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Hematological and lipid profiles before and after Statin Treatment in Patients with Dyslipidemia at St. Paul's Hospital Millennium Medical College, Addis Ababa, Ethiopia: Retrospective Cohort and Cross-Sectional Study.

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This is to certify that the thesis prepared by **Serkalem Ayele**, entitled:" **Hematological and lipid profiles before and after Statin Treatment in Patients with Dyslipidemia at St. Paul's Hospital Millennium Medical College, Addis Ababa, Ethiopia: Retrospective Cohort and Cross-Sectional Study** and submitted in a partial fulfilment of the requirements for Master of Science degree in Clinical Laboratory Sciences (Hematology and Immunohematology) complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

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Abbreviations

ACS	Acute coronary syndrome
ASCVD	Atherosclerotic cardiovascular diseases
APoA1	Apo lipoprotein A1
Apo B	Apo lipoprotein B
AVS	Atorvastatin
CAD	Coronary artery disease
CBC	Complete blood count
CVD	Cardiovascular disease
DC	Dendritic cells
DRERC	Department Research and Ethics Review Committee
Ecs	Endothelial cells
EDTA	Ethylene demine tetra acetic acid
EPO	Erythropoietin
GFR	Glomerular filtration rate
HGB	Hemoglobin
HCT	Hematocrit
HDL-C	High-density lipoprotein cholesterol
HMG-CoA	Hydroxyl methyl glutaryl co-enzyme a reducates
LDL-C	Low-density Lipoprotein Cholesterol
Lp (a)	Lipoprotein a

MCH	Mean corpuscular hemoglobin
MCHC	Mean corpuscular hemoglobin concentration
MPV	Mean platelet volume
NHANES	National Health and Nutrition Examination Survey
NLR	Neutrophil lymphocyte ratio
NTpro-BNP	N-terminal pro-brain natriuretic peptide
NO	Nitric oxide
OPD	Outpatient Department
RBC	Red blood cell
RDW	Red cell distribution width
SOPs	Standard Operating Procedures
SVS	Simvastatin
TC	Total cholesterol
TG	Triglycerides
VCAM-1	Vascular cell adhesion molecule-1
VCS	Volume, conductivity, and scatter
VLDL	Very low density lipoproteins
WBC	White blood cell count

Abstract

Background: Dyslipidemia is a plasma lipid abnormality. Statins are a widely used lipid-lowering drug. Previous studies have demonstrated the association of hematological parameters with lipid profiles and the effect of statin on these parameters. However, the results are still controversial and their application in clinical practice is limited, so this study was conducted to improve the significance of hematological parameters in dyslipidemia patients.

Objective: To determine the effect of statin treatment on hematological parameters in patients with dyslipidemia from August to November, 2023 at St. Paul's Hospital Millennium Medical College, Addis Ababa, Ethiopia

Methods: A cross-sectional study from August to November 2023 was conducted at St. Paul's Hospital Millennium Medical College, Ethiopia. Patients with dyslipidemia, 158 who have been taking statin for at least 3 months and 158 controls who monitor their serum lipids by lifestyle modification were enrolled in the study. They were selected by a convenient sampling method. Hematological and lipid profiles were analyzed by Beckman Coulter and Cobas 6000 analyzers, respectively. The data was analyzed using SPSS version 23. The normality of the data was checked by the Kolmogrov-smimov test. A parametric or non-parametric test was used for data analysis. All statistical significance was considered at $p < 0.05$.

Result: A total of 316 dyslipidemia patients, 158 on statin treatment for at least three months, and 158 statin naive controls were participated in the study. The majority of them had diabetes and hypertension. After the treatment, total cholesterol, low-density lipoprotein (LDL-C), and triglyceride were reduced significantly. White blood cell (WBC), red cell distribution width (RDW), and mean platelet volume decreased (MPV) (from 7.01±.1.6 to 6.7±1.6, 14.1±1.08 to 12.8±0.7, and 10.04±1.15 to 9.70±.99, respectively) with a p-value < 0.05. The change observed in RDW showed a correlation with the change that occurred in LDL-C.

Conclusion: the study showed difference before and after Statin Treatment in Patients with Dyslipidemia on WBC, MPV and RDW parameters values; hence, these parameters could be used as additional tools to monitor patient status after statin treatment.

Key words: hematological profile, dyslipidemia, statin

1. Introduction

1.1. Background

Dyslipidemia is a plasma lipid amount abnormality. It could be because of high low-density lipoprotein cholesterol (LDL-C), reduced high-density lipoprotein cholesterol (HDL-C), elevated total cholesterol (TC) and elevated triglyceride (TG), occurring either singly or in combinations (1, 2). In medical practice guidelines, TC ≥ 200 mg/dL or LDL-C values ≥ 130 mg/dL are considered as diagnostic of dyslipidemia (3). A raised level of LDL-C is among the leading modifiable risk factors and one of the most closely associated biomarkers of atherosclerotic cardiovascular diseases (ASCVD) (4).

Atherosclerotic cardiovascular diseases are the consequences of atherosclerosis. It includes acute coronary syndrome (ACS), myocardial infraction, stable or unstable angina, coronary artery disease, transient ischemic attack and ischemic stroke (5). Atherosclerosis is characterized by vascular lipid deposition, arterial luminal narrowing, plaque expansion and instability (6). Its pathophysiology involves persistent inflammation and failure to resolve the inflammation (7). Inflammatory cells (monocytes and T lymphocytes) recruited, transmigrate and differentiate in macrophage in the area of the atherosclerotic lesion due to the expression of vascular cell adhesion molecule-1 (VCAM-1) by endothelial cells (ECs) and by chemoattractant (8).

Within the intima macrophage, phagocyte modified lipoproteins (oxidized LDL-C) lead to the accumulation of cholesterol ester in the cytoplasm of macrophage forming foam cells (lipid laden macrophages), which is the early stage of atherosclerosis (9). Adaptive immune response, particularly involving T lymphocytes, exert a decisive regulatory effect on innate immune cell monocytes while B lymphocytes have an inhibitory effect on atherogenesis (10). Other immune cells like neutrophils, platelet neutrophil aggregates, and eosinophils, are also found in the plaque (11).

Impaired post inflammatory responses in atherosclerosis cause sustained and maladaptive inflammation, which promotes plaque progression and triggers thrombo-occulasive events. Platelets are inactive state in the blood circulation of healthy subjects. However, in thrombo-occulasive event they transform from a static dish into a globular swelling shape resulting in an increase in mean platelet volume (MPV) (12).

Hematological parameter like white blood cell count (WBC), monocytes, red cell distribution width (RDW), neutrophil (absolute count and percentage), lymphocyte (absolute count and percentage), platelet count, MPV, and neutrophil lymphocyte ratio (NLR) have been described as substantial and individual predictor of adverse cardiovascular disease (CVD) (13, 14), due to the inflammation and systemic hypoxemia that occurred in the pathophysiology (15).

Statins are a widely used lipid-lowering drug that inhibits cholesterol biosynthesis by down regulating hydroxyl methyl glut aryl co-enzyme a reductase (HMG-CoA) (16). Inhibition active site of HMGA-CoA prevents its substrate access, blocking the conversion of HMGA-CoA to mevalonic acid in the liver. This reduces hepatic cholesterol synthesis, leading to increased production of microsomal HMG-CoA reductase and increased cell surface LDL-C receptor expression (17). The increased receptor level, promotes the uptake of blood LDL-C, reducing its blood concentration (18).

Statins other than their cholesterol-lowering effect have pleiotropic effect like reduction in inflammation, reduction in smooth muscle cell proliferation, decreasing LDL-c oxidation, plaque stabilization, inhibiting platelet aggregation and improvement of endothelial dysfunction, which decrease cardiovascular risk (19). Statins are classified into natural statin and synthetic statin. Lovastatin, pravastatin and simvastatin (SVS) are natural statin. While atorvastatin (AVS) and fluvastatin are completely synthetic products (20).

1.2. Statement of the Problem

Dyslipidemia is a major risk factor for CVD mortality and morbidity in the world. It is the cause of 2.6 million deaths and 29.7 million disability-adjusted lives (21). In Africa, the prevalence of dyslipidemia is increasing because of economic growth, urbanization and unhealthy dietary patterns. Its prevalence ranges from 5.2 to 89.9% (22). A study in Addis Ababa, Ethiopia, revealed that 77.3% of the study participants had at least one type of dyslipidemia (among four hundred fifty respondents who had at least one type of dyslipidemia, 433 (96.2%); were newly diagnosed) (23). Although dyslipidemia is a major risk for CVD, it can be modified with treatment and healthy diet. Thus, providing an additional and easily available way for the physician to monitor the progress of dyslipidemia patient would be helpful.

Statin treatment lowers CVD risk and mortality through its effect on lipid profile and pleiotropic effect (24). Some of their pleiotropic effects include anti-inflammatory effects, decreasing platelet activation, and platelet aggregability (25). This could explain their possible effect on hematological parameters. Most studies conducted to determine the effect of statin on hematological parameters focus on MPV, RDW, and NLR (12, 26, 27, 28, 29). However, studies on other hematological parameters are limited, even if different studies shows a significant association with dyslipidemia. (30). Thus, in our study, the effect of statin on these hematological parameters was determined by adding some parameters to the previous studies.

There were previous studies conducted to see the effect of statin treatment on hematological parameters. However, there is a great deal of heterogeneity in their founding, and their application in clinical practice is limited. Since statin is a widely used lipid-lowering drug, it was better to understand the effect of this drug on hematological parameters to further improve their significance in monitoring the drug response of dyslipidemia patients.

1.3. Significance of the study

This study was aimed to improve the significance of hematological parameters in dyslipidemia patients, who are taking statin therapy, indicating an additional tool to the clinician for monitoring the patients' drug response. By using a readily available and cost effective hematology analyzer, the study presented another parameter to be measured besides serum lipid measurement, this would help the patient financially. In addition to this, the study has implications for policymakers, health institutions, and administrators to improve dyslipidemia patients' management and follow- up systems, by incorporating this routine test. This research can be used as a reference for future studies to be conducted in this area of field.

2. Literature review

There have been different literatures focusing on the effect of statin on hematological parameter in dyslipidemia. One was a quasi-experimental study conducted from January 2017 to December 2017 by Butt et al. in Pakistan to determine the effect of AVS on hematological parameters in patients with dyslipidemia. 100 dyslipidemia patients with no statin treatment were prescribed 20 mg of AVS per day for 12 weeks and were evaluated for changes on hematological parameters. After treatment, TC, LDL-C, TG and HDL-C decreased significantly. Levels of MPV reduced from 10.37±1.27fl to 9.35 ± 0.38fl, RDW levels fell from 14.46±0.51% to 14.24 ± 0.46% and NLR value decreased from 2.82 ± 0.25 to 2.73 ± 0.25. (for all p value is <0.001) (26).

Yu et al. conducted a study in 2015 in China with the objective investigating the effects of SVS and AVS on the biochemical and hematological markers in patients with risk CVD. One hundred and fifty outpatients from the department of cardiology, who were diagnosed with coronary heart disease, hypertension, hyperlipidemia, or other CVD, were included in the study and treated with AVS or SVS for 4 weeks. After treatment, all the lipid parameters were changed. For hematological parameters, only MPV was significantly decreased in both SVS group and AVS group. However, there was no significant correlation between MPV and lipid parameters before and after treatment (12).

Another observational, prospective study was conducted by Anand et al. in Gujarat, India. Fifty-four patients affected by hyperlipidemia without any other disease were participated in the study. The result revealed the effect of statins (AVS or rosuvastatin) in reducing RDW, MPV and

WBC. Statin treatment for 8 weeks also showed a significant reduction in LDL-C and TG. HDL-C was increased significantly (31).

A prospective study was conducted at Adnan Menderes University Medical Faculty, Division of Hematology, and Aydin, Turkey. Forty-four patients with primary hypercholesterolemia were treated for 24 weeks with atorvastatin. The outcome of the study indicates that statin treatment significantly decreased TC, LDL-C, and TG. It increased the level of HDL-C significantly. However, in this study statin did not show a significant effect on hematological parameters (32).

A retrospective study conducted in Ankara, Turkey, by Colak et al. to see the effectiveness of statin treatment in reducing RDW and MPV in patients with stable coronary artery disease (CAD). One hundred twenty-one statin naive patients who underwent angiography with suspected CAD between June 2012 and June 2013 were retrospectively enrolled in the study. The results showed a notable reduction in TC, LDL-C, and TG. After the treatment, RDW value fell significantly. However, no significant change was observed for MPV and other hematological parameters. In this study, there was no significant correlation found between RDW and serum cholesterol levels both before and after statin treatment. In addition, there was no significant correlation between the change in RDW and the change in each lipid parameters (27).

Another study in Turkey incorporated 30 participants with uncontrolled primary dyslipidemia with hypolipidemic diet treatment and 30 normolipidemic healthy participants, showed rosuvastatin treatment for 12 weeks significantly reduced Levels of MPV. The changes in MPV levels with treatment were not correlated to changes in plasma lipids (28).

Similarly, a retrospective study conducted in Turkey by Sivri et al. in 2013 showed that both AVS and rosuvastatin lowered MPV. The two statins revealed a comparable effect on the reduction of TC, LDL, HDL, and TG. The MPV of patients did not show any significant association with lipid parameters before and after treatment. There was no significant correlation between changes in MPV and change in HDL-C, LDL-C or TG after one month treatment (33).

In the Slovak Republic, a cohort study conducted by Kucera et al. in 2016. The study determined the influence of AVS on plasma atherogenic biomarker of 40 patients with hypercholesterolemia, without previous lipid lowering treatment. TC, LDL-C, HDL-C, and TG declined after 40 for 3

months of atorvastatin treatment. Hematological parameters such as MPV and RDW were measured before and after treatment. No significant changes were observed for the selected hematological parameters (MPV and RDW). However, the platelet count was decreased significantly. At baseline, a strong correlation between HDL-C, TG with MPV was found (29).

According to a retrospective study conducted in Turkey in 2015 by Gungoren et al., statin therapy from 10 to 80 mg atorvastatin or 10 to 40 mg rosuvastatin for 24 weeks did not affect NLR and MPV in patients with hypercholesterolemia who had CAD or were at high CV risk (34). However, a cohort study conducted in 2019 showed that rosuvastatin (40 mg/day) and atorvastatin (80 mg/day) significantly decreased the levels of NLR ($p = 0.001$) at the end of one-month therapy in patients with acute myocardial infarction. Rosuvastatin produced a significant decrease in platelet count, but the effect of AVS platelet and monocytes counts were insignificant (35).

Similarly, Akin et al. in Turkey, conducted a study on hypercholesterolemic patients recruited from December 2010 to May 2012 and normocholesterolemic healthy controls. It demonstrated that 24- week AVS treatment had an effect on MPV, platelet count, and NLR. However, the treatment did not impact RDW (36).

From the National Health and Nutrition Examination Survey (NHANES) from 1999 to 2004 in U.S.A., Yoon et al. conducted a study aimed to see the effects of statin on inflammatory markers. The study contains four analytical groups. Statin user, antibiotic group and nonuse group. The findings of this study showed that statin use was significantly associated with low WBC when compared to the other group (37).

Despite the literature available, their founding is inconclusive. Although many researchers in their studies revealed that statin has effects on MPV, NLR, and RDW, there is limited literature about the effect of this drug on another hematological parameter of dyslipidemia patient. In addition, to the best of my knowledge, there was no study in the study area on this topic, so this study would fill this gap.

3. Objectives

3.1. General Objective

- To determine hematological Parameters before and after Statin Treatment in patients with dyslipidemia from August to November, 2023 at St. Paul's Hospital Millennium Medical College, Addis Ababa, Ethiopia

3.2. Specific objectives

- To compare hematological profile at before and after 3 months of initiation of statin treatment in patients with dyslipidemia.
- To determine the correlation between hematological profile change and lipid profile change.

4. Methods and materials

4.1. Study area

This study was conducted at cardiac and diabetic clinic of St. Paul's Hospital Millennium Medical College which is found in Addis Ababa city. The hospital was established in 1968. The medical college opened in 2007 during the Ethiopian Millennium celebration.it has 700 admission beds, more than 2000 outpatient and emergency client visit the health facility daily. The cardiac clinic runs two days per week and is visited by average 40-50 patients per day. The diabetic clinic runs 4 days per week.

4.2. Study design and Study period

A retrospective cross-sectional study was conducted from August to November 2023 using primary and secondary data to determine the effect of statin treatment on hematological parameters in patients with dyslipidemia at St. Paul's Hospital Millennium Medical College, Addis Ababa, Ethiopia

4.3. Population

4.3.1. Source population

All patients suffering from dyslipidemias who have attended at St. Paul's Hospital Millennium Medical College, Addis Ababa, Ethiopia

4.3.2 Study population

4.3.2.1. Case group

Dyslipidemia patients who monitor their serum lipids by taking statin with lifestyle modification, who fulfilled inclusion criteria and have the willingness to participate in this study.

4.3.2.2. Control group

Dyslipidemia patients who monitor their serum lipid without taking statin. Selected from a similar setting and matched with other factors, they fulfilled inclusion criteria and were willing to participate in this study.

4.4. Inclusion and exclusion criteria

4.4.1. Inclusion criteria

4.4.1.1. Case group

- Dyslipidemia patients who have taken statin for at least three months.
- Dyslipidemia patients who have regular follow up from August 2021-June 2023.

4.4.1.2. Control group

- Dyslipidemia patients who monitor their serum lipids by lifestyle modification.
- Dyslipidemia patients who have regular follow up during the study period

4.4.2. Exclusion criteria

4.4.2.1. Case group

- Patients who have liver disease
- Patients with kidney disease
- Patients who have an acute infection
- Patients with hematological disease
- Patients who did not have baseline information.
- Pregnant women
- Patients who are taking other lipid lowering-drugs

4.4.2.1. Control group

- Patients who have liver disease
- Patients with kidney disease
- Patients who have an acute infection
- Patients with hematological disease
- Patients who did not have baseline information.
- Pregnant women
- Patients who are taking other lipid lowering-drugs

4.5. Sample size and sampling technique

4.5.1. Sample size determination

The sample size was determined using the following formula:

$n \geq (Z_{1-\frac{\alpha}{2}} + Z_{1-\beta})2.\sigma l2 + \sigma 2\ 2/M_1\text{-}M_2$ (38).

Where $Z_{1-\alpha/2}$ = is critical value and a standard value for the corresponding level of confidence (in this study considering 95% confidence interval, α = 0.05, $Z_{1-\alpha/2}$ = 1.96)

$Z_{1-\beta}$ = Is the desired power (in our case, for 80% power, β = 0.2, Z 1−β = 0.84)

σ_1= is the standard deviation of statin group = 30, σ_2 is the standard deviation of control group = 43 and the mean difference of TC 12 taken from previous study (39).

$N=(1.96+0.84)^2*(30)^2+(43)^2$

N ≥ 150 for each study group, by adding 5% non-response rate 316 participants were enrolled in the study.

4.5.2 Sampling technique

Non probable convenient sampling technique was used

4.6. Variables

4.6.1. Dependent variable

Hematological parameters

4.6.2. Independent variable

Age

Sex

Smoking status

Alcohol consumption

Hypertension

Diabetic mellitus

Duration of statin treatment

Medication other than statin

Regular Physical exercise

Residence

Occupation

Educational level

Lipid profile

4.7. Data collection and laboratory analysis

4.7.1 Data collection procedure

After giving a brief explanation of the aim of the study and obtaining informed consent, data on the socio-demographic and past medical history of the study participants including history of hypertension, diabetes mellitus, and history of medication was collected from interview and medical records by a senior nurse using a validated structured questionnaire and checklist prepared by the principal investigator. The questionnaire has been developed in English, and then it has been translated to Amharic. All pre statin hematological parameters and serum lipids data were collected retrospectively from patients' medical records from August 2021-June 2023 for comparison to the post statin value. For the value after the treatment, about 5 ml of blood was collected from the patients and their CBC and serum lipid analyzed using Beckman Coulter and Cobas 6000 automated analyzers, respectively.

4.7.2 Laboratory analysis

4.7.2.1 Specimen collection and processing

The patient was asked to fast at least for 12 hour then the data collector (laboratory technologist) collected about 5ml of blood from each participant in accordance with standard operating procedures (SOPs). The blood was divided into tube containing anticoagulant (EDTA) and into a tube without anticoagulant (a serum separator tube). The EDTA tube was immediately inverted to mix the anticoagulant with the blood so the blood did not clot. Within 2 hours of sample collection, the blood placed in the serum separator tube was centrifuged after it was clotted.

After the collection and processing, the specimen was taken to hematology and chemistry workstation to be analyzed. The principal investigator, together with senior laboratory technologist, checked the quality of the specimen, and those accepted were analyzed using Beckman Coulter automated hematology analyzer for CBC and Cobas 6000 automated chemistry analyzer for lipid profile.

4.7.2.2. Test principle of laboratory analytes

4.7.2.2.1. Complete blood count

The CBC of participants were analyzed by Beckman Coulter hematology analyzer.

Detection principle of Beckman Coulter hematology analyzer

The whole blood delivered in to RBC chamber and WBC. In the RBC chamber, RBC and platelets are discriminated and counted by electrical impedance (coulter) method. In the WBC chamber, a reagent lyse the RBC and release hemoglobin. The analyzer uses VCS (volume, conductivity, and scatter) technology for WBC and 5 differential counts. The technology include the volumetric sizing of cells by impedance, conductivity measurement of cells, and laser light scatter, all performed simultaneously for each cell. After the WBC count, the WBC dilution is passed to the hemoglobinometer and the HGB concentration is determined by light transmittance. MCV is taken from volume distribution data. HCT, MCH and MCHC are calculated from measured and derived data. RDW is directly calculated from the RBC histogram(40).

4.7. 2.2.2. Lipid profile

Detection principle of Cobas 6000

Cobas 6000 analyzer combined photometric, ion selective electrode (ISE) and electrochemiluminescence technology. The ISE quantifies electrolytes like sodium, potassium, and chloride. The photometric system can measure colometric or immunoturbidometric reaction utilizing end point or kinetic rate absorbance measurement.

A. Total cholesterol

Principle of the test

TC is measured by enzymatic colometric method. In the presence of cholesterol ester hydrolase, cholesterol esters are cleaved and release free cholesterol and fatty acid. 3-OH group of cholesterol is oxidized by cholesterol oxidase. One of the reaction by products, H_2O_2 is measured quantitatively in a peroxidase catalyzed reaction that produces a color. Absorbance is measured at 505 nm. The method uses an endpoint reaction that is specific for cholesterol (41). The reaction sequence is as follows:

Cholesterol ester hydrolase

Cholesterol esters + H_2O ----------> cholesterol + fatty acid

Cholesterol oxidase

Cholesterol + O_2 ----------> cholest-4-en-3-one + H_2O_2

Peroxidase

$2H_2O_2$ + 4-aminophenazone + phenol ----------> 4-(p-benzoquinonemonoimino)-phenazone + 4 H_2O

B. Low-density lipoprotein cholesterol

Principle of the test

Most of the circulating cholesterol is found in three major lipoprotein fractions: very-low-density lipoprotein cholesterol (VLDL-C), LDL and HDL(41).

TC = VLDL-C+ LDL-C + HDL-C

LDL-C is calculated from measured values of TC, TG, and HDL-C as follows:

LDL-C = TC - HDL-C- TG/5

Where TG/5 is an estimate of VLDL-C.

C. High-density lipoprotein cholesterol

Principle of the test

HDL-C is analyzed by enzymatic colorimetric method like that of TC. Lipoproteins that contain apoB excluded from the analysis, Sulfated alpha-cyclodextrin in the presence of Mg+2, forms complexes with apoB containing lipoproteins, they becomes non-reactive to the enzymatic cholesterol reagent. Polyethylene glycol-coupled cholesterol esterase and cholesterol oxidase are hydrolyzed for the HDL-C measurement (41). Absorbance is measured at 600 nm.

- ✓ ApoB containing lipoproteins + α-cyclodextrin + Mg^{+2} + dextran SO_4 ---> soluble non-reactive complexes with apoB-containing lipoproteins
- ✓ HDL-C esters +PEG-cholesterol esterase ---- > HDL-unesterified cholesterol + fatty acid
- ✓ unesterified cholesterol +O_2 PEG-cholesterol oxidase ----- > cholestenone +H_2O_2
- ✓ H_2O_2 + 5-aminophenazone + N-ethyl-N-(3-methylphenyl) ----- >N-succinyl ethylene diamine+ H_2O + H^+ peroxidase ----- > qunoneimine dye +H_2O

D. Triglyceride

Principle of the test

TG are measured enzymatically in the serum or plasma. It is hydrolyzed to produce glycerol. Glycerol is then oxidized using glycerol oxidase, and H_2O_2, one of the reaction products, is measured as at 500 nm (41). The reaction sequence is as follows:

Lipase

Triglycerides + $3H_2O$ ----------> glycerol + fatty acids

Glycerokinase

Glycerol + ATP ----------> glycerol-3-phosphate + ADP

Glycerophosphate oxidase

Glycerol-3-phosphate +O_2 ----------> dihydroxyacetone phosphate + H_2O_2

Peroxidase

H_2O_2+ 4-aminophenazone + 4-chlorophenol ----------> 4-(p-benzoquinone-monoimino)-

Phenazone + $2H_2O$ + HC

4.8. Data quality assurance

To assure quality of research, it is important to maintain the quality of the data used as input. For that reason, an experienced nurse was involved in data collection. Before the data collection, orientation was given on the objective of the study, approaching to interview, reviewing medical history and recording of data. Questionnaires, which have been validated by reviewing literature and translated into the local language, were used. To check the accuracy and consistency of the translated questionnaire, it was re-translated into English. Any unclear point regarding the questioner and data collection procedure were clarified before collecting data. The principal investigator supervised the data collector and also participated in the data collection process. Concerning laboratory analysis, all pre-analytical, analytical, and post-analytical phases' quality was ensured by working according to SOPs.

4.8.1 Pre analytical

In this phase, data collector strictly followed SOPs to collect the blood sample in a properly labeled EDTA tube and in a plain tube. The sample was evaluated for proper labeling, presence of clots, volume, and so on. Based on sample rejection and acceptance criteria, any sample that did not meet the acceptance criteria was rejected. When there was a delay in the analysis, it was stored at 2℃-8℃. Before analyzing the patient sample, reagents was checked for their integrity and expiry date.

4.8.2. Analytical

Quality controls were run to ensure the accuracy and precision of the sample result. All specimens were analyzed within 6 hours of collection. The sample was analyzed according to SOPs after the quality control result passed.

4.8.3. Post analytical

In this phase, results were reviewed by comparing them with reference interval. When there were any unexpected or flagged results, they were confirmed by repeating the test or smear review accordingly. To avoid clerical error, print out results that were generated by the automated analyzer were used. After collection, data was checked for completeness, and the presence of outliers so they were either corrected or removed. Data was entered using a double entry method into EPI software to check clerical errors.

4.9. Data analysis and interpretation

After checking the completeness of the data, it was entered into Epi Data Software, and exported to Statistical package for the social sciences Software (SPSS) version 23 for analysis. For categorical variables, frequency and percentage were calculated. Kolmogrov-smimov test was used to test normality. For normally distributed data, mean± standard deviation was used to express the data. Median with interquartile range taken for data that fail to have normal distribution. Paired student t-test and Wilcoxon signed rank test were used to compare pre-statin and post-statin paired value. An independent t-test and a Mann-Whitney U test were used to compare the value between groups. Pearson correlation coefficient was employed to determine the correlation between hematological and lipid profile before and after the treatment. The relationship between hematological and lipid profile change was determined by linear regression analysis. $P < 0.05$ (two-tailed) was considered significant.

4.10. Ethical consideration

The study was conducted after obtaining ethical clearance from the Department of Research and Ethics Review Committee, Department of Medical Laboratory Sciences, College of Health Sciences, Addis Ababa University and getting permission from St. Paul's Hospital Millennium Medical College. Written, informed consent was obtained from all participants. All clinical data and laboratory findings were kept confidential and used for the sole purpose of the study.

4.11. Operational definition

Drinking alcohol: Those who reported having more than 2 glasses of drink containing alcohol per week during the past 30 days was considered alcohol consumer.

Dyslipidemia: TC > 200 mg/dl, LDL-C >130, each value alone or in combination.

Habit of Regular exercise: performing physical exercise for 30 minute or longer per day was considered as a habit of regular exercise.

Khat chewer: Those who chew khat currently.

Lifestyle modification: Having habit of exercise along with diet modification

Regular follow up: Patients who attend regular checkups and take statin according to physician orders.

Smoker: All current smokers (cigarettes and any usage of tobacco products) and those who quit smoking less than one year.

5. Result

5.1. Socio-demographic and clinical characteristics of study groups

The socio-demographic and clinical characteristics of study participants, 158 patients who have dyslipidemia, on statin treatment for at least three months and 158 statin naive controls, are presented in Table 1. The cases and controls were comparable in terms of gender distribution, with slightly more females (51.3% and 54.4% respectively) in both groups. Regarding age, 74.7% of the cases and 71.5% of the controls were between 41-60 years old. A significant proportion of participants had completed diploma and higher education. In terms of occupation, the majority of the participants were self-employed. About 63.3% and 50.6% of the cases and 55.7% and 48.7% of controls had a history of diabetes mellitus and hypertension, respectively. Habits of smoking, alcohol consumption, and chat chewing reported by small percentage. The majority of the participants had a habit of regular physical exercise. The duration of statin treatment varies, with the majority receiving treatment for 6-12 months. 84.8% of cases and 82.3% of controls were reported taking other medication (Table 1).

Table 1. Socio-demographic and clinical characteristics of dyslipidemia patients on statin treatment and statin naïve control at St. Paul's Hospital Millennium Medical College, Addis Ababa, Ethiopia, 2023 (n = 316)

Variables		Cases	Control
		Frequency (%)	Frequency (%)
Gender	Male	77 (48.7)	72 (45.6)
	Female	81 (51.3)	86 (54.4)
Age	20-40	9 (5.7)	13 (8.2)
	41-60	118 (74.7)	113 (71.5)
	>61	31 (19.6)	32 (20.3)
Educational status	Unable to read and write	14 (8.9)	11(7)
	Primary school	31 (19.6)	39 (24.7)
	High school	44 (27.8)	29 (18.4)
	Diploma and above	69 (43.7)	79 (50.0)
Occupation	Governmental employee	32 (20.3)	42 (26.6)
	Se1f employee	48 (30.4)	60 (30)
	Daily worker	44 (27.8)	27 (17.1)
	Other	31(19.6)	26(16.5)
	Private business owner	3 (1.9)	3(1.9)
Diabetes mellitus history	Yes	100 (63.3)	88 (55.7)
	No	58(36.7)	70 (44.3)
Hypertension	Yes	80 (50.6)	77 (48.7)
	NO	78 (49.4)	81 (51.3)
Habit of alcohol	Yes	38 (24.1)	46 (29.1)
	No	120 (75.9)	112 (70.9)
Habit of smoking	Yes	18 (11.4)	22 (13.9)
	No	140 (88.6)	136(86.1)
Habit of chat chewing	Yes	11(7.0)	21(13.3)
	No	147 (93.0)	137(86.7)

Duration of statin treatment /follow up period(Month)	3-6 6-12 >12	50 (31.6) 60 (38.0) 48 (30.4)	53 (33.5) 64 (40.5) 41 (25.9)
Taking other medication	Yes No	134 (84.8) 24 (15.2)	130 (82.7) 28 (17.7)
Habit of Physical activity	Yes No	121(76.6) 37 (23.4)	128 (81.0) 30 (19.0)

Note: Other occupation: - house wife, farmer…., duration of treatment is for case, follow up period is for controls, other drug: - medication for diabetes mellitus and hypertension

Note: p-value taken from independent t-test or Mann-Whitney U test.

5.2. Hematological parameters of study participants at before and after statin treatment

In this study, there was a significant decrease in means of WBC from 7.0±1.6 to 6.7±1.6, MPV (10.04±1.15 to 9.7±.99), and RDW (14.1±1.08 to 12.8±.7) from pre to post treatment ($p<0.05$) for those statin groups. But, for the control group, there was no significant change observed for this parameter. There was no significant change observed in the other hematological parameter for both groups (table 3).

Table 2Table 2. Hematological parameters at before and after statin treatment in dyslipidemia patients, on statin treatment and statin naive control at St. Paul's Hospital Millennium Medical College, Addis Ababa, Ethiopia, 2023

Variables	Statin group (n = 158)			Control group (n = 158)		
	Pre-statin	Post-statin	p-value	Pre-L	Post-L	p-value
WBC	7.02±1.62	6.71±1.67	0.00	6.61±1.54	6.51±1.57	0.294
RBC	4.73±0.43	4.77±0.43	0.355	4.79±0.41	4.70±0.41	0.34
Hgb	14.01±1.40	14.15±1.37	0.594	13.99±1.51	13.88±1.44	0.422
Hct	42.90(5.07)	43.80(5.40)	0.058	43.90(6.43)	43.90(5.28)	0.851

MCV	92.18±5.38	92.06±5.87	0.827	93.23±4.44	93.01±4.48	0.587
MCH	30.05(3.30)	29.7(2.90)	0.340	29.55(3.55)	29.50(2.90)	0.435
MCHC	32.70(3.32)	32.40(2.85)	0.243	31.70(3.75)	31.45(3.80)	0.281
RDW	14.12±1.09	12.80±0.71	0.000	13.33±1.0	13.2±0.96	0.400
NET (%)	58.11±6.95	58.29±6.70	0.737	58.58±6.37	58.35±6.24	0.594
LYM (%)	31.03±6.05	30.87±6.30	0.743	29.81±6.02	30.29±5.7	0.258
MON (%)	6.35(3.40)	6.70(2.70)	0.826	6.35(3.60)	7.30(3.50)	0.051
EOS (%)	3.50(2.10)	3.50(2.00)	0.582	3.00(1.72)	3.00(2.13)	0.762
BAS (%)	0.70(0.60)	0.60(0.50)	0.56	0.2(0.4)	0.3(0.4)	0.315
NET (A)	3.85(1.96)	3.82(1.85)	0.822	3.85(1.96)	3.81(1.91)	0.203
LYM (A)	1.95(0.72)	1.98(.81)	0.459	1.88(0.79)	1.93(0.73)	0.401
MON (A)	0.42(0.22)	0.41(0.21)	0.583	0.49(0.23)	0.46(0.22)	0.060
EOS (A)	0.21(0.16)	0.21(0.16)	0.301	0.21(0.13)	0.20(.12)	0.865
BAS (A)	0.05(.04)	0.04(0.04)	0.51	0.02(0.02)	0.02(0.02)	0.452
PLT-C	224.5(100)	220(102)	0.088	214.5(65)	223.5(51)	.629
MPV	10.05±1.15	9.68±0.996	0.000	9.86±1.01	9.77±1.13	0.294

Note:-Pre-L-before life style modification, post-L Post life style modification, P-value taken from dependent t-test or wilcoxon signed rank test

5.3 Lipid profile of study participants before and after statin treatment

TC and LDL-C were significantly reduced in both study groups ($p<0.05$). In addition, TG level showed a significant reduction after statin treatment. However, no significant changes were observed in the HDL-C level in both study groups (table 4).

Table 3Table 3. Lipid profile at before and after treatment in dyslipidemia patients, on statin treatment and without statin control at St. Paul's Hospital Millennium Medical College, Addis Ababa, Ethiopia, 2023

Variables	Statin group (n = 158)			Control group (n = 158)		
	Pre-statin	Post-statin	p-value	Pre L	Post-L.	p-value
TC	251.79±43.00	187.40±35.25	0.000	230.74±28.24	209.7±29.46	0.000

LDL-C	171.2(40.05)	118.4(50.0)	0.000	151.8(28.45)	130.65(30.02)	0.000
HDL-C	47.28±12.04	47.55±10.84	0.433	45.59±1.51	45.72±11.0.	.714
TG	178.95(80.8)	166.60(80.8)	0.000	138.3(87.18)	133.3(81.60)	0.051

Note:-Pre-L-before life style modification, post-L Post life style modification, p-value taken from dependent t-test or Wilcoxon signed rank test.

5.4. Correlation between hematological parameters and serum lipids

Before statin treatment, selected hematological parameters (WBC, RDW and MPV) did not show significant correlation with TC, HDL-C, LDL-C, and TG (all $p > 0.05$). After treatment, it was also the same, no correlation was found between the variables (table 5).

Table 4Table 4. Correlation between hematological profile and lipid profile at baseline and after statin treatment in dyslipidemia patients at St. Paul's Hospital Millennium Medical College, Addis Ababa, Ethiopia, 2023 (n = 158)

Parameters	Before statin treatment				After statin treatment			
	TC	LDL-C	HDL-C	TG	TC	LDL-C	HDL-C	TG
WBC (r,p)	-0.07, 0.383	-0.01, 0.948	-0.09, 0.219	-0.04, 0.608	-0.04, 0.549	0.04, 0.665	-0.16, 0.51	-0.10, 0.192
MPV (r,p)	-0.04, 0.590	-0.01, 0.976	-0.01, 0.991	-0.03, 0.968	-0.27, 0.061	0.23, 0.053	-0.02, 0.797	-0.10, 0.196
RDW (r,p)	-0.14, 0.080	0.01, 0.893	-0.03, 0.746	-0.2, 0.811	-0.55, 0.49	0.75, 0.348	-0.07, 0.401	-0.18, .051

Note: (r, p) Pearson correlation coefficient, p-value

The change in RDW showed a significant correlation with the change in LDL-C ($\beta = 0.186$, p-value < 0.05). However, there was no a significant correlation between other hematological (MPV and WBC) and lipid profiles, as shown in (Table 6)

Table 5Table 5 . Correlation between the change in MPV, RDW, and WBC with the change of TC, HDL, LDL-C, and TG values of dyslipidemia patients on statin treatment at St. Paul's Hospital Millennium Medical College, Addis Ababa, Ethiopia, 2023 (n = 158)

Mean difference between pre and post treatment values		Coefficient(β)	Standard error	t-value	p-value
MPV	TC	.106	.002	1.333	.185
	HDL-C	-.015	.020	-.194	.847
	LDL-C	-0.27	.003	-.338	.736
	TG	.001	.003	.008	.994
RDW	TC	.097	.002	1.216	.226
	HDL-C	-.052	.022	-.656	.512
	LDL-C	.186	.003	2.363	<0.05
	TG	-.079	.003	-.993	.322
WBC	TC	-.109	.002	-1.364	.175
	HDL-C	-.034	0.19	.428	.276
	LDL-C	-.113	.002	-1.416	.159
	TG	-.073	.003	-.916	.361

5.5 Hematologic and lipid parameters before and after in patients with dyslipidemia among different durations of treatment.

The Change of TC, LDL-C, and RDW showed a significant difference among different durations of treatment. (Table 7). Post hoc analysis was done for the parameters that have significant variation

Table 6Table 6. Hematologic and lipid parameters before and after in patients with dyslipidemia among different durations of treatment, at St. Paul's Hospital Millennium Medical College, Addis Ababa, Ethiopia, 2023 (n = 158)

ANOVA			Post hoc analysis	
Duration	MD	P-value	Paired group	p-value
	RDW			
3-6	1.41	0.03	3-6 vs 6-12	0.690
6-12	1.21		6-12 vs >12	0.023
>12	1.91		3-6 vs >12	0.563
	MPV			
3-6	0.38	0.879		
6-12	0.32			
>12	0.42			
	WBC			
3-6	0.18	0.292		
6-12	0.48			
>12	0.23			
	TC			
3-6	57.59	0.013	3-6 vs 6-12	0.421
6-12	60.04		6-12 vs >12	0.040
>12	89.82		3-6 vs >12	0.001
	LDL-C			
3-6	50.31	0.042	3-6 vs 6-12	0.783
6-12	55.08		6-12 vs >12	0.129
>12	64.5		3-6 vs >12	0.016

Note: duration: - duration of treatment in months, MD: - mean difference between pre and post treatment value

6. Discussion

This study was carried out in 316 dyslipidemia patients, 158 who have been taking statin for at least 3 months and 158 statin naive control groups. Females were more dominant than male ratio. The majority of the participants had comorbidities, 63.3% and 50.6% of the cases and 55.7% and 48.7% of the controls had diabetes mellitus and hypertension, reflecting their known association with dyslipidemia. The association between them is mediated by a complex interaction between metabolic, inflammatory and vascular pathways (42).

According to our study, the value of TC, LDL-C, and TG fell after stain treatment. Our study findings were in line with a study conducted in Pakistan, which showed that the administration of statin decreased TC, LDL-C, HDL-C and TG (26). Similarly, a study conducted in China reported a similar finding (12). Another study in India indicated, statin therapy is capable of reducing TC, LDL-C, and TG (31). The reduction in TC and LDL-C was in agreement with previous research and well agree with the established role of statin as a cholesterol-lowering agent, particularly LDL-C (17). HDL-C value did not show any significant change in this study, which is unlike to the previous studies (12, 26, 31). This difference in finding could be due to the variation in statin dosage and durations, since it is not fixed in our study. It requires further study.

We observed that TC and LDL-C showed a significant reduction in patients receiving statin therapy for 6-2 and over 12 months than patient receiving treatment for 1-3 months. This suggests that statin lower TC and LDL-C progressively with a longer duration of treatment. Population-based cohort study, in Korea, revealed that longer duration of statin treatment decreased the risk of major adverse cardiovascular event (43). Our findings reflects continuing use of statin is more effective in lowering lipid profile than taking it for a short period of time, which leads to decrease load in the atherosclerosis plaque through time.

In this study, RDW fell from 14.2 to 12.8. This finding is consistent with previous studies conducted in Pakistan by Butt et al. and in India, respectively (26, 31). It is also similar to a study conducted by Colak et al. in Turkey (27). In contrast to this study, few studies showed that statin had no effect on RDW (12, 29, 32). Difference in the findings might be due to the different study design used. RDW is an indicator of RBC size variation, in past mostly used to classify anemia (44). However, recent studies associate high RDW with intensified inflammation in the vascular wall, CVD and mortality (45, 46). The observed decrement in RDW value can be attributed by anti-inflammatory properties of statin, which possibly improved erythrocyte maturation and decreased anisocytosis (20).

Moreover, the significant correlation between the change in RDW and LDL-C in this study, suggests there is a potential interaction between erythropoiesis and lipid metabolism. It is indicated in animal study conducted by Lu et al. reduced cholesterol blocked erytroid proliferation (47). Translocate protein 2 (TSPO2), which is expressed specifically in late erythroblast, was able to bind to cholesterol and participated in intracellular cholesterol redistribution during RBC synthesis (48). In addition, one experimental study reported, increased intracellular cholesterol could cause abnormal reticulocyte maturation (49).

In our study, WBC count was reduced significantly after statin treatment. The result agrees with previous finding in India, which reported, statin treatment reduced WBC in hyperlipidemia patient (31). In addition, study conducted by Yoon et al. in the general population of USA demonstrated that low WBC was associated with statin usage (37). The possible mechanism of statin lowers WBC could be by its anti-inflammatory effect (20). Iwata et al. study demonstrated, the potential of satin to decrease cytokine production in human epithelial bronchial cells via mevalonic cascade, independent of cholesterol synthesis cascade. This inhibition of pro-inflammatory cytokine production results the reduction of WBC recruitment and activation (50).

In this study, the change observed in WBC did not correlate with the change observed in lipid profile. This indicates that the effect of statin treatment on WBC is irrespective of their effect on cholesterol synthesis. In this regard, studies on human are limited. however, in vitro statin decreased inflammation with absence of lipid concentration change (51). Moreover, statin showed anti-inflammatory property in an inflammatory disorder that are unrelated to lipid (52, 53).

As for MPV, the study revealed that stain reduced MPV effectively, which is in line to previous studies (12, 28, 31, 54). Similar results were gained from the study conducted by Akin et al. (36). Which, showed that Atorvastatin treatment for 24 week was able to decrease MPV in hypercholesterolemia patient. In contrast, the findings of our study are not consistent with retrospective study conducted by Gungoren et al. (34). Which, demonstrated that statin were in effective in changing MPV of hypercholesterolemia patients. The possible difference may be due to the difference in patient characteristics, 261 hypercholesterolemia patients who have CAD or high CV risk were participated in their study. However, our study did not assess the presence of CAD or CV risk. Also, another study participated patient suspected for CAD reported statin treatment did not affect the MPV. MPV is an indicator of platelet size and activity. Higher MPV is associated with increased platelet reactivity and cardiovascular risk (55). The reduction of MPV observed in this study may suggest a potency of statin to modulate platelet activity and aggregation. The finding of this study supported by study conducted by Barale et al, in their study, maximal platelet aggregability to adenosine diphosphate (ADP), collagen and arachidonic acid (AA) was reduced by simvastatin treatment. In addition, platelet activity markers, soluble P-selectin (sP-selectin) and CD40 ligand (sCD-40L) are also decreased after the treatment (39).

The change occurred in MPV did not correlate with the change occurred in lipid profile. This finding is coherent with two studies conducted in turkey that reported, the reduction of MPV did not correlate with the reduction of TC, LDL or TG (28, 33). This indicates that the inhibition of platelet aggregation in dyslipidemia patients, receiving statin treatment is not related to lipid lowering effect of statin. Several experimental studies showed statin exerts anti-thrombus effect by enhancing nitric oxide (NO) bioactivity, by action independent of lipid lowering, such as up regulation of eNOS (56, 57). In a study conducted in normocholesterolemic smokers demonstrated that the endothelium dependent vasodilation independent of serum lipid change (57). In another study in hypercholesterolemia patient, statin treatment caused a significant improvement in endothelial activity in 3 days before an effect occurred on LDL-C occurred (58).

7. Strength and limitation

7.1. Strength of the study

Our study assessed several hematological parameters instead of focusing on selected hematological parameters.

7.2. Limitation of the study

The study had some limitation. First, it did not integrate direct measure of patient adherence to exercise and diet modification because of the retrospective study design. This information may introduce difference in lifestyle factors among participant. In addition to this, the finding of the study cannot be generalized to the population because it is an institutional based study.

8. Conclusion and Recommendation

8.1. Conclusion

Statin therapy showed significant change on some hematological and lipid parameters. WBC, RDW, and MPV were reduced significantly after the statin treatment. Regarding lipid profile, TC, LDL-C and TG were reduced after the treatment. The change occurred in WBC and MPV did not show a correlation with the change occurred in TC, LDL-C and TG. However, the change observed in RDW showed a significant correlation with the change observed in LDL-C.

8.2. Recommendation

In parallel to lipid profile hematological parameters, particularly RDW, WBC and MPV of dyslipidemia patients should be checked during follow-up period for improving treatment strategies. The parameters could be used as an additional tool for monitoring statin response. Further, longitudinal studies should be conducted by monitoring patient adherence to exercise and diet.

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Annexes

Annex I: Principle and Procedure of Tests

Venous Blood Collection

Material needed

- Test requisition
- Tourniquet and disposable gloves
- Alcohol (70%) and gauze square or alcohol wipes
- Sterile disposable needles with syringe

Procedure

- Identify the patient
- Prepare all necessary equipment
- Label EDTA containing tube
- Apply the tourniquet on the upper arm of the patient look large, well anchored vein
- Allow the site to dry
- Insert the needle with attached syringe or evacuated tube
- steadily draw the blood
- Release the tourniquet, remove the needle and press firmly on the vein puncture site with a piece of cotton.
- Mix tubes with anticoagulant by inverting the tubes several times

If a syringe was used, carefully remove the needle before dispensing the blood into a test tube. Blood should never be forced back through the needle, and the syringe plunger should be slowly depressed. Discard the used needle into an appropriate safety container

2. DxH 800 Beckman Coulter Hematology Analyzer

Principle

The aspirated whole blood divided into two aliquots, add each mixed with an isotonic diluent. The first dilution delivered to RBC aperture chamber, and the second delivered to WBC chamber.in the RBC chamber RBC and platelet discriminated and counted by electrical impedance (coulter) as the cells are passing through sensing aperture.in the WBC chamber, a reagent to lyse RBC and release hemoglobin is added before WBC count and differentiation. Beckman coulter uses VCS (Volume, conductivity, and scatter) technology for total WBC count and 5 differential counts. The VCS technology include the volumetric sizing of cells by impedance, conductivity measurement of cells, and laser light scatter, all performed simultaneously for each cell. After WBC count The WBC dilution is passed to the hemoglobinometer for the determination of hemoglobin concentration (light transmittance read at wavelength of 525 nm). MCV taken from volume distribution data. HCT, MCH and MCHC are calculated from measured and derived data. RDW is directly calculated from RBC histogram as coefficient of variation of the RBC volume distribution.

Specimen requirements

About 3-4 ml of venous blood collected into EDTA tubes.

Procedure

1. Turn ON the power switch on the front side of the analyzer.

2. Perform quality control analysis on 3 levels of control blood material (low, normal and high) to verify that the instrument is performing within the specified ranges of the quality control material. 3. Slide each sample firmly into the cassette. Ensure the bar-codes are facing up within the cassette window

4. Place the cassettes into the input buffer to the right of the SPM. The SPM automatically begins cycling the cassettes.

5. After the SPM cycles the samples, review the sample results at the System Manager

Reagents of DxH 800 Beckman Coulter

COULTER DxH Diluent Is a cyanide-free, isotonic buffered saline solution. It dilutes the specimen, is used for rinsing SPM components between sample analyses, and provides a sheath stream to transport the sample through the flow cell.

COULTER DxH Cell Lyse Is a cyanide-free CBC lytic reagent that lyses red blood cells for the white blood cell count, and works in conjunction with COULTER DxH Diluent to generate a stable hemoglobin measurement. And also used to lyse the red blood cells and discriminates nucleated red blood cells from white blood cells

DxH Diff Pack Consists of the Erythrolyse Lytic reagent and StabiLyse preservative reagent. The Erythrolyse Lytic Reagent is a cyanide-free lytic reagent that dilutes the blood sample, and lyses red blood cells in preparation for white blood cell measurement in the flow cell. The StabiLyse Preservative reagent neutralizes the Diff lytic reagent and preserves the white blood cells for measurement in the flow cell. Together, Erythrolyse and StabiLyse provide the five part differential.

DxH Retic Pack Consists of a reticulocyte stain and a reticulocyte-clearing reagent. The reticulocyte stain reagent is a cyanide-free reagent that uses a dye to stain reticulocytes. The reticulocyte-clearing reagent is a cyanide-free reagent that stabilizes the dye-reticulum complex to enhance discrimination of reticulocytes from mature red blood cells utilizing the VCS technology.

DxH Cleaner Is a cyanide-free, aldehyde-free cleaning agent that degrades residual materials so that they may be flushed from the system with the diluent

3. Cobas 6000 chemistry analyzer

Detection principle of cobas 6000

Cobas 6000 is analyzer is fully automated, random access, software controlled system for immunoassay and photometric analyses of variety of tests. The analyzer combined photometric, ion selective electrode (ISE) and electrochemiluminescence technology. The ISE quantify

electrolyte like sodium, potassium and chloride. The photometric system can measure colometric or immunoturbidometric reaction utilizing end point or kinetic rate absorbance measurement. The Cobas 6000 can utilize both manual and barcode based bidirectional interface for data entry and execution.

A. Total cholesterol

Cholesterol is measured by enzymatic colometric method. In the presence of Cholesterol ester hydrolase, cholesterol esters are cleaved and release free cholesterol and fatty acid. 3-OH group of cholesterol is oxidized by cholesterol oxidase. One of the reaction by products, H_2O_2 is measured quantitatively in a peroxidase catalyzed reaction that produces a color. Absorbance is measured at 505 nm. The method used endpoint reaction that is specific for cholesterol. The reaction sequence is as follows:

Cholesterol ester hydrolase

Cholesterol esters + H_2O ----------> cholesterol + fatty acid

Cholesterol oxidase

Cholesterol + O_2 ----------> cholest-4-en-3-one + H_2O_2

Peroxidase

$2H_2O_2$ + 4-aminophenazone + phenol ----------> 4-(p-benzoquinonemonoimino)-phenazone + 4 H_2O

B. Low-density lipoprotein cholesterol

Principle of the test

Most of the circulating cholesterol is found in three major lipoprotein fractions: very low density lipoproteins cholesterol (VLDL-C), LDL and HDL(41).

TC = VLDL-C+ LDL-C + HDL-C

LDL-C is calculated from measured values of TC, TG and HDL-C as follows:

LDL-C = TC - HDL-C- TG/5

Where TG/5 is an estimate of VLDL-C.

C. High-density lipoprotein cholesterol

Principle of the test

HDL-C is analyzed by enzymatic colorimetric method like that of TC. Lipoproteins that contain apoB excluded from the analysis, Sulfated alpha-cyclodextrin in the presence of Mg+2, forms complexes with apoB containing lipoproteins, they become non-reactive to the enzymatic cholesterol reagent. Polyethylene glycol-coupled cholesterol esterase and cholesterol oxidaseis hydrolyzed for the HDL-C measurement. Absorbance is measured at 600 nm.

ApoB containing lipoproteins + α-cyclodextrin + Mg^{+2} + dextran SO_4 ---> soluble non-reactive complexes with apoB-containing lipoproteins

HDL-C esters +PEG-cholesterol esterase ---- > HDL-unesterified cholesterol + fatty acid

unesterified cholesterol +O_2 PEG-cholesterol oxidase ----- > cholestenone +H_2O_2

H_2O_2 + 5-aminophenazone + N-ethyl-N-(3-methylphenyl) ----- >N-succinyl ethylene diamine+ H_2O + H^+ peroxidase ----- > qunoneimine dye +H_2O

D. Triglyceride

Principle of the test

Triglycerides are measured enzymatically in the serum or plasma. Dyslipidemia is hydrolyzed to produce glycerol. Glycerol is then oxidized using glycerol oxidase, and H_2O_2, one of the reaction products, is measured as at 500 nm. The reaction sequence is as follows:

Lipase

Triglycerides + $3H_2O$ ----------> glycerol + fatty acids

Glycerokinase

Glycerol + ATP ----------> glycerol-3-phosphate + ADP

Glycerophosphate oxidase

Glycerol-3-phosphate +O_2 ----------> dihydroxyacetone phosphate + H_2O_2

Peroxidase

H_2O_2+ 4-aminophenazone + 4-chlorophenol ----------> 4-(p-benzoquinone-monoimino)-

Phenazone + $2H_2O$ + HC

Annex II: English and Amharic Versions of Participant Information Sheet and Consent form

Addis Ababa University, College of Health Sciences, Department of Medical Laboratory Sciences

You are invited to participate in a study to be conducted by MSc student Serkalem Ayele at Addis Ababa University, College of Health Sciences, Department of Medical Laboratory Science. Please read the following statements and ask any unclear points before you agree to participate.

Introduction

The topic of this study is the effect of statin on Hematological parameters in patient with Dyslipidemia at St. Paul's Hospital Millennium Medical College, Addis Ababa, Ethiopia.

Participation in this study is exclusively voluntarily. If you are not interested to participate, there will be no consequences and you will get all the services provided in the hospital with no problems. If you decide to participate, you have to sign on the consent permission template form.

What is expected from me as participant of the study?

As a participant of this study, you will be requested to give small amount of blood. Blood will be collected from your arm using sterile syringe. If you are agree to give sample you will be also requested to answer for questionnaire.

Potential Risks and Discomforts

There will be some pain during collection of blood from your arm but this does not produce serious pain and not harmful to your health .The result of the study will be beneficial to put a new strategy for monitoring dyslipidemia patient progress. Hence, you are indirectly benefiting other patients and the society in this respect

Potential benefits to participant and/or to the society

The result of the study will be beneficial to put a new strategy for monitoring dyslipidemia patient progress. Hence, you are indirectly benefiting other patients and the society in this respect.

Compensation for participation

You will not receive any payment for your participation in this research study .

Confidentiality

On the request paper your name or your identities will not be mentioned. Samples and information given by the participants will serve only for this research not for any other purpose.

Person to contact

Please direct any questions or problems you may encounter during this study to the principal investigator:

Serkalem Ayele

Department of Medical Laboratory Sciences, College of Health Sciences

Addis Ababa University

Cell phone: +251920091476

Email:serkalemayele1@gmail.com

Consent Form

ID. No:

This page contains an agreement signature to participate in the study entitled "The effect of statin on Hematological parameters in patients with Dyslipidemia at St. Paul's Hospital Millennium Medical College, Addis Ababa, Ethiopia." So please read the following points and sign your signature at the end in the space provided.

I understand the objective of the study in "The effect of statin treatment on Hematological parameter of patients with Dyslipidemia at St. Paul's Hospital Millennium Medical College, Addis Ababa, Ethiopia.". I know that the information and sample (blood) that I gave are going to be used for this study only. I understand that, all the information given for the study and the results are confidential. I understand that I will not get any money for my participation. Therefore, with full understanding of the situations I agree to give the entire necessary information and blood sample for laboratory analysis.

Signature of the participant: …….. Date:

Amharic Version Participant Information Sheet and Consent Form

አዲስ አበባ ዩኒቨርሲቲ ጤና ሳይንስ ኮሌጅ የህክምና ላብራቶሪ ት/ክፍል

በአዲስ አበባ ዩኒቨርሲቲ ፣ በጤና ሳይንስ ኮሌጅ ፣ በሕክምና ላብራቶሪ ሳይንስ ዲፓርትመንት ውስጥ በኤምሲሲ ተማሪ ሰርካለም አየለ በሚካሄድ ጥናት ላይ እንዲሳተፉ ተጋብዘዋል.።እባክዎን የሚከተሉትን መግለጫዎች ያንብቡ እና ለመሳተፍ ከመስማማትዎ በፊት ማንኛውንም ግልጽ ያልሆኑ ነጥቦችን ይጠይቁ.።

መግቢያ

የዚህ ጥናት ርዕስ በ ቅዱስ ፓውሎስ ሆስፒታል ሚሊኒየም ኮሌጅ ውስጥ የሚተከሙየ ዲስሌፒዲሚያ ህመምተኞች የስታቲን ሕክምና ከመጀመሩ በኋላ የለዉ የሄሞቶሎጂ ልኬት ለዉጥ ነው። በዚህ ጥናት ውስጥ ሚሳተፉት በፈቃደኝነት ብቻ ነው. ለመሳተፍ ፍላጎት ከሌለዎት ምንም መዘዝ አይኖርም እና ምንም ችግር ሳይኖርብዎት በሆስፒታል ውስጥ የሚሰጡትን አገልግሎቶች ሁሉ ያገኛሉ።.ለመሳተፍ ከወሰኑ በፍቃድ ፈቃድ አብነት ቅጽ ላይ መፈረም አለብዎት።

የጥናቱ ተሳታፊ በመሆኔ የሚጠበቅብኝ ምንድንነዉ?

የዚህ ጥናት ተሳታፊ እንደመሆንዎ መጠን አነስተኛ መጠን ያለው ደም እንዲሰጡ ይጠየቃሉ ፣ አዲስ መርፌን በመጠቀም ደም ከእጅዎ ይሰበሰባል ናሙና ለመስጠት ከተስማሙ በተጨማሪ ለጥያቄዎች መልስ እንዲሰጡ ይጠየቃሉ።

በዚህ ጥናት መሳተፍ የሚያስከትሊኣቸው ችግሮች

ከእጅዎ ደም በሚሰበሰብበት ጊዜ አንዳንድ ህመም ሊኖር ይችላል ነገር ግን ይህ ከባድ ህመም አያመጣም እንዲሁም ለጤናዎ ጎጂ አይደለም ።

በዚህጥናት መሳተፍ የሚያስገኛቸው ጥቅሞች

የጥናቱ ውጤት የ ዲስሌፒዲሚያ ታካሚ ሂደትን ለመቆጣጠር አዲስ ስትራቴጂ ለማስቀመጥ ጠቃሚ ነው። ስለሆነም በተዘዋዋሪ መንገድ ሌሎች በሽተኞችን እና ህብረተሰቡን በዚህ ረገድ እየተጠቀሙ ነው.።

ለተሳትፎ ክፍያ

በዚህ የምርምር ጥናት ውስጥ ለመሳተፍ ምንም ክፍያ አይቀበሉም

ምስጢራዊነት

በጥያቄ ወረቀቱ ላይ ስምዎ ወይም ማንነትዎ አይጠቀሱም. በተሳታፊዎች የተሰጡ ናሙናዎች እና መረጃዎች ለዚህ ምርምር ዓላማ ብቻ ያገለግላሉ።

ሰው ለማነጋገር

እባክዎን በዚህ ጥናት ወቅት ሊያጋጥሟቸው የሚችሏቸውን ማናቸውም ጥያቄዎች ወይም ችግሮች ለዋና መርማሪው ይምሩ-:

ሰርካለም አየለ

የህክምና ላብራቶሪ ሳይንስ ትምህርት ክፍል ፣ የጤና ሳይንስ ኮሌጅ

አዲስ አበባ ዩኒቨርሲቲ

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መጠየቅያ ቅጽ

የጥናቱ ተሳታፊ መለያ ያቁጥር፡

ይህ ገጽ በ ቅዱስ ፓውሎስ ሆስፒታል ሚሊኒየም ኮሌጅ ውስጥ የሚተከሙየ ዲስሌፒዲሚያ ህመምተኞች የስታቲን ሕክምና ከመጀመሩ በኋላ የለዉ የሄሞቶሎጂ ልኬት ና ከ ሊፒድ ልኬት ለዉጥ ነው። ጥናት ላይ ለመሳተፍ የስምምነት ፊርማ ሚፈርሙበት ነዉ ። ስለዚህ እባክዎን የሚከተሉትን ነጥቦች ያንብቡ እና በመጨረሻው ቦታ ላይ ፊርማዎን ይፈርሙ

በ ቅዱስ ፓውሎስ ሆስፒታል ሚሊኒየም ኮሌጅ ውስጥ የሚተከሙየ ዲስሌፒዲሚያ ህመምተኞች የስታቲን ሕክምና ከመጀመሩ በኋላ የለዉ የሄሞቶሎጂ ልኬት ለዉጥ ነው። የጥናት ዓላማውን ተረድቻለሁ ።

የሰጠሁት መረጃ እና ናሙና (የደም) ለዚህ ጥናት ብቻ ጥቅም ላይ እንደሚውል አውቃለሁ።

ለጥናቱ የተሰጠው መረጃ እና ውጤቶቹ ሁሉ ሚስጥራዊ መሆናቸውንተረድቻለሁ።

ለተሳትፎዬ ምንም ገንዘብ እንደማላገኝ ተረድቻለሁ።

ስለዚህ ስለሁኔታዎቹ ሙሉ ግንዛቤ በማግኘት የላቦራቶሪ አስፈላጊ መረጃዎችን እና የደም ናሙና ለላቦራቶሪ ምርመራ ለመስጠት እስማማለሁ።

የተሳታፊው ፊርማ ፡ ቀን፡

Annex III: English and Amharic Versions Questionnaire

English version of questionnaire to be filled by data collector

Part one: general information

Card number…….. Patient identification numbers …… date……..

Part two: sociodemographic and clinical questions		
No	Questions	Response
1	age	…….
2	Sex	A .Male B. Female
3	Educational level	A. Unable to read and write B. Primary school C. High school D. Diploma and above
4	Residence	A. Urban B. Rural

5	Occupation	A. Governmental employee B. Se1f employee C. Daily worker D. Private business owner E. Other, specify
6	Are there any medication that you are taking other than statin/ lipid drug lowering drug?	A .Yes B. No
7	If you say yes for question no 6, which type of drug are you taking?	……..
8	For how long you have been taking statin/ lipid lowering drug/you started follow up ?	……..
9	Which type of statin/lipid lowering drug are you taking?	……..
10	Do you have diabetes?	A. Yes B .No
11	Do you have hypertension?	A .Yes B .No

Part three : your nutritional habit and life style

12	Did you take cigarette in past 1 year?	A .Yes B .No
13	Do you drink alcohol?	A .Yes B .No
14	IF you say yes for question number 13, how many glasses per week do you drink?	…….
15	Do you chew khat currently?	A .Yes B .No
16	Have you been engaged in physical activity?	A .Yes B .No
17	If yes, for question number 16, for how long do you exercise per day	….
18	Do you have a Fasting habit?	A .Yes B .No
19	If Yes, how is your fasting habit?	A. Eating vegetable food-only

		B. Complete abstinence from food then eating all kinds of food C. Complete abstinence from food then eating vegetable food-only.			
20	put" 0" for your choice Food types	Once/day	More than Once/ day	Occasionally (holidays)	never
	Roots				
21	Legumes				
22	Cereals				
23	Vegetables				
24	Fruits				
25	Meat				
26	Egg				
27	Milk and milk product				
28	Coffee and tea				

Amharic Version of Questionnaire

ክፍል1. አጠቃላይ መረጃ

ካርድ ቁጥር ፡ መለያ ቁጥር፡

ቀን፡

ክፍል2. ጠያቂ ስለ ማህበራዊ ስነህዝብ እና ክሊኒካዊ ሁኒታዉች		
ቁጥር	ጥያቄ	ምላሽ
1	እድሜ	……

2	ጾታ	…….
3	የትምህርትደረጃ	ሀ.ማንበብ ና መጻፍ የማይችል ለ. የመጀመሪያ ደረጃ ሐ. ሁለተኛ ደረጃ ና ከዛ በለይ መ. ዲፕሎማ ና ከዛ በለይ
4	የመኖሪያ ቦታ	ሀ. ከተማ ለ.ገጠር
5	ሥራ	ሀ. የመንግስት ሠራተኛ ለ. የግል ተቀጣሪ ሐ. የግል ሰራ መ. ሌላ ካለ ይግለፁ……..
6	ከ ስታቲን(የ ኮሌስትሮል መቀነሳ) ዉጭ ሌላ መድሃኒት ይወስዳሉ?	ሀ. አዎ ለ. አይደለም
7	ጥያቀ ቁትር 6 መልሶ አዎ ከ ሆነ፤ ምነ ዓይነት መድሃኒት ይወስዳሉ ?	…….
8	ስታቲን (የ ኮሌስትሮል መቀነሳ) መዉሰድ ወይም ለኮሌስትሮል ክትትል ከ ጀመሩ ምን ያህል ጊዜ ሆኖት?	……
9	የትኛውን ዓይነት . ስታቲን ነው እየወሰዱ ያሉት	……
10	የስኪር በሽታ አለበዎት	ሀ. አዎ ለ. አይደለም
11	የደም ግፊት ህመም አለብዎተ	ሀ. አዎ ለ. አይደለም

ክፍል3. የ አመጋገብ እና የህይወት ልምድዎ

12	ባለፈው 1 አመት ዉስጥ ሲጋራ ወስደዋል?	ሀ. አዎ

		ለ. አይደለም
13	አልኮል ያለው መጠጥ ይጠጣሉ ?	ሀ. አዎ ለ. አይደለም
14	ለ ጥያቀ ቁትር 13መልሶ አዎ ከ ሆነ፤ በ ሳምንት ምን ያህል ብርቸጭቆአልኮል ያለው መጠጥ ይጠጣሉ?	……
15	ጫት ይቅማሉ?	ሀ. አዎ ለ. አይደለም
16	የ አካል ብቃት ህንቅስቃ ያደርጋሉ?	ሀ. አዎ ለ. አይደለም
17	ለ ጥያቀ ቁትር 16 መልሶ አዎ ከ ሆነ፤ በ ከቀን ለምን ያህል ጊዜ ይንቀሳቀሳሉ	……..
18	የ መፆም ልምድ አለዎት?	ሀ. አዎ ለ. አይደለም
19	መልስዎአዎንከሆነ፣የመፆምልምድዎእንዴትነው	ሀ. አትክልቶችን ብቻ መመገብ ለ. በአጠቃላይ ከምግብ መታቀብ ከዚያም ያገኙትን መመገብ ሐ.በአጠቃላይከምግብመታቀብከያም አትክልቶችን መመገብ

በ መረጡት ቦታ ላይ የ “0 “ምልክት ያሰክመቱ	የምግብዓይነት	በቀንአንድጊዜ	በቀንከአንድጊዜ በላይ	አልፎአልፎ	ተጠቅሜአላውቅም
20	ሥራሥር				
21	አዝርት				

22	ጥራጥሬ				
23	አትክልት				
24	ፍራፍሬ				
25	ሥጋ				
26	ወተትና የወተት ተዋፅዎ				
27	እንቁላል				
28	ሻይ እና/ወይም ቡና				

Annex IV: Declaration

I, the undersigned agree to accept responsibility for the scientific ethical and technical conduct of the research project and for provision of required progress reports as per terms and conditions of the research publications office.

M.Sc. candidate: Serkalem Ayele (B.Sc.)

Signature: _____

Date of submission: _____

This thesis has been submitted with our approval as advisors.

Advisor: Fekadu urgessa (MSc, PhD Fellow)

Signature: _____

Date: _____

Place: Addis Ababa, Ethiopia.

Advisor: Melatwork Tibebu (MSc, PhD Fellow)

Signature: _____

Date: _____

Place: Addis Ababa, Ethiopia.