማና ቀን _____ _____

11.6. Annex 6: Questionnaire (Amharic version)

ውድ ተሳታፉዎች ቀጥል ያለውንመ ጠይቅ ለመሙላት ስለተባበሩን እናመሰግናለን::የመጠይቁ መለያ

ቁጥር_____

1	ጾታ	1. ወንድ 2. ሴት
2	ዕድሜ	---------ዓመት
3	የጋብቻ ሁኔታ	1. ያገባ 2. የሞተባቸው 3. ያላገባ 4. የፈታ
4	መኖሪያ ቦታ	1. ገጠር 2. ከተማ
5	የትምህርት ደረጃ	1. ማንበብናመጻፍ 2. አንደኛደራጃ 3. ሁለታኛደራጃ 4. ኮሌጅ
7	ሃይማኖት	1. ኦርቶዶክስ 2. ሙስሊም 3. ፖሮቴስታንት 4. ሌላ
8	ከዚ በፊት ደም ለግሰው ያውቃሉ	1. አዎን 2. አይ
9	መልሶት አዎን ከሆነ ምን ያህል ጊዜ	-----
10		
11	ክብደት(በኪ.ግ.)	
12	ቁመት(በሜትር)	
13	የሰውነት ክብደት ልኬት(ኪ.ግ. / ሜ2)	
14	የወገብ ዙሪያ(በሴ.ሜ.)	
15	የዳሌ ዙሪያ(በሴ.ሜ.)	
16	ወገብ-ለ-ዳሌንጽጽር	