Training Report

Internship Training Report

Submitted to

Lovely Professional University, Punjab

in partial fulfillment of the requirements

For the degree of

Master of Science in Clinical Biochemistry

Submitted by:
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(Reg. No 11400527)

SCHOOL OF PHYSIOTHERAPY AND PARAMEDICAL SCIENCES
LOVELY PROFESSIONAL UNIVERSITY, PUNJAB, INDIA
May, 2016

CERTIFICATE

This is to certify that **Mr./*Ms Ajay Kumar*** bearing **Registration Number *11400527*** has completed his/her Master of Science in Clinical Biochemistry internship under our guidance and supervision. This report is record of the candidate own work carried out by him/her under my supervision. I certify that the matter embodied in this report is original and has been not submitted anywhere for the reward of any other degree.

Internal Supervisor	***External Supervisor***
Dr. Pranav Kumar Prabhakar	Dr. Satish Arora
Date:	Date:
Lovely Professional University	Arora Nursing Home,
Phagwara,Punjab	Phillaur, Punjab,

DECLARATION

I hereby declare that the work embodied in this internship report was carried by me under the supervision of Dr. Pranav Kumar Prabhakar (Internal Supervisor), Lovely Professional University and Dr Satish Arora (External Supervisor), Arora Nursing Home. This work has not been submitted in part or in full in any other university for any degree or diploma.

Name: Ajay Kumar

Date: …….

Place: Lovely Professional University,

Phagwara Punjab.

ABSTRACT

OBJECTIVE

Life style modifications have direct impact on reducing the risk of cardiovascular diseases. No long term data available which comparing the impact of life style modifications alone or with medication. Higher consumption of whole grains and complex carbohydrates, vegetables, less fat in food and low fat dairy products shows significant reduction in lipid level. We studied 105 subjects to examine impact of dietary modification and exercise alone and with medication on the level of various lipids like cholesterol, triglyceride, LDL-C, VLDL-C, HDL-C, cholesterol: HDL-C ratio.

STATE OF PURPOSE

The purpose of research is: about testing the actual impact of dietary changes and daily light exercise on the level of lipids alone or also in the presence of medication to reduce the risk of cardiovascular diseases.

This study is good for the betterment of society also; as if we found significant reduction with life style modifications only then we can save lot of lives from death by CVDs.

To compare the amount of changes alone by life style modification or with combination of medication and life style changes.

To proved given hypothesis for applying it to the real situations.

HYPOTHESIS

To reduce the risk of CVDs we need to reduce level of lipids in the circulation. For that I designed a study in which I'll select some subjects and categorize them into three groups. First group is of control subjects. Subjects in second group will follow life style modification like dietary modifications and physical exercise. Subjects of group three follow life style modification along with medication. Control group show no or very little changes in level of lipids, whereas group II and group III will show reduction in the level of total cholesterol, triglyceride, LDL-C, VLDL-C, cholesterol: HDL-C ratio and increase in the level of HDL-C. Changes in group III will be more as compare to group II due to medication along with life style modification.

MATERIAL AND METHODS

All the kits used for the estimation of Total cholesterol, triglyceride and High density lipoprotein cholesterol purchased from Medsource Ozone Biomedicals Pvt. Ltd., Faridabad, India. Subjects for this study were searched by visiting from one village to another and total 163 volunteer individuals participated. Out of these 163 only 105 selected. 58 subjects rejected because of more Age, heavy alcoholic, and heavy smoker. All the candidates fall in age group of 40-80 years. After selection of all the subjects, their samples were collected to check level of lipids in their blood. Then on the basis of level of lipids, selected individuals were divided into three groups. All normal 22 healthy subjects shifted to group I (control healthy subjects), 36 subjects selected in group II (following dietary modification and physical exercise) and 47 subjects in group III (taking medicine of statin family along with dietary modifications and physical exercise). Then level of lipids checked in their blood after the interval of 20-25 days, four times in 16 weeks. Biochemical examinations were performed on in all four phase to examine change in the level of lipids by collecting blood samples. CHOD-POD/ phosphotungstate method was used for the estimation of total cholesterol and HDL-C in blood. Triglyceride was estimated in serum by GPO-POD method with TBHA as chromogen. VLDL-C, LDL-C, and cholesterol: HDL-C was calculated by using calculative formula. From the collected data mean and S.D. of all phases in different lipids was calculated using MS excel 2007 and then this data presented graphically using MS excel 2007.

RESULT

After the period of 16 weeks subjects of group I, group II and group III had great percentage of changes in the level of various lipids like total cholesterol (-0.37% in group I from 189.83 to 189.13 mg/dl, -11.74% in group II from 237.33 to 209.47 mg/dl, and -14.70% in group III from 247.98 to 211.57 mg/dl), triglyceride (-0.66% in group I from 131.65 to 130.78 mg/dl, -17.21 % in group II from 201.81 to 167.08 mg/dl, and -19.21% group III from 244.82 to 197.78 mg/dl), LDL-C (+0.38% in group I from 177.53 to 178.21, -17.13% in group II from 129.86 to 107.61 mg/dl, and -24.82% in group III from 143.04 to 107.54 mg/dl), VLDL-C (0% in group I from 26.30 to 26.30, -16.95% in group II from 40.47 to 33.61 mg/dl, and -21.87% in group III from 50.65 to 39.57 mg/dl), HDL-C (+2.75% in group I from 61.87 to 63.57 mg/dl, +0.82% in group II from 68.22 to 68.78 mg/dl, and, +18.7% in group III from 54.28 to 64.43 mg/dl), and

cholesterol: HDL ratio (-3.87% in group I from 3.10 to 2.98 mg/dl, -11.88% in group II from 3.45 to 3.04 mg/dl, and -30.44% in group III from 4.73 to 3.29 mg/dl).

CONCLUSION

From this study it's clear that life style modification shown its impact on reduction level of cholesterol, triglyceride, LDL-C and VLDL-C, but it fail to increase level of HDL-C. On addition of medicine along with life style modification the reduction in the level of cholesterol, triglyceride, LDL-C and VLDL-C increased sharply and it also increases level of HDL-C.

So if a person improves his/her lifestyle they can reduce the risk of CVD.

ACKNOWLEDGEMENT

It is my pleasure to be indebted to various people, who directly or indirectly contributed in the development of this work and who influenced my thinking, behavior, and acts during the course of the study.

I express my sincere gratitude to our head of department **of Lovely Professional University** Mr. Gurinder Singh for providing an opportunity to undergo this project work at **Arora Nursing Home**, Phillaur, Punjab.

I am thankful to Dr Satish Arora (M.S. Surgeon) and all the staff of Arora Nursing Home for their support, co-operation and motivation provided to me during this session for constant inspiration, presence and blessings.

I also extend my sincere appreciation to Dr. Pranav Kumar Prabhakar who provided his valuable suggestion and precious time in accomplishing my project report.

Lastly, I would like to thank all the almighty and my parents for their moral support and my friends with whom I shared my day to day experience and received lots of suggestion that improved my quality of work.

Ajay Kumar

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LIST OF ABBREVIATION

1. CVD ……Coronary Heart Disease
2. ER ……...……Endoplasmic Reticulum
3. VLDL ……..Very Low Density Lipoprotein
4. LDL ……..Low Density Lipoprotein
5. HDL …….High Density Lipoprotein
6. TG …….Triglyceride
7. HMG CoA ……..3-hydroxy-3-methylglutaryl-coenzyme A
8. AHA ……American Heart Association
9. TC ……..Total Cholesterol
10. POD …….Peroxidase
11. CHOD ……..Cholesterol Oxidase
12. CHE ……Cholesterol Esterases
13. H_2O_2 ……..Hydrogen Peroxide
14. AAP …….Amino Antipyrine
15. LPL …….Lipoprotein Lipase
16. GK ……...Glycerol Kinase
17. ATP ……Adenosine Triphosphate
18. G-3-P ……..Glycerol-3-Phosphate
19. GPO …….Glycerol-3-Phosphate Oxidase
20. HCL ……Hydrochloric Acid
21. SD ……..Standard Deviation

CHAPTER 1
INTRODUCTION

1.1CARDIOVASCULAR DISEASE: A LIFE THREATENING DISEASE

Cardiovascular disease is a main cause of death worldwide especially in USA. According to data of National Center for Health Statistics, out of total 24,71,984 deaths, 6,16,828 were caused by heart disease in the United States in 2008 (1). Hyperlipidemia is a common risk factor for the development of CVD (cardiovascular disease). Hyperlipidemia is not only mean rise in cholesterol and triglyceride; it also includes other lipids which rarely produce harmful effects. Prolong hyperlipidemia can lead to atherosclerosis (2). Mainly cholesterol and triglyceride is responsible for CVD. Normally cholesterol involved in structure and function of the cells whereas triglyceride used as immediately for energy or stored in fat cells of body (3).

1.2ORIGIN OF TRIGLYCERIDES IN BODY

Diet and liver these are two main sources from where body gets triglyceride. Triglyceride in diet is hydrolyzed by pancreatic lipase into monoacylglycerol and fatty acids, which along with bile salt form micelles which then enter into enterocytes by simple diffusion. This then form triglyceride in ER (endoplasmic reticulum) and then packed by Golgi bodies to form chylomicron. Triglyceride in enterocytes along with cholesterol, phospholipids and Apo protein B48 form chylomicron, which enter into blood stream via lymphatic system (figure 1) (4).

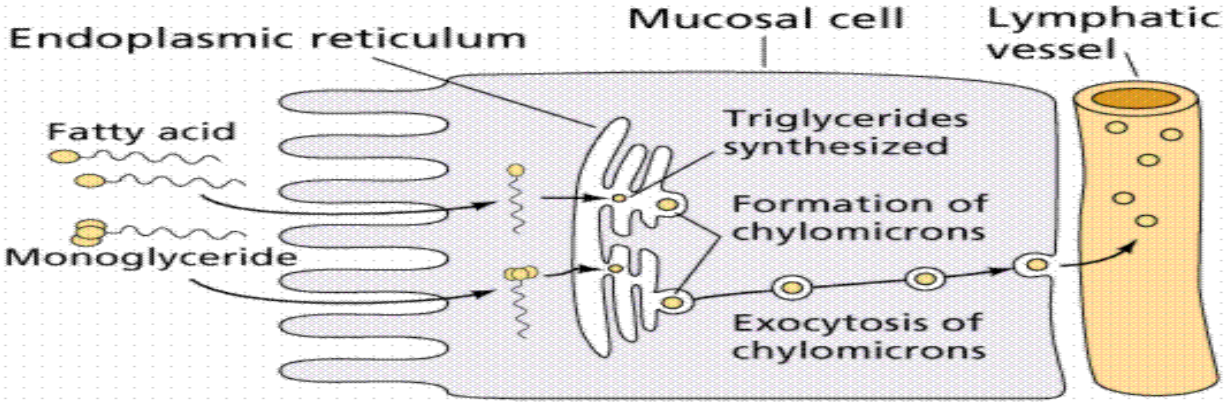

Figure 1: Transportation of fatty acids and monoacylglycerol into enterocytes, synthesis of TG in endoplasmic reticulum, formation of chylomicron, and transportation of chylomicron via lymphatic system.

The synthesis of triglyceride in liver was described more than 40 years ago by Kennedy and coworkers. In liver triglyceride synthesized from an intermediate product of carbohydrate metabolism which is glycerol 3 phosphates. This glycerol-3-phosphate form phosphatidic acid which then converted into triglyceride (5). In first step glycerol-3-phosphate is converted into lysophosphatidic acid in the presence of enzyme glycerol-3-phosphate acyltransferase in mitochondria and endoplasmic reticulum (6).This lysophosphatidic acid then esterifies into phosphatidic acid and reaction catalysed by lysophosphatidate acyltransferase (figure 2). This enzyme found in ER. Then this phosphatidic acid converted into triacylglycerol (7).

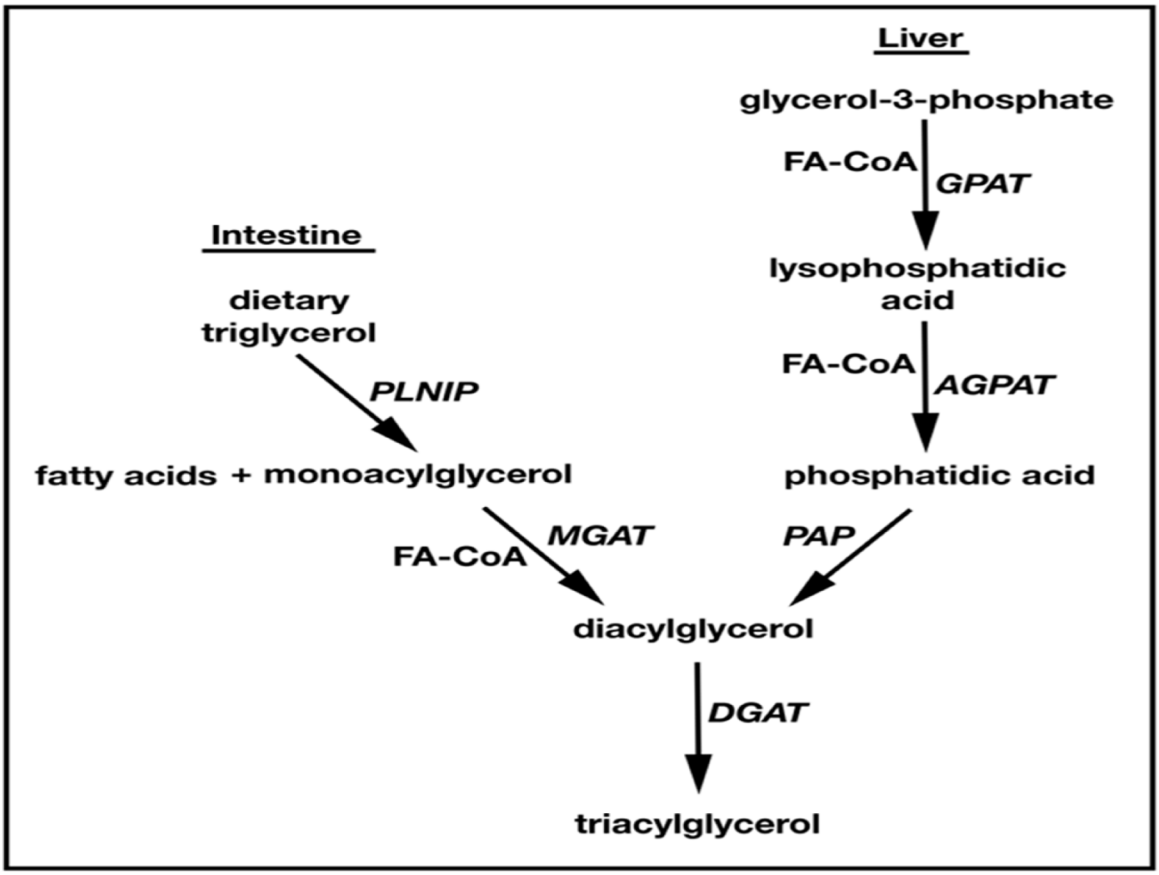

Figure 2: Synthesis of triglyceride in liver from carbohydrate metabolite glycerol-3-phosphate and entry if dietary triacyglycerol into circulation.

1.3ORIGIN OF CHOLESTEROL IN BODY

However, cholesterol is an important component of our body but increased level of circulating cholesterol increases risk of cardiovascular diseases. It account for 1 out of 3 deaths in USA (8). Liver play important role in homeostasis of all types of cholesterols.

Diet, extra hepatic tissues and liver are the main sources of cholesterol supply to body for its routine functioning. Other lipoproteins such as VLDL-C (very low density lipoprotein), LDL-C (low density lipoprotein) and HDL-C (High density lipoprotein) synthesized in liver using phospholipids, cholesterol, triglyceride and Apo proteins as a precursor (figure 3).

Unhealthy lifestyle including more consumption of cholesterol and saturated fat in diet and less physical activities can lead to change in the level of various types of cholesterols (like HDL, LDL, & VLDL) and triglyceride (9). Not only this, heavy intake of alcohol, overweight, diabetes, and underactive thyroid gland, can also increase cholesterol level in circulation (10). Three main factors responsible for hyperlipidemia are: Nutrition, metabolic diseases and genetics.

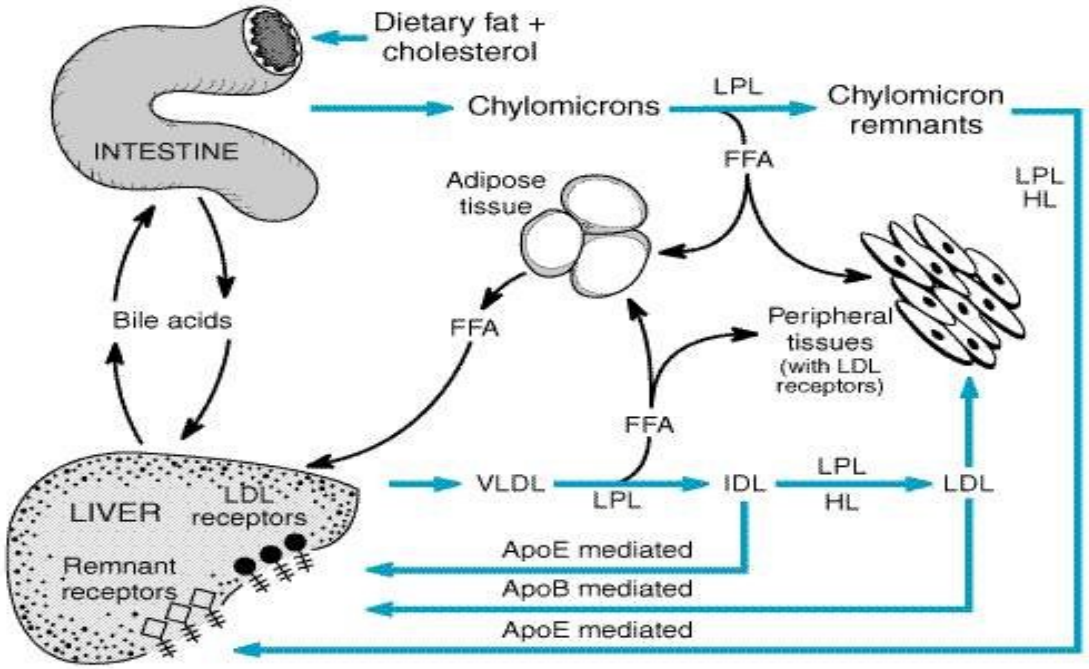

Figure 3: Synthesis of chylomicron and its transportation, synthesis of VLDL, and LDL and its removal from circulation.

1.4PATHOPHYSIOLOGY OF HYPERLIPIDEMIA CAUSING CVD

Pathophysiology of hyperlipidemia classified into two types: primary hyperlipidemia and secondary hyperlipidemia. Primary hyperlipidemia is caused due to genetic defect in lipid metabolism due to deficiency of lipoprotein lipase and absence of surface apoprotein Cll. This defect in lipid metabolism can lead to hypertriglyceridemia and hyperchylomicronemia (11).

Secondary hyperlipidemia caused due to secondary diseases like diabetes and hypothyroidism. These secondary diseases lower activity of lipoprotein lipase which increase level of VLDL in circulation. Hypothyroidism also lowers hepatic degradation of cholesterol which further increases cholesterol level in blood (12-13).

1.5 THERAPEUTIC TREATMENT OF HYPERLIPIDEMIA

Presently five different classes of drugs are used to treat hyperlipidemia are: statin, nicotinic acid derivatives, fibric acid derivatives, bile acid binding resins and cholesterol absorption inhibitors (14-15).Out of them statin is most commonly used drug which inhibit enzyme HMG CoA reductase and lower synthesis of cholesterol in liver. Moreover, statin also promotes excretion of cholesterol via liver (16). It promotes excretion of LDL by up regulation of LDL receptors which bind to the apoB on the surface of LDL particles and promote internalization of LDL to liver for excretion (17). Statin also lower TG level as statin reduces synthesis of Triglyceride and also increase excretion of TG rich LDL. By reducing LDL indirectly, statin also lower triglyceride level as shown in figure 4.

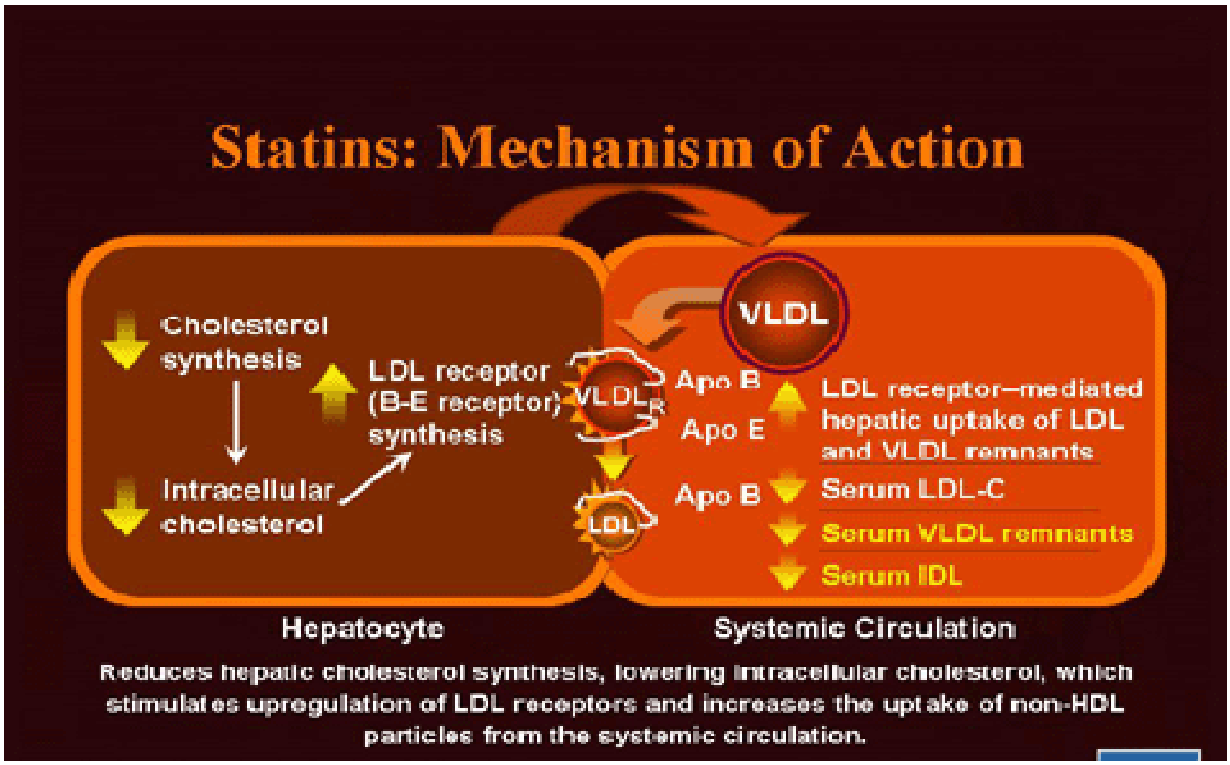

Figure 4: Mechanism of action of statin drug on cholesterol synthesis, on synthesis of LDL receptor and its overall impact on level of various lipids.

1.6LIFE STYLE MODIFICATION SHOWS REDUCTION IN LEVEL OF LIPIDS

Not only drugs but life style modifications also have significant role in reduction of lipid level in blood. These life style modifications included modification in dietary components and routine exercise (18). According to the Canadian cholesterol guidelines, lifestyle changes remain the cornerstone for the treatment of dyslipidemia and prevention of CVD (19). Lifestyle modifications are good, safe and cost effective method of lipid lowering.

Recently, various researches also directed towards understanding of different dietary compositions and their impact on lipid profiles to lower CVD risk. Based on these trends, American Heart Association (AHA) providing dietary guidelines that endorse the higher consumption of whole grains and complex carbohydrates, vegetables and low fat dairy products shows significant reduction in lipid level. Also recommended is the consumption of foods which are lower in fat content (20-21).

Even weight loss alone is sufficient to lower risk of CVD by lowering triglyceride level and raising HDL level. Body weight can be reduced with change in dietary habits with or without physical activities, but physical activity increases the rate of recovery from high triglyceride and cholesterol (22).Caloric restriction is the best way to lower body weight than restriction macronutrients. As research shows that restricted carbohydrate diet has similar effect on body weight when compared with low carbohydrate diet. So there is no need of restricting full diet (23-24). Some studies also reported significant reduction in lipid level with low fat and high protein containing diet (25-26).

Most of plant food consists of fibers, either soluble or insoluble. Study shows that soluble fibers are more effective in reducing LDL-C level. Soluble fibers reduce the amount of bile reabsorbed be intestine and excreted this bile into stool. To compensate this loss liver started to make more bile, for this purpose liver used cholesterol. This cholesterol is taken from LDL-C. To get more and more LDL-C liver increases expression of specific receptors for LDL-C. In this way soluble fiber reduces LDL-C level (27).

As we know VLDL and LDL synthesized in liver by using triglyceride and cholesterol, but due to fewer intakes of dietary fat and carbohydrate triglyceride and cholesterol are not available in sufficient amount to synthesize LDL and VLDL.

Due to reduction in the level of chylomicron and VLDL now level of cholesterol also reduced in extra hepatic tissues. The remaining cholesterol which is already present in extra hepatic tissue is also transported by HDL-C to liver and reduced level of free cholesterol in circulation.

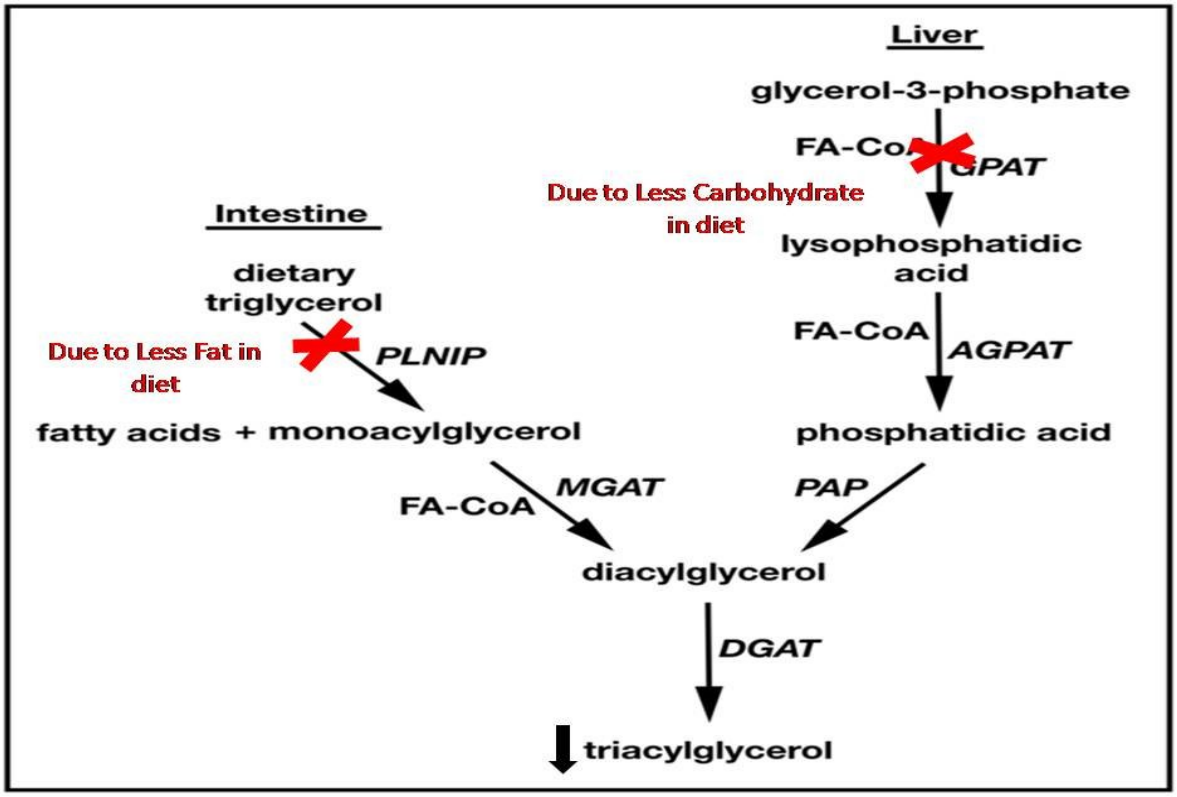

Figure 5: Mode of action of life style changes in reducing triglyceride level by blocking its synthesis.

Regular physical activities alter metabolism of cholesterol in positive way. Exercise enhances synthesis and activity of several enzymes which enhances reverse transport of cholesterol. Exact mechanism is still unclear, but evidence shows that physical activity along with other factors like dietary modifications, weight loss affects enzyme and hormone activity to alter rate of lipid synthesis, and reverse transport of cholesterol from blood for its clearance (28).

CHAPTER 2

REVIEW OF LITERATURE

2.1EPIDEMIOLOGY

A study was conducted by Melanie Nichols et al; in 2014 about epidemiology of CVD in Europe. They collected comparable data from various European and international data sources. This study gives information that more than four million Europeans die every year just because of CVD (29).

Another study on epidemiology of cardiovascular disease in UK 2014 was conducted by Prachi Bhatnagaret al; in 2014. This study also shown high burden of CVD in UK. Rate of death due to CVD is high in women than men. 28% women died just because of CVD but not the men (30).

2.2HISTORY OF CVD

Nikolai N. Anichkov (1885–1964) first demonstrates the role of cholesterol in development of atherosclerosis. This is one of the greatest discovery in 20th century (31). Felix Marchand first introduces the term atherosclerosis in 1904 and also mentioned that atherosclerosis causes blockage of arteries (32)

In 1910, Adolf Windaus proved that atheromatous lesions show 6 and 20 times more free and esterified cholesterol than normal arteries. Then in 1913 Nikolai N. Anichkov showed changes in vascular walls caused by cholesterol (32).

To find out historical evidences of atherosclerosis Adel H. Allam, MD et al; conducted a study on Egyptian mummies to find atherosclerosis in them. They performed whole body computed tomography scanning of 52 mummies and 20 out of them showed evidences of atherosclerosis (33).

2.3CAUSES OF CVD

Cholesterol and triglyceride both are major risk factors for cardiovascular diseases. A study was conducted by Juha Pekkanen, M.D., PhD et al; to find out relation between total cholesterol and CVD. They selected 2541 men and observed then for more than 10 years.

They found persons having high cholesterol level have 3.45 times high risk of death due to cardiovascular diseases than a person having desirable cholesterol level (34).

Plasma triglyceride level is a risk factor for cardiovascular diseases. This was shown by John E. Hokanson et al; in his study.

2.4IMPACT OF DIET AND EXERCISE ON LIPID LEVEL

A study was conducted by David J Decewicz et al; on 73 participants (34 women and 39 men), out of them 36 were diseased and 37 were control. All these individuals gone through diet change, exercise, stress management, and group support. In this study only diseased individuals were selected which diagnosed with coronary artery disease, stable angina, angioplasty, evidence of 50% narrowing of lumen of blood vessels, acute myocardial infarction, stunt placement, bypass surgery, high blood pressure and cholesterol level more than 200 mg/dl. A low fat containing vegetarian diet was given to them along with 180 min/week moderate exercise for 52 weeks. All life style interventions reduced level of all parameters responsible for CVD. After 52 weeks total 8.3% LDL reduction found (35).

Another study was conducted by R. James Barnard, PhD et al; on 4587 patients, who attended 3 week life style modification program having high amount of complex carbohydrate, high fiber, low fat, and low cholesterol diet with daily exercise. At the end 23% reduction reported in total cholesterol. More reduction was observed in males than in females, as total cholesterol in male -24.4% or in female 20.8% and LDL-C in males 25% or in females 19.4%. 33% reduction in serum triglyceride also reported (36).

Study was conducted by Umamaheswari Kannanet al; on 52 obese adults. All adults performed simple exercise of 20 minutes each day in at least 5days of a week for 15 weeks. At the end researches found significant reduction in LDL-C (37).

A study was conducted in diabetic clinic of J.A. Hospital, G.R. Medical College, Gwalior, M.P, India by Neetu Mishra on 67 diabetic patients. They classified them into three categories. First healthy control (50 patients), second group contain 17 subject who controlled their diabetes with exercise and third group contain 50 patient controlling their diabetes with medicine. At the end they found significant glycemic control and reduction in lipid levels (table 1) (38).

Table 1: Showing reduction in the level of various lipids during a study by Neetu Mishra in 67 subjects.

	Group 1	Group 2	Group 3
Triglyceride	1.44 %	2.30 %	2.37 %
Total Cholesterol	4.79 %	6.06 %	6.08 %
HDL-C	1.01 %	0.68 %	0.75 %
VLDL-C	0.65 %	1.05 %	1.10 %
LDL-C	3.13 %	4.36 %	4.19 %

Hegsted, D. M et al. conducted a study on 2 groups of men with serum cholesterol within 200 – 300 mg/dl to measure Quantitative effects of dietary fat on serum cholesterol in man. They gave equal amount of fat to all the men and then at the end of four weeks they estimated cholesterol level in blood. They found 100 mg of fat in the diet increased up to 5% cholesterol level (39).

Another study by Howard BV et al; in 1993-1998 also explained that, dietary intervention, intended to be low in fat and high in vegetables, fruits, and grains can reduce CVD risk. 48.835 postmenopausal women joined this study. 19,541 [40%] subjects assigned to intervention or 29,294 [60%] comparison group. A low-density lipoprotein cholesterol level was significantly reduced by 3.55 mg/dl; levels of high-density lipoprotein cholesterol, and triglycerides did not significantly differ in the intervention vs. comparison groups. Thus this study shows that, diet had no significant effects on incidence of CHD (40).

A study was conducted by to evaluate the effects of the National Cholesterol Education Program's dietary interventions on major cardiovascular disease risk factors by Shaomei Yu-Poth et al; Dietary interventions significantly decreased plasma lipids and lipoproteins. Plasma total cholesterol (TC), LDL cholesterol, triacylglycerol, and TC: HDL cholesterol decreased by 0.63 mmol/L (10%), 0.49 mmol/L (12%), 0.17 mmol/L (8%), and 0.50 (10%) (41).

CHAPTER 3

OBJECTIVES

The general objectives of the research are highlighted in the following points:

- As title of the research suggest, the major motto of the research is to inculcate the impact of the dietary changes and light workout daily on the cholesterol level in an individual. Collectively, it's a project to highlight if an individual can reduce the chances of CVD by the dietary changes and light exercise rather than the depending completely on the medication.
- Besides, the project can also be enlightened for the objective of comparing before and after result of dietary changes and exercise.
- To describe the regulation of different factors of lipids in the body, generally the metabolism and their importance and adverse effects in human body simultaneously.
- To spread the knowledge regarding effects of cholesterol/ lipid in human health, the methods of their management and the implementation of procedure of its management.
- To elaborate the information on CVDs and their causes, prevention and treatment.
- To illustrate the mechanical effects of exercise on the metabolism and regulation of cholesterol level in the body.
- To define different dietary management that can be effective in the control of irregular lipid profile that can lead to the CVDs.
- To summarize every details used in the project for the extraction of proper result and conclusion.

CHAPTER 4

METHOD AND MATERIALS

All the kits used for the estimation of Total cholesterol, triglyceride and High density lipoprotein cholesterol purchased from Medsource Ozone Biomedicals Pvt. Ltd., Faridabad, India. Disposable syringes for the collection of samples were purchased from Nihal Healthcare, Solan, Himachal Pradesh, India. All other required plasticware and instruments provided by Arora Nursing Home, Phillaur, Punjab, India.

4.1SELECTION OF SUBJECTS

This project work was divided into four phases. In each phase samples were collected at regular interval to access change in the level of lipids. Subjects for this study were searched by visiting from one village to another and total 163 volunteer individuals participated. These subjects belongs to different categories like normal healthy subjects, Some with known high cholesterol and TG history, Some with unknown high TG and cholesterol history, and Some with cardiac surgeries. Both males and female candidates involved in it. All the candidates fall in age group of 40-80 years. Out of these 163 only 105 selected. 58 subjects rejected because of more Age, heavy alcoholic, and heavy smoker.

Data of all 163 patients was collected on a form with their signature on consent, which included their name, Age, Gender, address, weight and occupation in first section. Then information related to their diseases, history of surgery, medicines and diet they are taking was collected. Then consent was taken weather they are interested to follow all the diet and exercise plans or not with their signatures. Out of which 56 females and 49 males were selected. Each subject allotted with unique identity number. After selection of all the subjects, their samples were collected to check level of lipids in their blood. Then on the basis of level of lipids, selected individuals were divided into three groups. All normal 22 healthy subjects shifted to group I, 36 patients selected in group II and 47 subjects in group III. Group I included normal healthy individuals, group II included those patient which are having slightly high cholesterol and TG and previously not taking any kind of medicine and group III contain those individuals which are having either high TG, high cholesterol or both. Out of these 47 subjects some were already taking lipid controlling medicine and rest were prescribed by physician.

Almost all the subjects were taking tablets of statin family to control their lipid level. A Diet plan and exercise was planned for all the subjects of group II and group III. Diet plan included consumption of less fat, oil, non veg., more intake of high fiber food, high protein diet and less carbohydrate than as usually. Normal milk was also replaced with skimmed milk. Diet contains boiled vegetables, fruits, rice (once in a day), grains, pulses without oil, and dairy products prepared from skimmed milk.

All subject of group II and group III were instructed to do moderate aerobic exercise including walking and cycling at least 30 minutes in a day or 6 days of a week. Group II is only following Diet plan and exercise but group III also taking medicine along with diet plan and exercise. Then level of lipids checked in their blood after the interval of 20-25 days, four times in 16 weeks. And then whole data was arranged in tabular form. Samples were collected by personal visits to each subject. Subjects were also observed on regular basis with personal visit weather they are actually following diet and exercise plan or not, regarding this information was also collected from their neighbors, family members and colleagues. All the samples were collected in total 4 phases. Samples collected in first phase before categorization of subjects into groups. Then samples of second, third and fourth phase at the interval of 20-25 days.

Plain fasting samples were collected and transported to the laboratory within 2-3 hour after the collection. Out of all, one subject skipped this study after first phase.

4.2BIOCHEMICAL STUDY OF LIPID LEVEL

Biochemical examinations were performed on 2^{nd}-4^{th} week in first phase, 6^{th}-8^{th} week in second phase, 10^{th}-12^{th} week in third phase, and 14-16^{th} week in last fourth phase to access change in the level of lipids by collecting blood samples.

Fasting plane blood samples were collected for examination and after collection placed in a box contains coolant. All the samples transported to the laboratory within 2-3 hour after collection, where serum was separated from whole blood samples by centrifugation at 2000-2500 rpm for 10 minutes after thawing at room temperature. Then level of Total Cholesterol, Triglyceride and HDL-Cholesterol was estimated using spectrophotometer. Level of LDL-Cholesterol, VLDL-Cholesterol and Cholesterol: HDL-Cholesterol ratio was estimated by using calculative formulas.

4.3ESTIMATION OF TOTAL CHOLESTEROL

CHOD-POD/ phosphotungstate method was used for the estimation of total cholesterol in blood. Mono reagent kit was used which contain lipid clearing factor to clear off all lipids up to 1000mg/dl with no interference from bilirubin upto 20mg/dl, hemoglobin up to 20 gm/dl, and triglyceride up to 1000 mg/dl. Kit reagent contains various enzymes which catalyzes various reactions to form red colored complex.

Cholesterol ester hydrolyzed enzymatically into free cholesterol and free fatty acids by cholesterol esterase enzyme. Then this free cholesterol is oxidized into cholest-4 ene 3-one and hydrogen peroxide and this reaction catalyzed by an enzyme Cholesterol oxidase (CHOD). Then enzyme peroxidase converts hydrogen peroxide into red colored quinoneimine dye by coupling with phenol and 4 aminoantipyrine (4AAP). All the reaction steps are given below:

$$\text{Cholesterol ester} + H_2O \xrightarrow{\text{CHE}} \text{Cholesterol} + \text{Free Fatty Acids}$$

$$\text{Cholesterol} + O_2 \xrightarrow{\text{CHOD}} \text{Cholest} - 4 \text{ ene 3-one} + H_2O_2$$

$$H_2O_2 + \text{Phenol} + \text{4- Aminoantipyrine} \xrightarrow{\text{POD}} \text{Red Quinoneimine complex} + H_2O$$

1 ml reagent was used to estimate cholesterol level in which 10 µl serum sample/cholesterol standard was added. Then incubated for 5 minutes at 37° C. then absorbance of standard and test was measured against distilled water at 505nm (Green Filter) (42).

4.4ESTIMATION OF HDL- CHOLESTEROL

Same reagent was also used for the estimation of HDL-cholesterol but the sample was treated with precipitating agent before use. On addition of precipitating reagent to the serum it's followed by centrifugation. This precipitating reagent removes all non HDL-Cholesterols from serum and only HDL-Cholesterol remains in supernatant.

Then this HDL supernatant was added to cholesterol reagent, then various enzymes catalyze various reactions to form red colored complex. Intensity of this color measured to calculate level of HDL-C (43).

To measure HDL-C level in serum 100 µl of supernatant and cholesterol standard was added to separate test tubes containing 1 ml cholesterol reagent and incubated for 5 minutes at 37°C. Absorbance of standard and test was measured against distilled water at 505nm (Green Filter). Absorbance was measured semi-automated biochemistry analyzer.

4.5ESTIMATION OF TRIGLYCERIDE

Triglyceride was estimated in serum by GPO-POD method with TBHA as chromogen. Triglycerides present in serum are catabolised into glycerol and free fatty acids in the presence of enzyme lipoprotein lipase. In the presence of enzyme glycerol kinase and ATP glycerol is converted into glycerol – 3 – phosphate on which enzyme glycerol-3-phosphate oxidase acted to form hydrogen peroxide and dihydroxyacetone phosphate. Hydrogen peroxide along with 4- Aminoantipyrine and phenolic compound TBHA in the presence of enzyme peroxidase form red coloured quinoeimine complex. Intensity of this complex measured at 505 nm. All the steps of reactions are given below:

$$\text{Triglyceride} \xrightarrow{\text{LPL}} \text{Glycerol + Fatty Acids}$$

$$\text{Glycerol + ATP} \xrightarrow{\text{GK}} \text{Glycerol - 3 – phosphate (G-3-P)}$$

$$\text{G-3-P + O2} \xrightarrow{\text{GPO}} H_2O_2 + \text{Dihydroxyacetone phosphate}$$

$$H_2O_2 + \text{4- Aminoantipyrine + TBHA} \xrightarrow{\text{POD}} \text{Red Quinieimine Complex} + H_2O + \text{HCl}$$

To estimate triglyceride level in 1 ml of TG reagent 10 µl of serum and TG standard was added and incubated for 10 minutes at 37°C. Then absorbance of test and standard was measured at 505nm spectrophotometrically at semi automated biochemistry analyzer (44).

4.6ESTIMATION OF VLDL-CHOLESTEROL

VLDL-Cholesterol was calculated by using calculative formula. Very low density lipoprotein (VLDL) is equal to one fifth of the triglyceride level. Thus VLDL calculated by using following formula (45).

$$\text{VLDL} = \frac{\text{Triglyceride (mg/dl)}}{5}$$

4.7 ESTIMATION OF LDL-CHOLESTEROL

Total cholesterol is mixture of VLDL, LDL and HDL. Thus from the known value of total cholesterol, HDL-Chol, and VLDL-Chol, it's easy to estimate level of LDL-Chol using simple calculative formula given below (46)

$$\text{LDL-C} = \text{Total Cholesterol} - (\text{HDL-C} + \text{VLDL-C})$$

4.8ESTIMATION OF TOTAL CHOLESTEROL: HDL-C Ratio

Cholesterol: HDL ratio was calculated by dividing total cholesterol by HDL-Cholesterol level as shown in formula (47).

$$\text{Total Cholesterol: HDL-C Ratio} = \frac{\text{Total Cholesterol}}{\text{HDL-Cholesterol}}$$

After estimating level of various lipids their values were recorded on data forms with date of test performed. Samples from all the subjects were collected four times during this study at the interval of 20-25 days and processes for estimation of lipid level.

4.9STATISTICAL ANALYSIS

Results are presented as mean ± standard deviation. Mean and S.D. of data from four different phases and 3 different groups was calculated using MS excel 2007. Then from mean value change in the level of various lipids in all three groups during four phases was calculated in %. All this data presented graphically using MS excel 2007.

CHAPTER 5

RESULT AND DISCUSSION

Based upon literature review diet was given to the selected subjects along with light physical activity to subjects in group II. Third group was also consuming medication of statin group along with diet modification and physical exercise. First group included normal healthy subjects.

In first phase blood samples were collected before starting of project and then on the basis of results all subjects categorized into three groups followed by diet modification and exercise with medication to one group. Then again samples were collected in three different phases at the interval of 20-25 days for the analysis of impact of diet modification and exercise alone and with medication on lipid levels. Impact of all this study on various groups is presented below:

5.1IMPACT ON TOTAL CHOLESTEROL LEVEL

Total Cholesterol consist of various different kind of cholesterols line High density Lipoprotein, Low density Lipoprotein and Very Low density lipoprotein. Total cholesterol was estimated in all three groups.

In group one which is having control subjects, no significant change in the level of cholesterol was noted. Mean ± S.D. value in phase 1, phase 2, phase 3, and phase 4 was 189.83 ±18.35, 189.04 ± 17.12, 188.13 ± 13.63 and 189.13 ± 15.65. In 16 weeks total cholesterol values were reduced by 0.37% (189.83 to 189.13 mg/dl).

This much less reduction in the cholesterol level is not having significant importance. In second phase cholesterol level was reduced by 0.41% (189.83 to 189.04 mg/dl). Then from second phase to third phase there was a reduction of 0.48% 9189.04 to 188.13), followed by an increase of 0.53% (188.13 to 189.13) in value from third phase to fourth phase (figure 6).

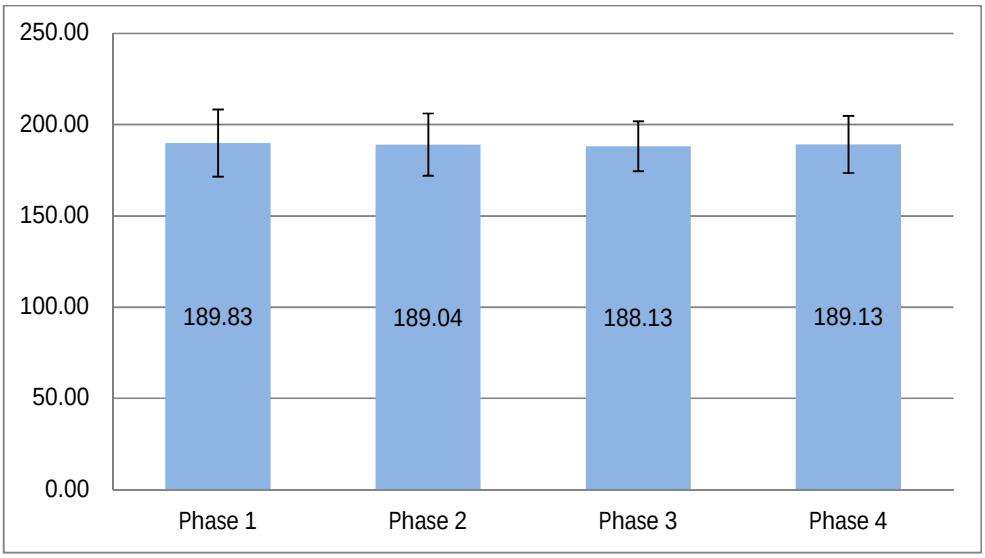

Figure 6 : Mean Level of total cholesterol with S.D. in the form of error bars in all four phases in subjects of group I

Second group (having subjects following Diet modification along with daily physical exercise) shown 11.74% (237.33 to 209.47 mg/dl) reduction in the level of total cholesterol. Initially reduction was only of 3.77% (237.33 to 228.39 mg/dl) in second phase which then reduced more by 4.77% (228.39 to 217.50 mg/dl) and 3.7% (217.50 to 209.47%) in third and fourth phase. Mean ± S.D. value in phase 1, phase 2, phase 3, and phase 4 was 237.33 ± 20.09, 228.39 ± 19.40, 217.50 ± 17.95, and 209.47 ± 17.03 as shown in figure 7

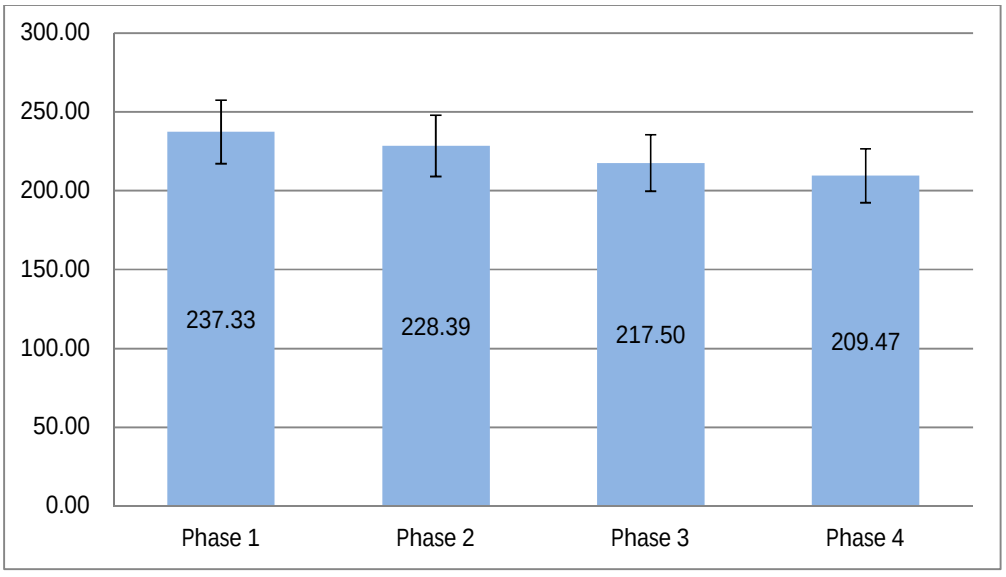

Figure 7 : Mean Level of total cholesterol with S.D. in the form of error bars in all four phases in subjects of group II

In third group (Consuming medicine along with diet modification and daily physical exercise) 247.98 ± 34.62, 237.13 ± 30.27, 224.59 ± 27.40, and 211.57 ± 27.17 mean ± S.D. value was obtained. Total 4.36% (247.98 to 237.17) cholesterol decreased in second phase followed by 5.30% (237.17 to 224.59 mg/dl) and 5.80% (224.59 to 211.57 mg/dl) in third phase and fourth phase (figure 8). Overall reduction in all those phases in 16 weeks was 14.7% (247.98 to 211.57 mg/dl).

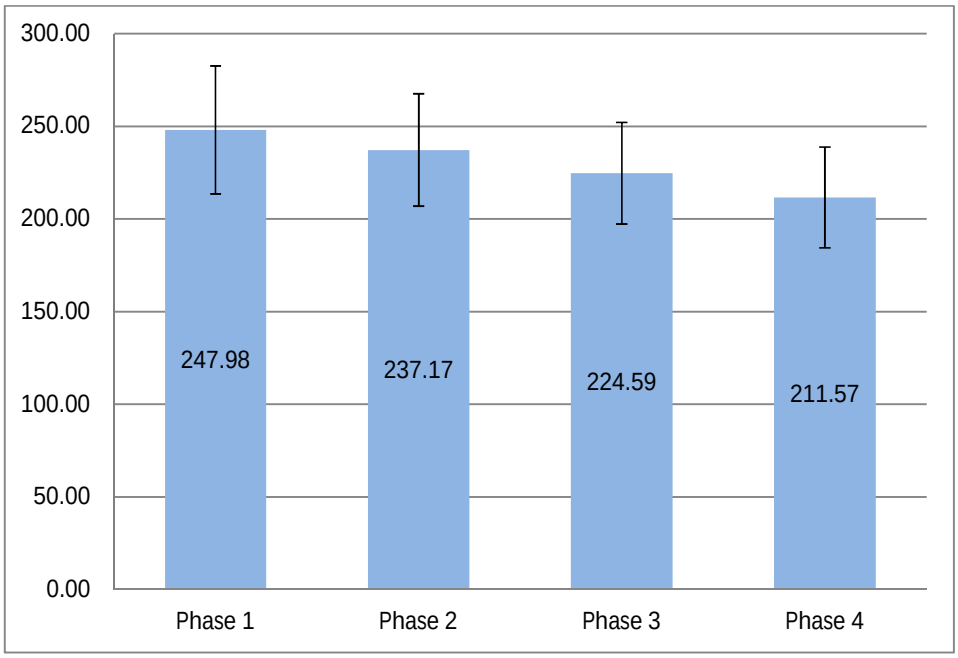

Figure 8 : Mean Level of total cholesterol with S.D. in the form of error bars in all four phases in subjects of group III

Reduction in the level of cholesterol is very less in control group than all other groups. Second group showing more reduction in the level of cholesterol than control group which means, diet modification and daily exercise has great impact on cholesterol level in body. If we compare second group with third group, then second group is lacking behind. In second group reduction was 11.74% whereas in third group it's 14.7% (figure 9). The only extra thing which third group is taking is medication, which means medication along with diet modification and daily exercise has much more positive impact on cholesterol level.

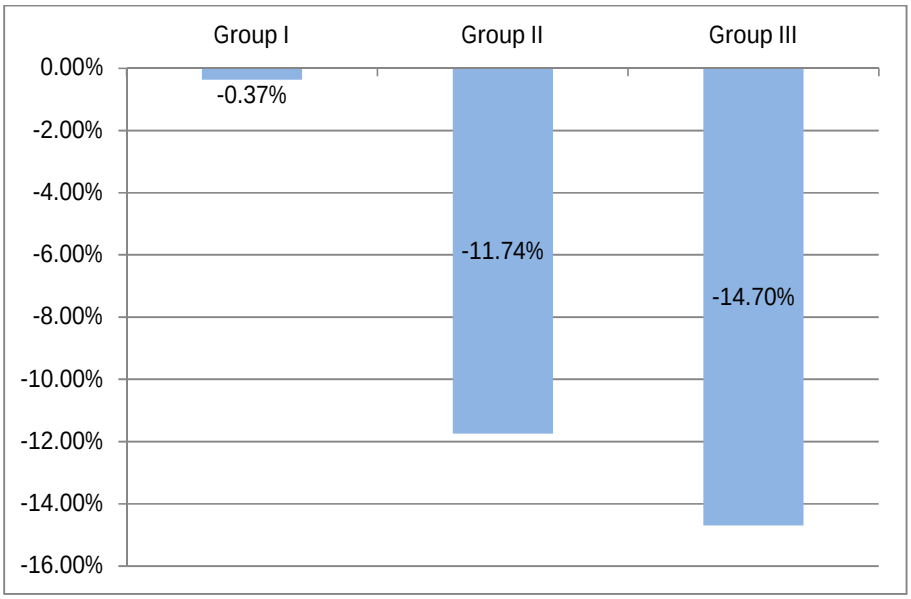

Figure 9 : Total overall percentage of reduction in the level of cholesterol in different groups

Figure 10 showing pattern with which cholesterol is reduction pattern of cholesterol. As in group I (control group) line is horizontally straight means there is not much change in the level of cholesterol in control group. Whereas line is coming downward in group II and group III. Decline is little sharp in group III than in group II which means medication along with lifestyle modification have more capability to reduce cholesterol level than life style modification alone.

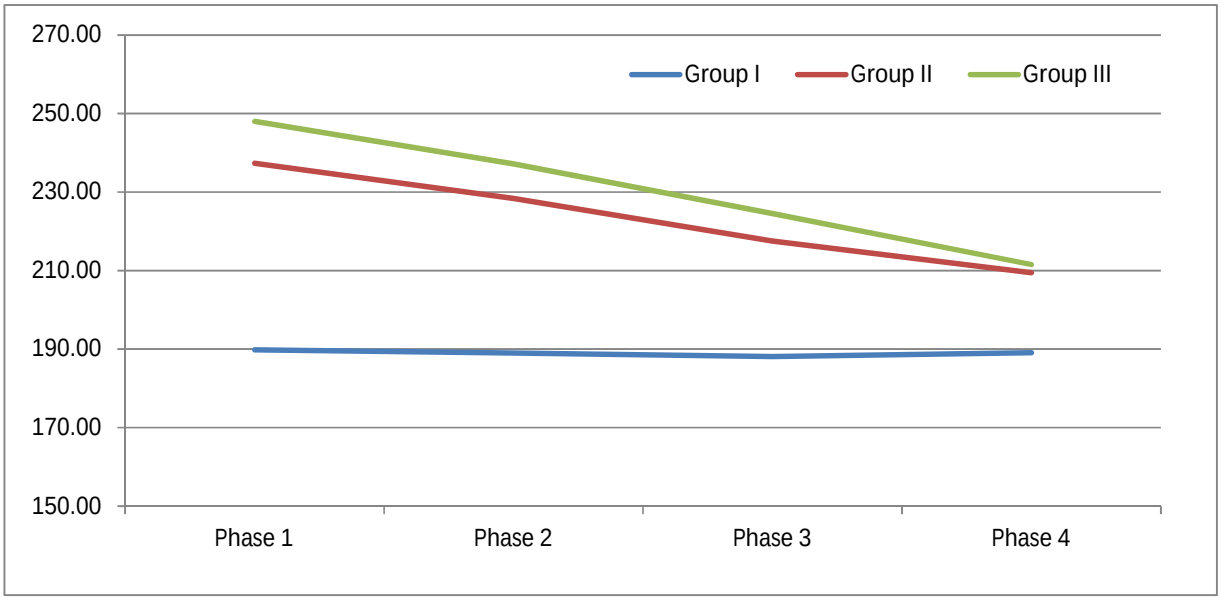

Figure 10 : Pattern of change in the level of cholesterol in 16 weeks in all three different groups

5.2 IMPACT ON TRIGLYCERIDE LEVEL

Level of TG also measured in all three groups. In group I phase 2 shows 131.65 mean ± 24.62 S.D., phase 2 shows 129.00 mean ± 26.55 S.D., Phase 3 shows 128.74 mean with 23.51 S.D. and phase 4 having 130.78 mean and ± 24.47 S.D. In time period of 16 weeks total 0.66% (131.65 to 130.78 mg/dl) TG level was reduced in control group. Up to phase 2 2.01% (131.65 to 129.00 mg/dl) reduction was reported, followed by again reduction of 0.20% (129.00 to 128.47) in phase 3. Then a rise of 1.58% (128.74 to 130.78) in the TG level was noted down. All these figures clearly explained in fig 11

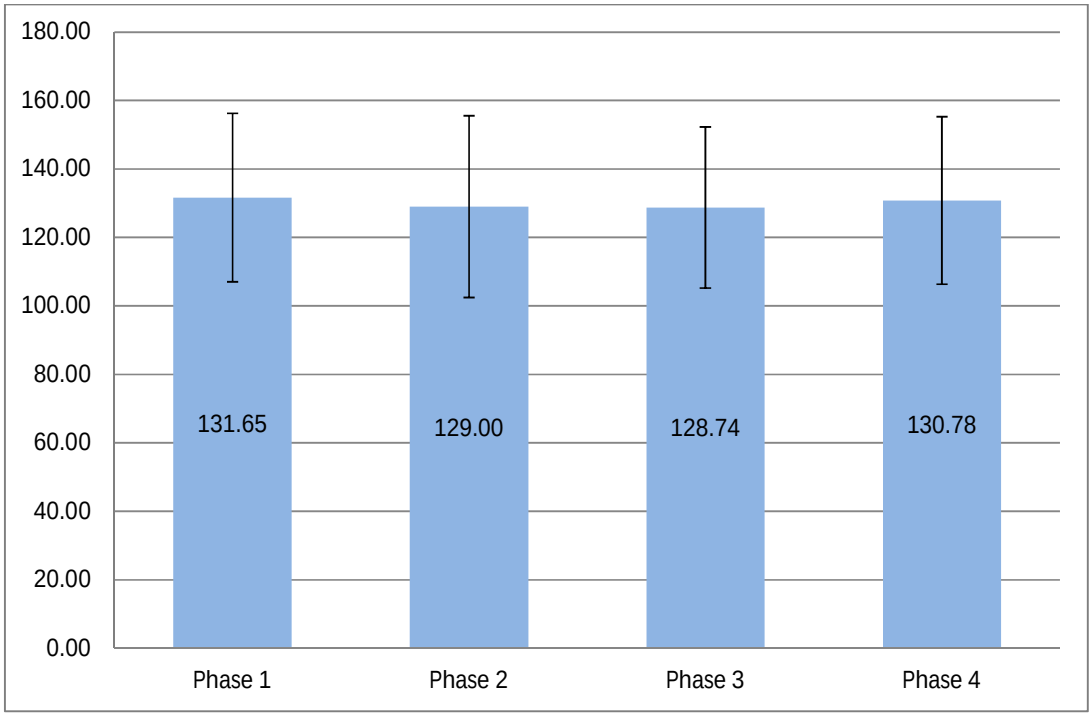

Figure 11: Mean Level of TG with S.D. in the form of error bars in all four phases in subjects of group I

In group II means ± S.D. values from phase 1 to phase 4 were 201.81 ± 22.62, 199.22 ± 53.77, 180.00 ± 20.72 and 167.08 ± 17.44. Total 17.21% TG level was reduced in this group by diet modifications and exercise. In phase 2 1.28% (201.81 to 199.22 mg/dl), in phase 3 9.64% (199.22 to 180.00 mg/dl) and in phase 3 7.18% (180.00 to 167.08 mg/dl) reduction was reported as mentioned in figure 12

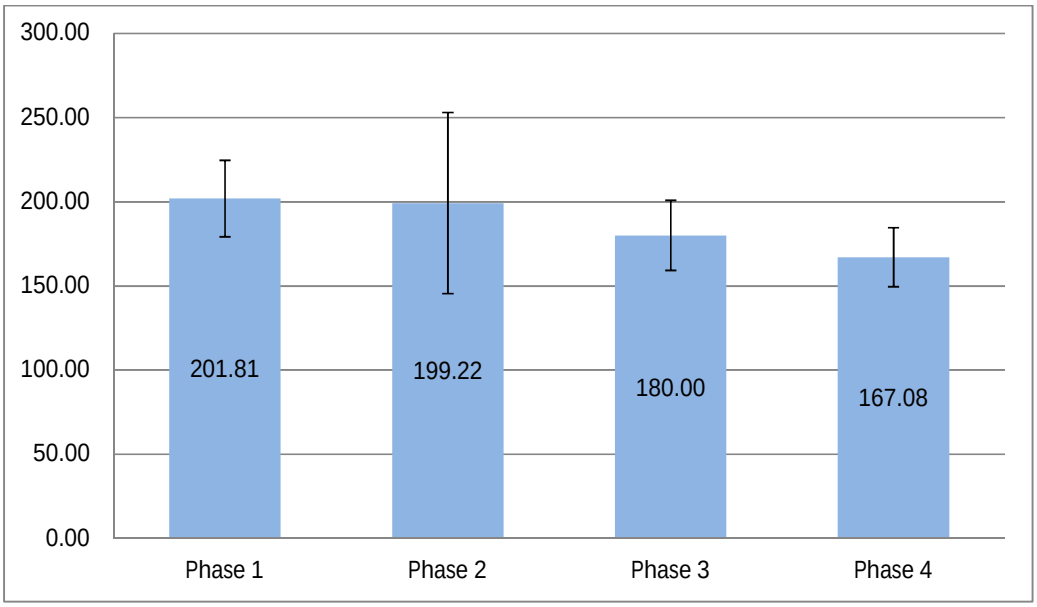

Figure 12: Mean Level of TG with S.D. in the form of error bars in all four phases in subjects of group II

Group III also shown 19.21% (244.82 to 197.78 mg/dl) overall reduction with 244.82 ± 64.07, 235.83 ± 52.99, 217.87 ± 50.59 and 197.78 ± 46.59 mean ± S.D. values in phase 1 to phase 4. Reduction was 3.67% (244.82 to 235.83 mg/dl) in phase 1 which was then increased up to 7.61% (235.83 to 217.87 mg/dl) in phase 3 and then again continuously increased up to 9.22% (217.87 to 197.78 mg/dl) in phase 4 (figure 13) under the influence of medicine, diet modification and exercise.

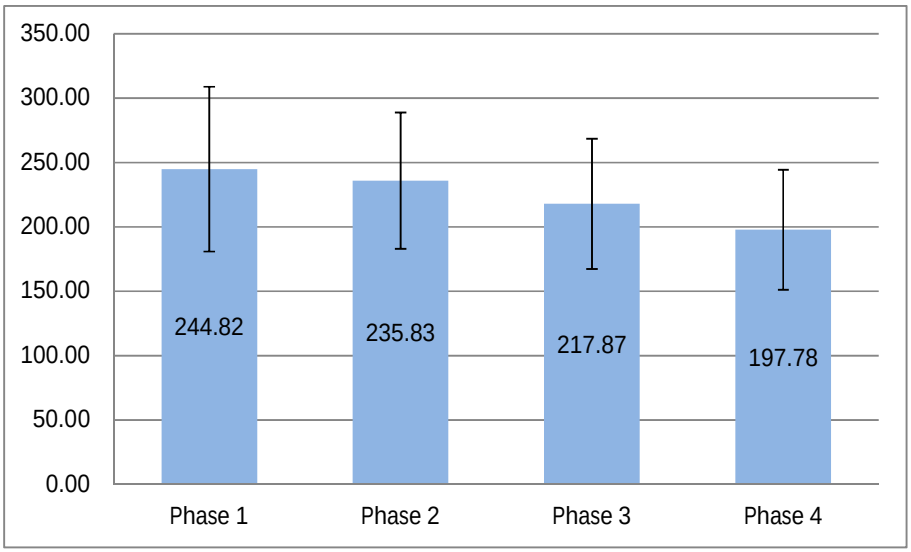

Figure 13: Mean Level of TG with S.D. in the form of error bars in all four phases in subjects of group III

Reduction in the level of TG is very less in control group than all other groups. Second and third group shows more reduction in TG level than control group, which means second group having much more reduction than control but less than group III. If we compare second group with third group then second group is lacking behind. In second group reduction was 17.21% whereas in third group it's 19.21% (figure14). The only extra thing which third group is taking is medication, which means medication along with diet modification and daily exercise has much more positive impact on cholesterol level. As it's clearly shown in figure 14

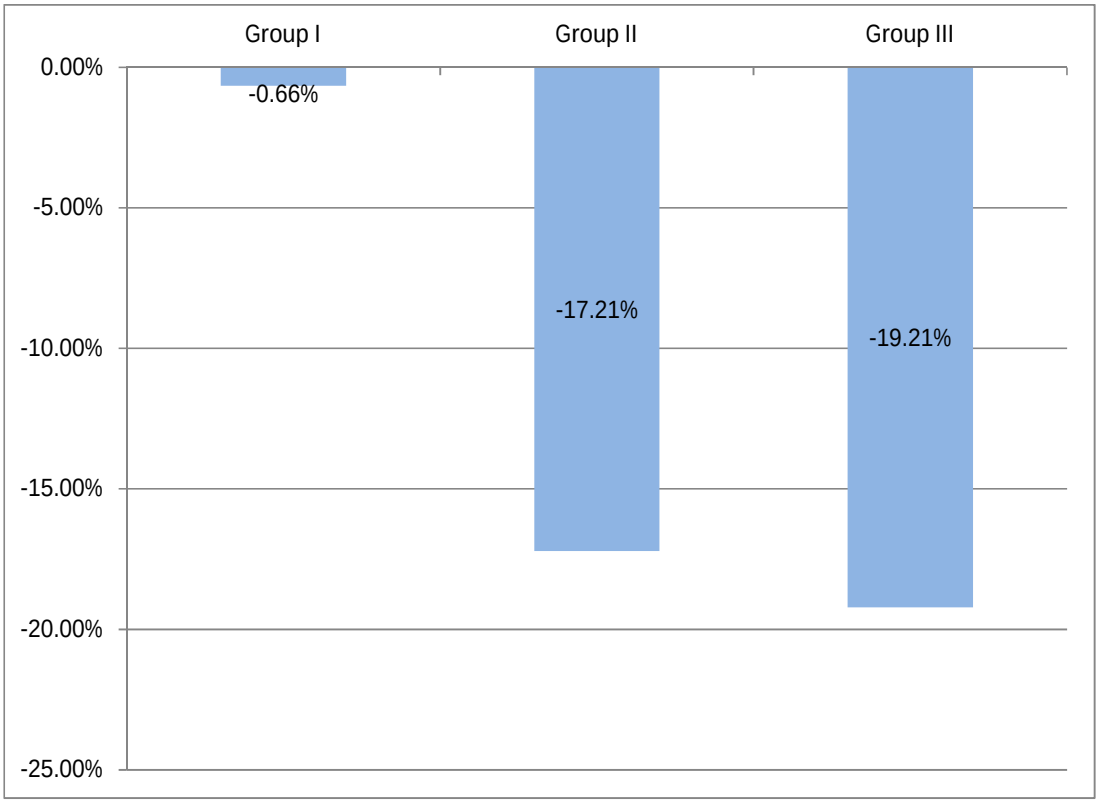

Figure 14: Total overall percentage of reduction in the level of TG in different groups

Following line graph in figure 15 shows overall changes in triglyceride level in subjects of all three groups. As group 1 (control) showing a straight line which means there is not more changes in the level of triglyceride whereas line of group II and group III showing great fall in the level of TG. Reduction is little bit more in group III than in group II which means medication along with lifestyle modification have more capability to reduce triglyceride level than life style modification alone.

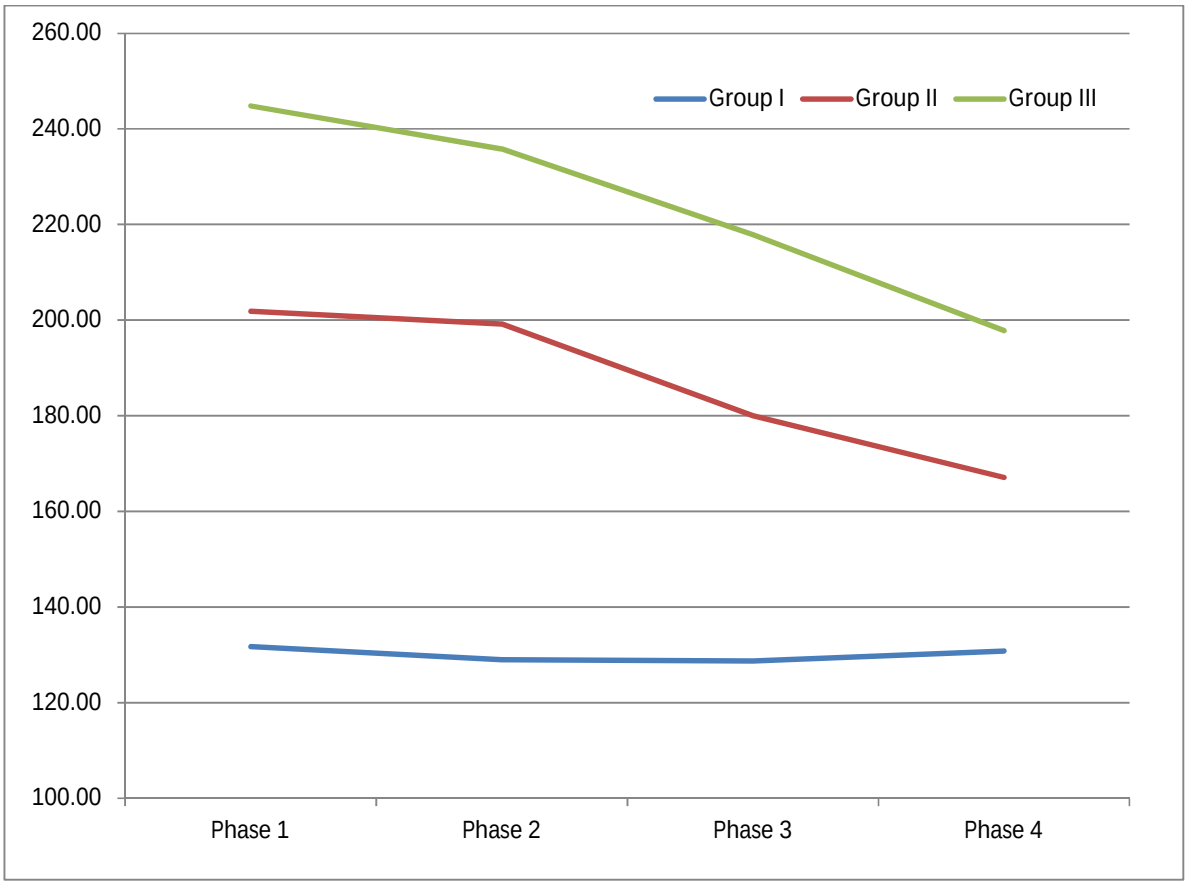

Figure 15: Pattern of change in the level of TG in 16 weeks in all three different groups

5.3 IMPACT ON LDL-C LEVEL

LDL is a low density lipoprotein having high content of lipids and less content of protein. Level of LDL-C was also measured in all subjects.

The mean ± S.D. of LDL-C level for all four phases in group I was 177.53 ± 17.26, 177.92 ±17.49, 177.03 ± 13.22, and 178.21 ± 15.76. If we talk about overall impact of 16 weeks on control group then a rise of 0.38% (177.53 to 178.21mg/dl) was found. In second phase LDL-C level rises by 0.21% (177.53 to 177.92 mg/dl), and then all fall of 0.50 % (177.92 to 1770.3 mg/dl) was reported in phase 3. Then an increase of 0.10 % (177.03 to 178.21 mg/dl) was reported in level of LDL-C in phase 4 as shown in figure 16

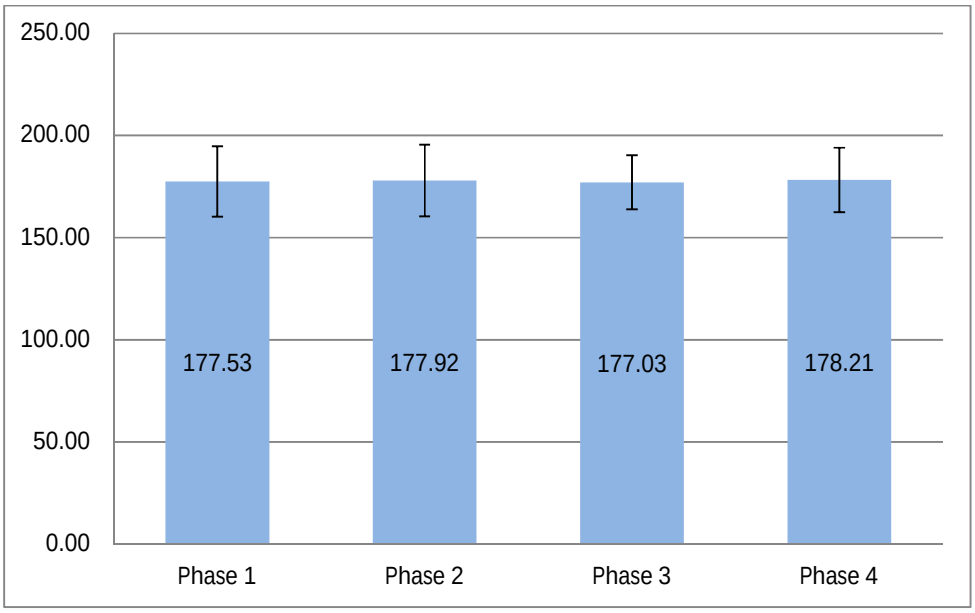

Figure 16: Mean Level of LDL-C with S.D. in the form of error bars in all four phases in subjects of group I

In group II a fall of 17.13% (129.86 to 107.61 mg/dl) was found. Mean ± S.D. value of LDL-C level in phase 1 to phase 4 was 129.86 ± 17.91, 122.22 ± 16.02, 113.33 ± 16.58 and 107.61 ± 15.70. Initially reduction in the level of LDL-C was 5.88% (129.86 to 122.22 mg/dl) in phase 2. In phase 3 and phase 4 reductions in LDL-C was 7.27% (122.22 to 113.33 mg/dl) and 5.05% (113.33 to 107.61 mg/dl) (figure 17).

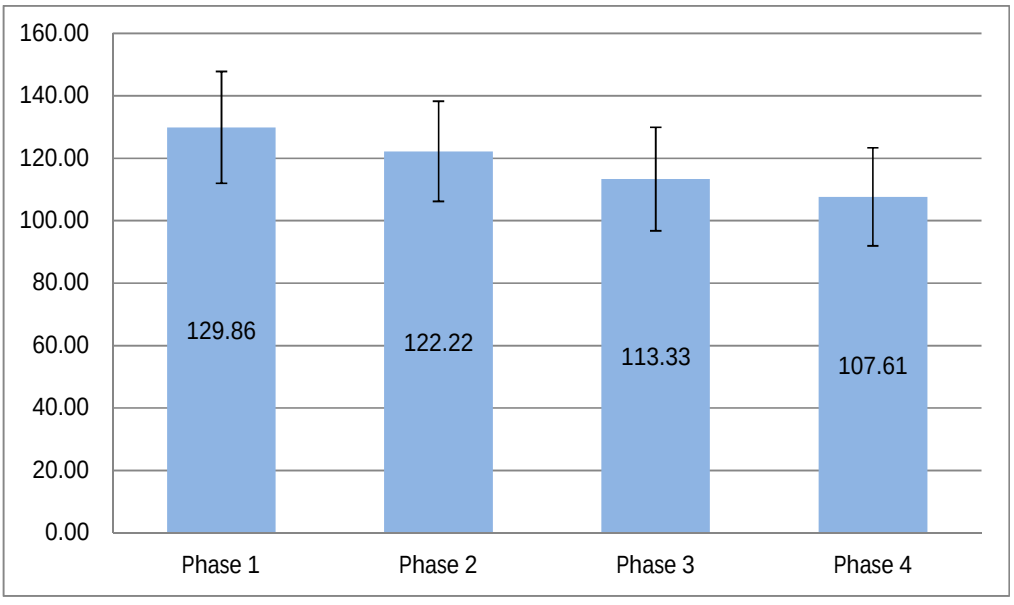

Figure 17: Mean Level of LDL-C with S.D. in the form of error bars in all four phases in subjects of group II

In group III highest reduction of 24.82% (143.04 to 107.54 mg/dl) was reported, which (reduction) increased step by step from start to end as in phase 2 reduction in LDL-C level was 7.35% (143.04 to 132.52 mg/dl) which is then in phase 3 it's 9.88% (132.52 to 119.43 mg/dl) and in last phase 4 it was 9.96% (119.43 to 107.54 mg/dl). The mean and S.D. value in phase 1 is 143.04 ± 35.84, in phase 2 it's 132.52 ± 32.24, in phase 3 value is 119.43 ± 29.13 and in phase 4 it's 107.54 ± 28.58 mg/dl as presented in figure 18.

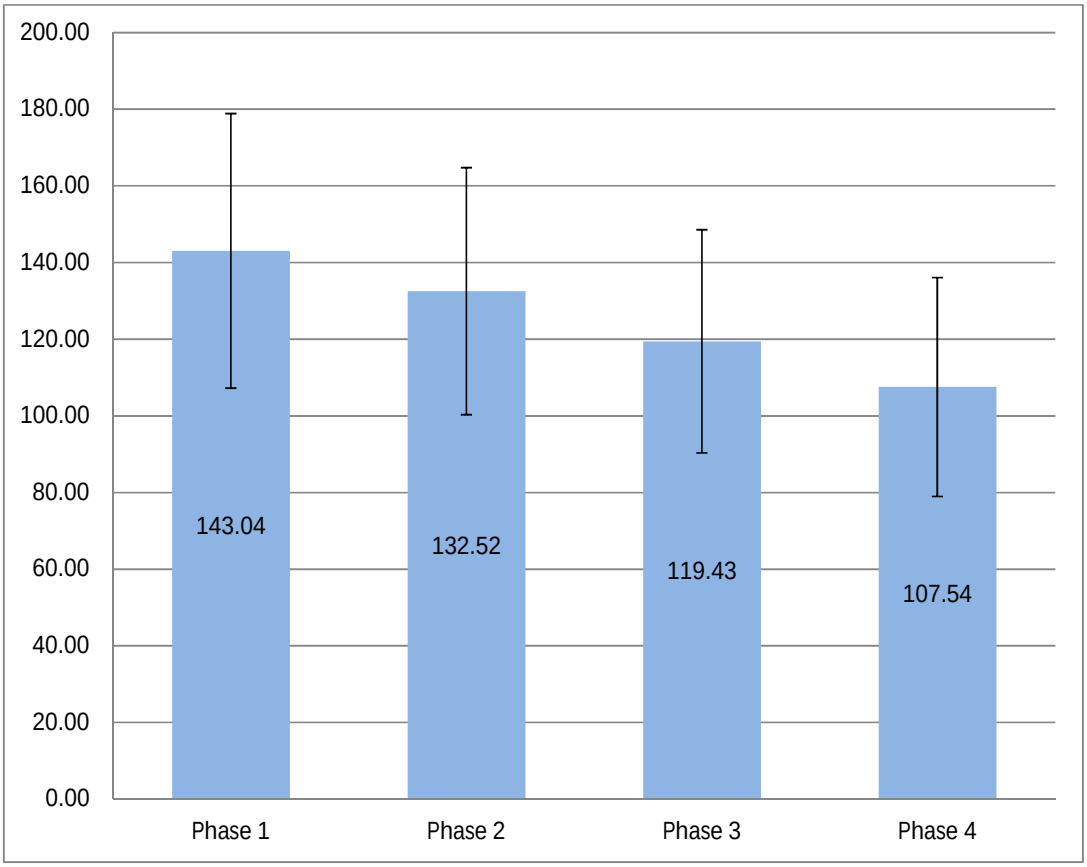

Figure 18: Mean Level of LDL-C with S.D. in the form of error bars in all four phases in subjects of group III

In group I a rise of 0.38% in the level of LDL-C was reported which is not having any significant importance due to slight change. In group II reduction in LDL-C level was reported which is 17.13%, this shows that life style modification including changes in diet and daily exercise have significant impact on LDL-C reduction. But reduction in group III (24.82%) is more than group II (figure 19). It clearly indicates addition of medicine along with lifestyle modification speed up the rate of LDL-C level reduction.

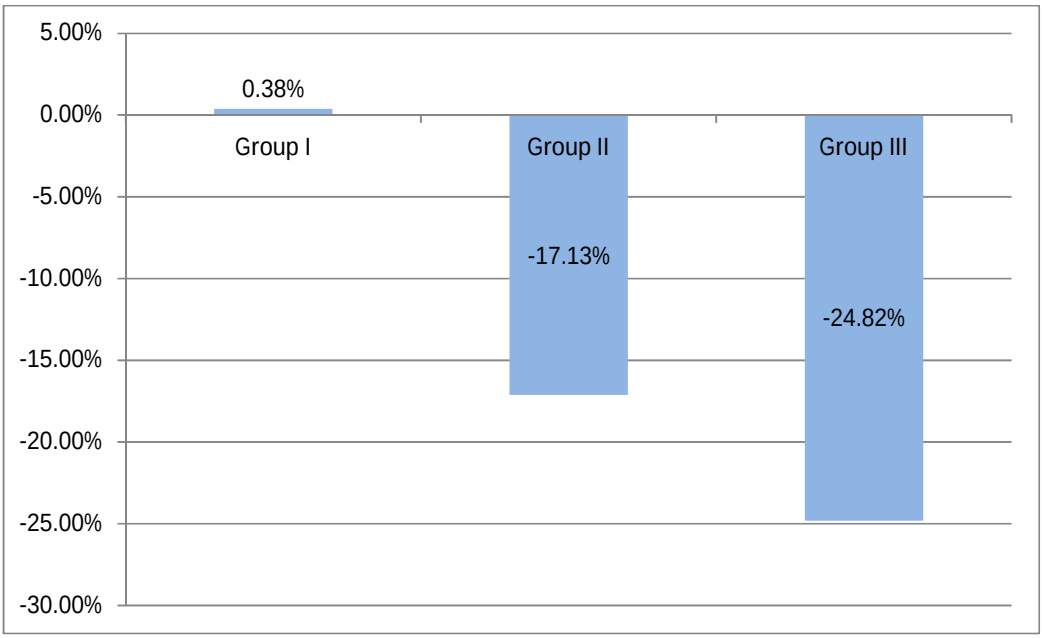

Figure 19: Total overall percentage of reduction in the level of LDL-C in different groups

From graph of figure 20 it's clear that change in LDL-C level is not significant as line is almost going straight. In group II and group III reduction is high as decline in the lines of graph is very sharp. Decline is high in group III than in group II, it means reduction in group III is more than group II, means medication along with lifestyle modification have more capability to reduce LDL-C level than life style modification alone.

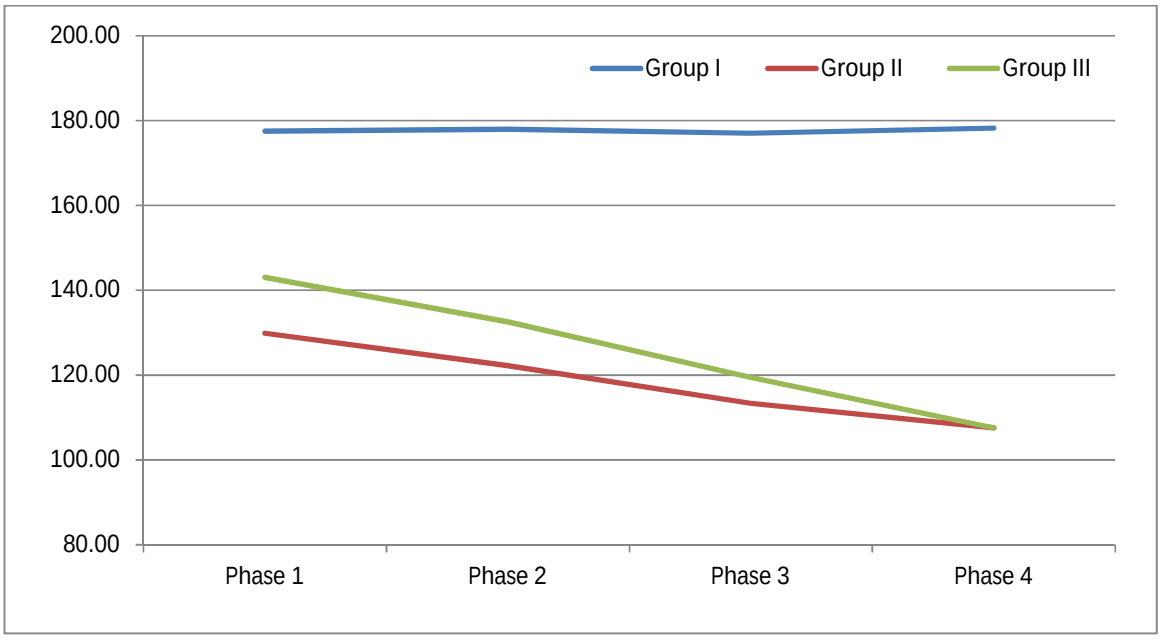

Figure 20: Pattern of change in the level of LDL-C in 16 weeks in all three different groups

5.4 IMPACT ON THE LEVEL OF VLDL-C

VLDL-C is a lipoprotein having very low concentration of protein and high amount of lipids in it. VLDL-C estimated in all subjects of three different groups.

In group I at the end of 16 weeks no change was reported in the level of LDL-C. Initially its level was reduced by 1.63 % (26.30 to 25.87 mg/dl) in phase 2 and then 0.50 (25.87 to 25.74 mg/dl) in phase 3. Then a rise of 2.17% (25.74 to 26.30 mg/dl) observed at the end in phase 4. This makes overall change 0%. If we talk about mean ± S.D. values of all phases then it was 26.30 ± 4.81 in phase 1, 25.87 ± 5.38 in phase 2, 25.74 ± 4.69 in phase 3 and 26.30± 4.68 in phase 4 (figure 21).

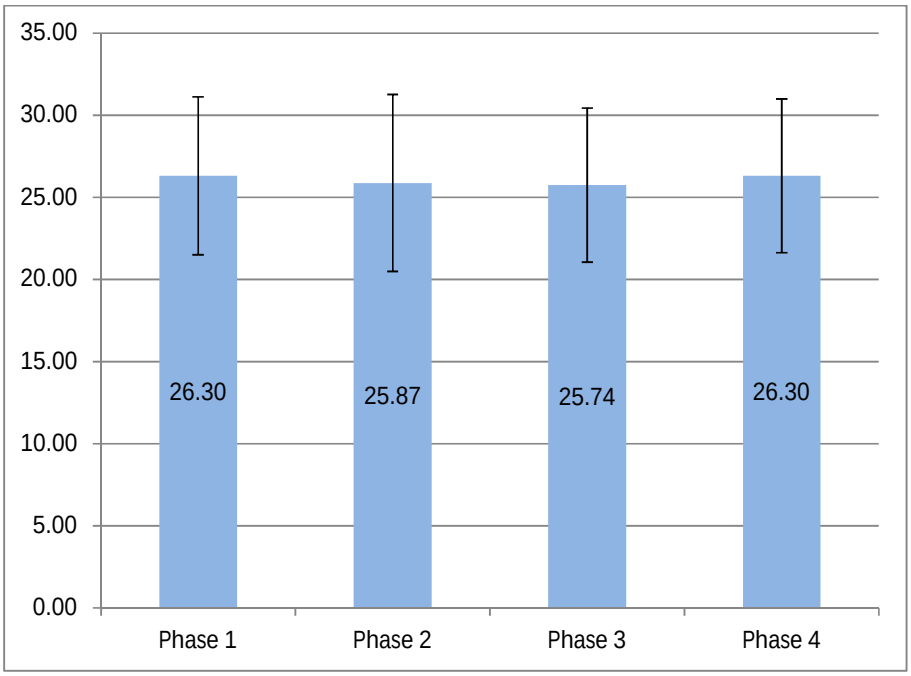

Figure 21: Mean Level of VLDL-C with S.D. in the form of error bars in all four phases in subjects of group I

Group II shown 16.95% (40.47 to 33.61 mg/dl) decrease in the level of VLDL-C in 16 weeks just under the influence of life style modifications. Overall mean ± S.D. of all phases was 40.47 ±4.63 in phase 1, 38.33 ± 4.52 in phase 2, 36.22 ± 4.27 in phase 3 and 33.61± 3.59 in phase 4. Initially decrease in the level of VLDL-C was 5.29% (40.47 to 38.33 mg/dl) in phase 1. This reduction was then increases continuously in phase 2 and phase 3 by 5.50% (38.33 to 36.22 mg/dl) and 7.21% (36.22 to 33.61 mg/dl) (figure 22).

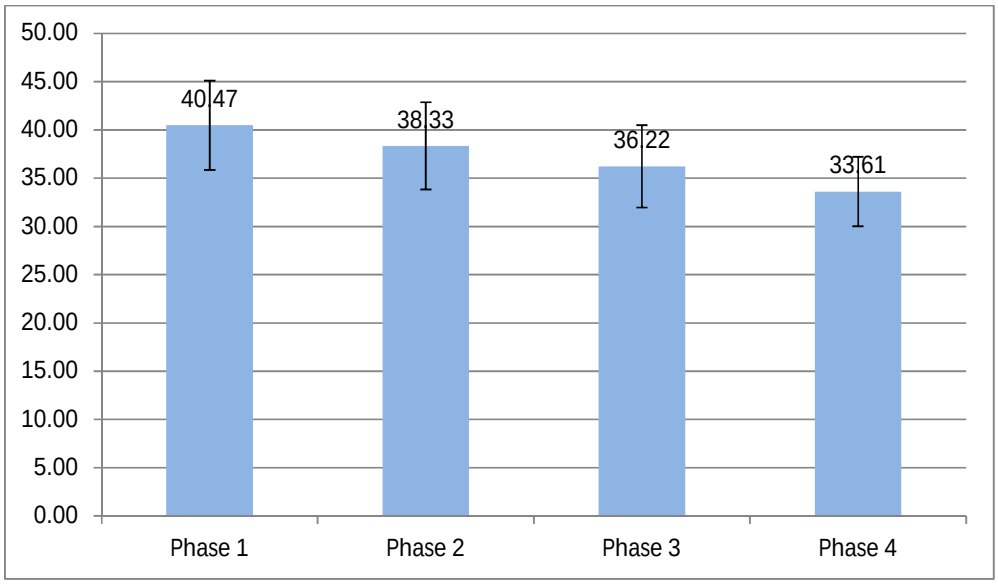

Figure 22: Mean Level of VLDL-C with S.D. in the form of error bars in all four phases in subjects of group II

Group III having 21.87% (50.65 to 39.57 mg/dl) decrease in the level of VLDL-C. This reduction was 6.73% (50.65 to 47.24 mg/dl) initially in phase 2, then 6.22% (47.24 to 44.30 mg/dl) in phase 3 and then heavy reduction of 10.68% (44.30 to 39.57 mg/dl) was reported in fourth phase. The mean ± S.D. value of all phases in this group was 50.65 ± 11.55 in phase 1, 47.24 ± 10.67 in phase 2, 44.30 ± 11.14 in group 3 and 39.57 ± 9.35 in phase 4 as represented in figure 23

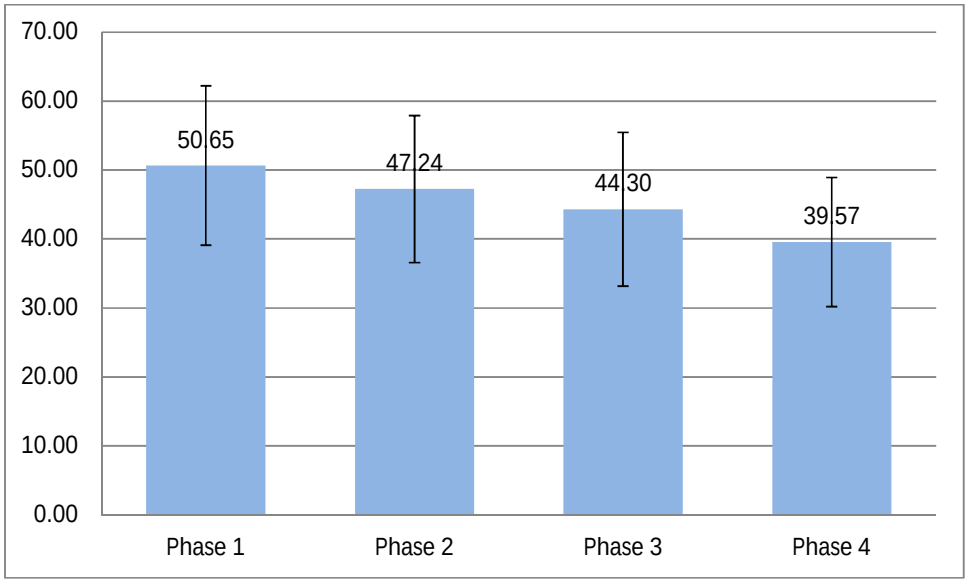

Figure 23: Mean Level of VLDL-C with S.D. in the form of error bars in all four phases in subjects of group III

If we talk about all three groups then in group I change in the level of VLDL-C was 0%, whereas in group II due to life style modifications 16.95% VLDL-C was reduced. If we see the reduction in group III, it was 21.87% (figure 24). It means if we add medicine along with life style modification then it can increase the rate of VLDL-C reduction.

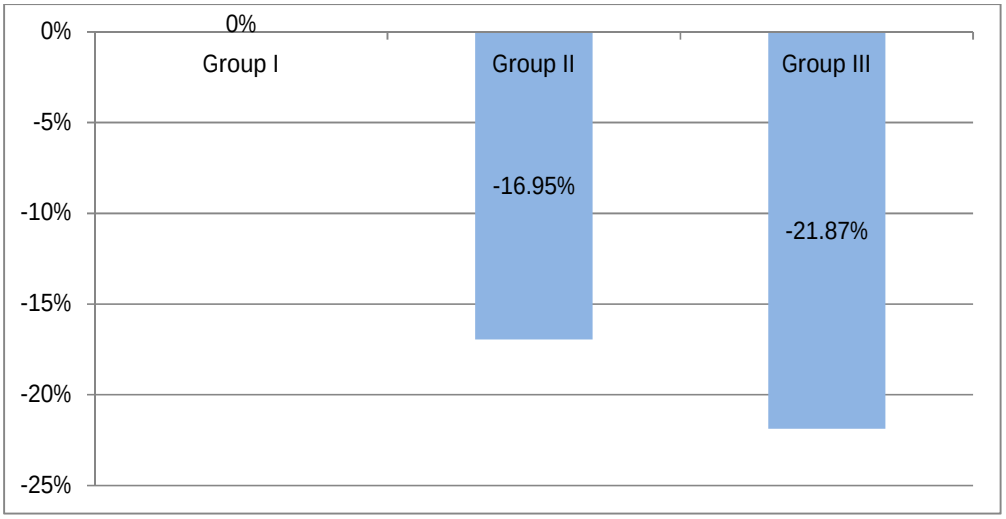

Figure 24: Total overall percentage of reduction in the level of VLDL-C in different groups

In graph given below it's clearly visible that group I there is no change in the level of VLDL-C level, whereas line which is showing data of group II and group III is going downward which is representing fall in the level of VLDL-C. Fall in VLDL-C level is slightly more in group III than group II. It means medication along with life style modification has better result than life style modification alone but the reduction we found in life style modification alone is also acceptable.

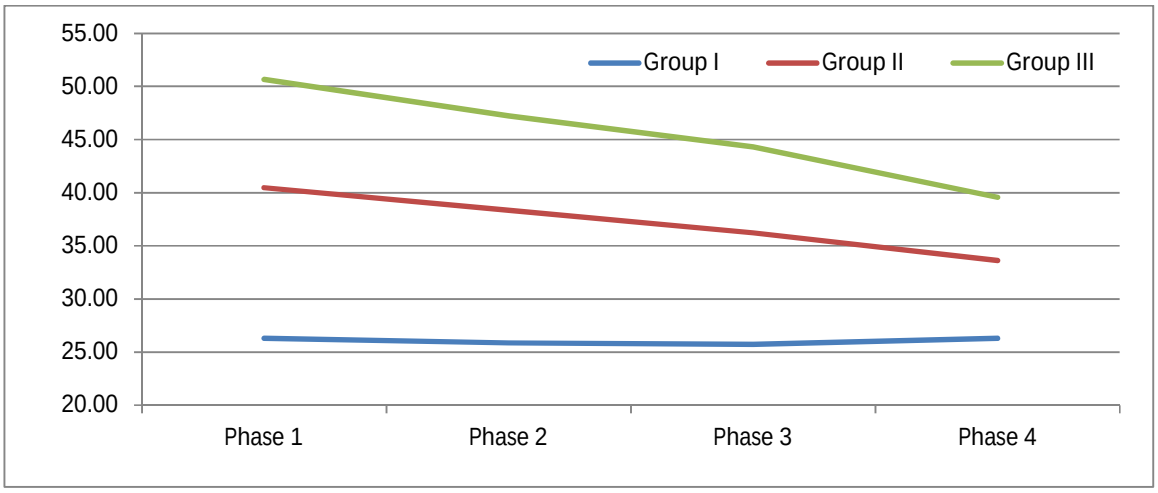

Figure 25: Pattern of change in the level of VLDL-C in 16 weeks in all three different groups

5.5IMPACT ON LEVEL OF HDL-C

HDL-C is only cholesterol which is also known as good cholesterol because it reduces risk of heart diseases.

In group I HDL-C raised by 2.75 % (61.87 to 63.57 mg/dl) in time period of 16 weeks. In phase 2 HDL-C level increased by 2.66% (61.87 to 63.25 mg/dl). In phase 3 increase in HDL-C level was 0.20% (63.52 to 63.65 mg/dl)) and in phase 4 HDL-C was reduced by 0.13% (63.65 to 63.57 mg/dl). Overall mean ± S.D. value in phase 1 was 61.87 ± 8.00, in phase 2 63.52 ± 6.51, in phase 3 63.65 ± 5.80 and in phase 4 it was 63.57 ± 5.49 mg/dl as presented in figure 26

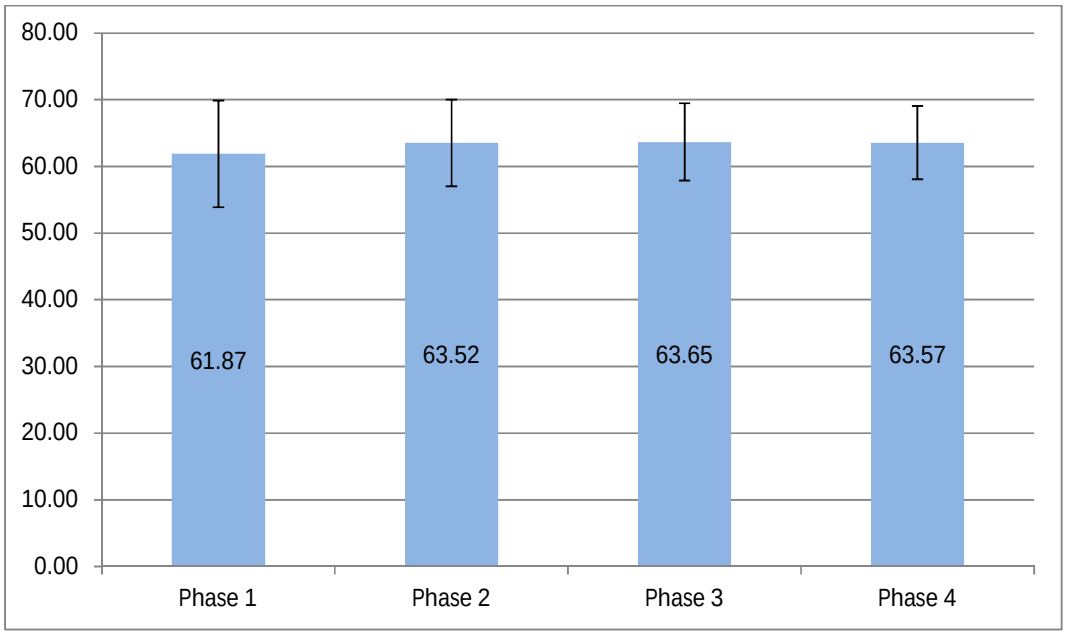

Figure 26: Mean Level of HDL-C with S.D. in the form of error bars in all four phases in subjects of group I

In group II HDL-C level was raised by 0.82% (68.22 to 68.78 mg/dl) which is very less than control group. This is quite different result we obtained from this group. HDL-C level raised by 0.98% (68.22 to 68.89 mg/dl) in phase 2 and 0.07% (68.89 to 68.94 mg/dl) in phase 4, but afterward reduction of 0.23% (68.94 to 68.78) found in phase 4. Mean ± S.D. value from phase 1 to phase 4 was 68.22 ± 6.99, 68.89 ± 5.81, 68.94 ± 4.34, and 68.78 ± 4.11 as shown in figure 27

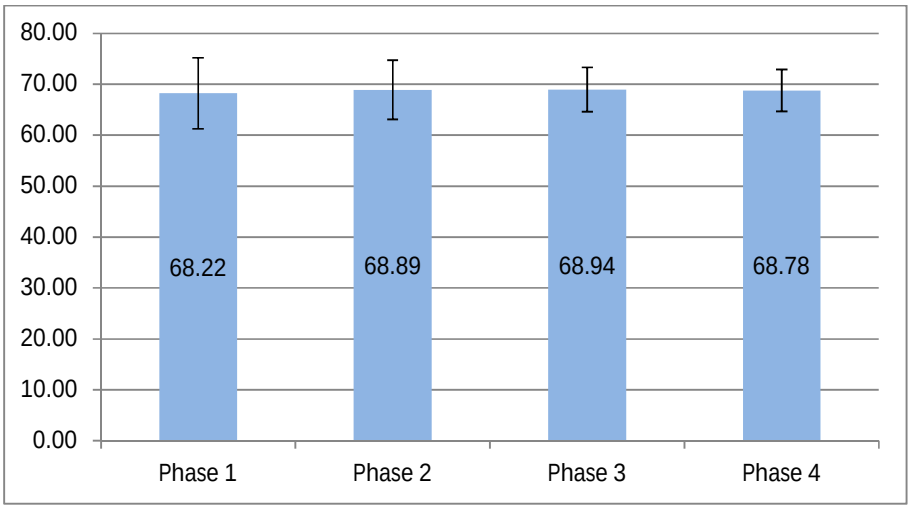

Figure 27: Mean Level of HDL-C with S.D. in the form of error bars in all four phases in subjects of group II

Group III shown significant increase of 18.70% (54.28 to 64.43 mg/dl) in HDL-C level, which is having significant importance. Mean ± S.D. of data in phase 1 was 54.28 ± 9.21, in phase 2, 3 and 4 was 57.41 ± 7.88, 61.50 ± 7.32, and 64.43 ± 6.55 mg/dl. In phase 2 HDL-C level raised up to 5.77% (54.28 to 57.41 mg/dl), which is again increased in phase 3 by 7.12% (57.41 to 61.50 mg/dl). In phase4 4.76% (61.50 to 64.13 mg/dl) HDL-C was raised as in figure 28.

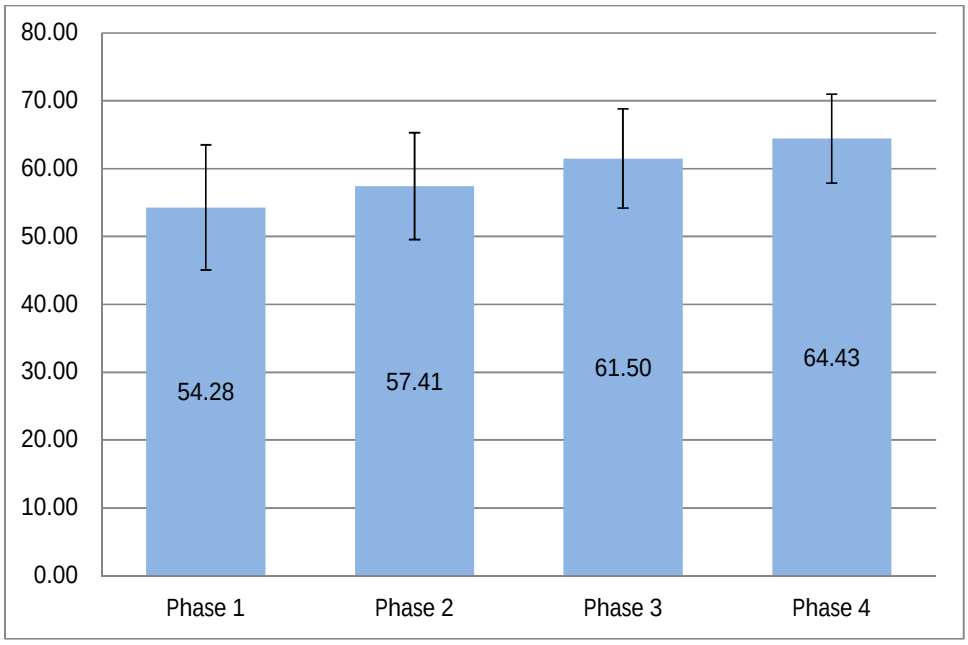

Figure 28: Mean Level of HDL-C with S.D. in the form of error bars in all four phases in subjects of group III

Increase in HDL-C was 2.75% in group I, 0.82% in group II and 18.7% in group III. This data means life style modification not having significant impact on HDL-C level as there is not any significant raised values was obtained from group II, but medication shown its full impact on level of HDL-C as HDL-C level raised by 18.7% in group III which is highly significant.

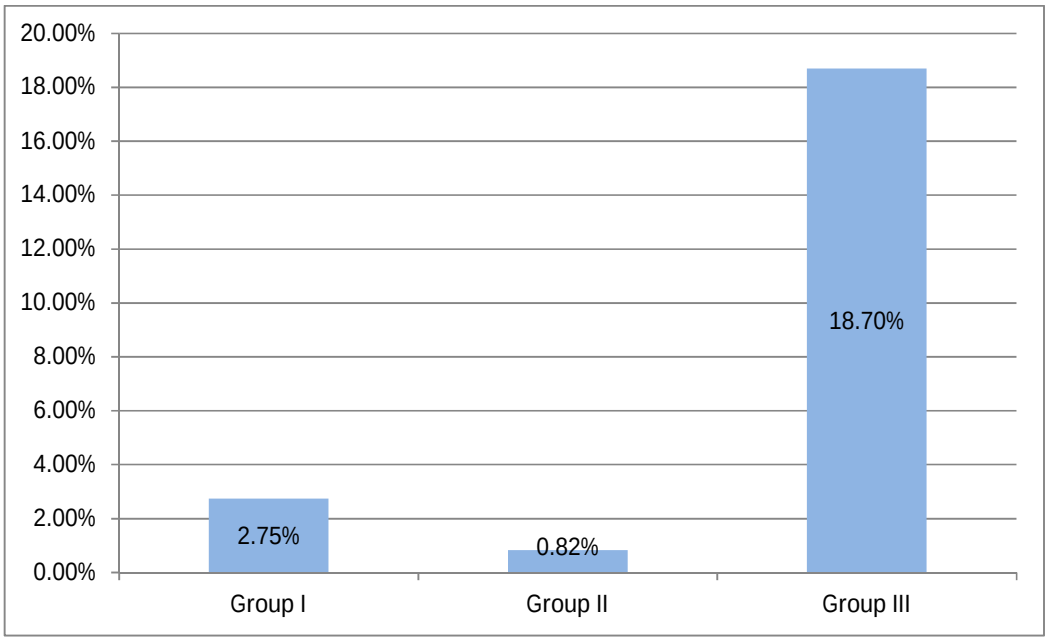

Figure 29: Total overall percentage of reduction in the level of HDL-C in different groups

As it's clearly visible in following graph (figure 30) blue line which is presenting group I is slightly going up it means little rise was found in group 1. Red line presenting group II is almost straight with a negligible increase in the level of HDL-C. This shows failure of life style modification in increasing level of HDL-C. Green line of group III is going upward showing incise in the level of HDL-C. It means medication increases level of HDL-C.

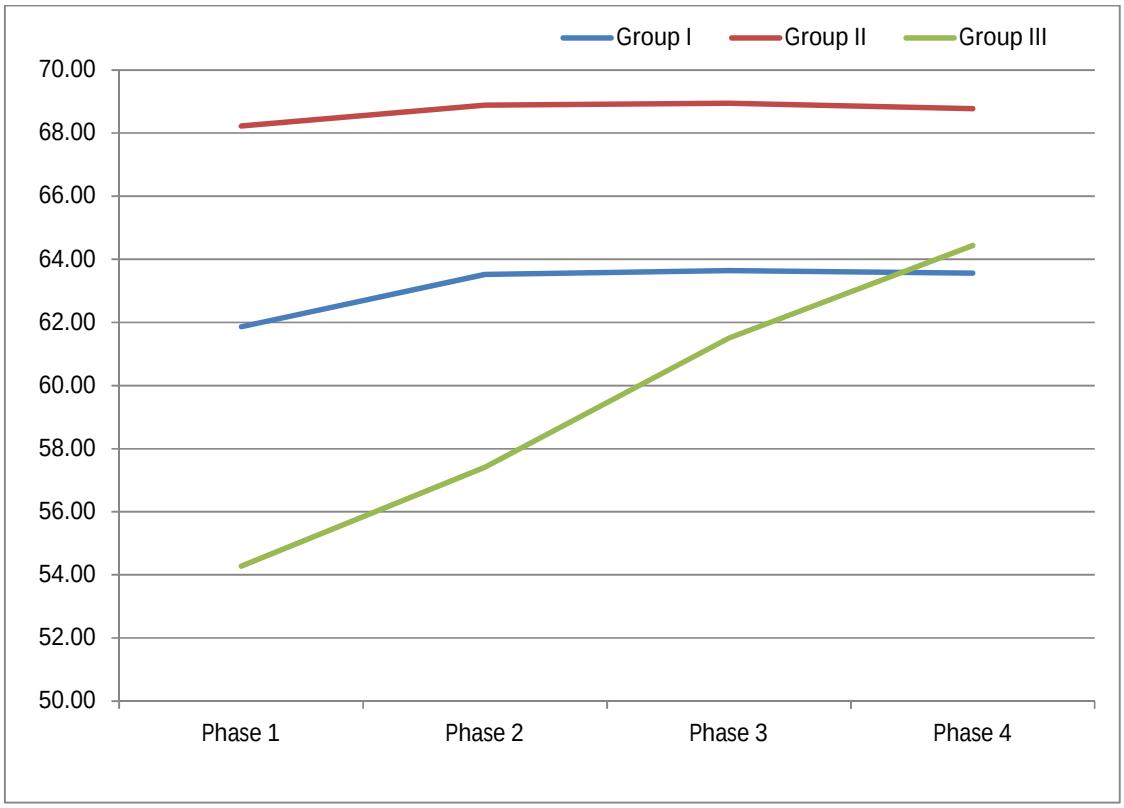

Figure 30: Pattern of change in the level of HDL-C in 16 weeks in all three different groups

5.6IMPACT ON LEVEL OF CHOLESTEROL: HDL-C RATIO

Cholesterol: HDL-C ratio was estimated in all three groups to determine risk of heart attack.

Group I Shows 3.87% (3.10 to 2.98 mg/dl) decrease in the Cholesterol: HDL-C ratio. This ration reduced in phase 2 by 2.90 % (3.10 to 3.01 mg/dl) and 4.19% (3.01 to 2.97 mg/dl) in phase 3. Then cholesterol: HDL-C ratio raised in phase 4 by 0.34% (2.97 to 2.98 mg/dl). Mean ± S.D. from phase 1 to phase 4 was 3.10 ± 0.51, 3.01 ± 0.42, 2.97 ± 0.30, and 2.98 ± 0.36 mg/dl.

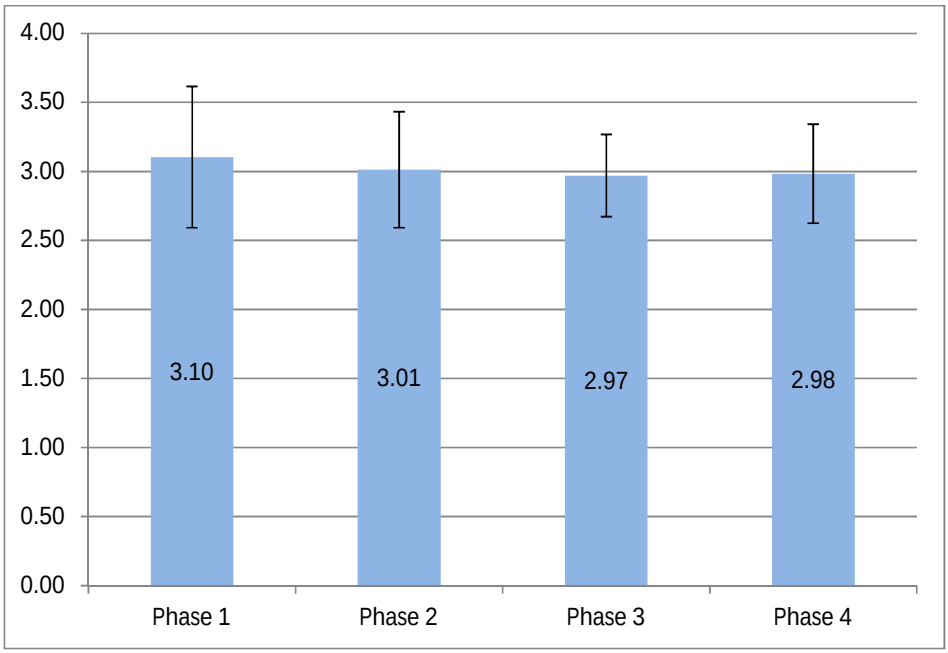

Figure 31: Mean Level of cholesterol: HDL-C ratio with S.D. in the form of error bars in all four phases in subjects of group I

In group II Cholesterol: HDL-C ratio was decreased up to 11.88% in 16 week time period by life style changes. In phase 2 this reduction was 3.77% (3.45 to 3.32 mg/dl), in phase 3 4.81% (3.32 to 3.16 mg/dl), and in phase 4 it was 3.80% (3.16 to 3.04 mg/dl). Mean ± S.D. values in all 4 phases was 3.45 ± 0.35, 3.32 ± 0.24, 3.16 ± 0.28 and 3.04 ± 0.27 mg/dl (figure 32).

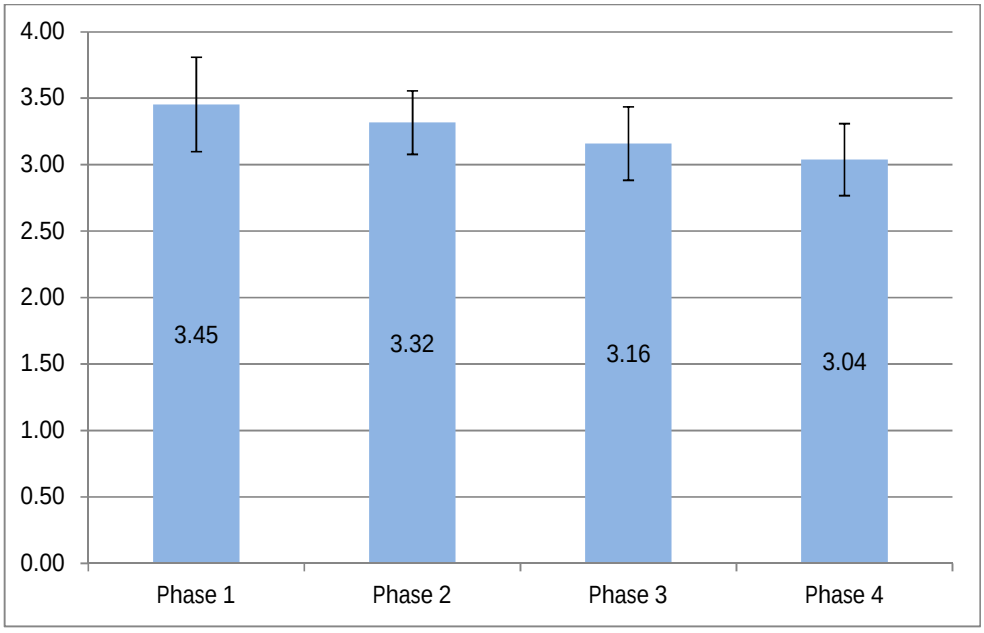

Figure 32: Mean Level of cholesterol: HDL-C ratio with S.D. in the form of error bars in all four phases in subjects of group II

Group III shows more reduction than group II. Cholesterol: HDL-C level was reduced by 30.44% 94.73 to 3.29 mg/dl) after 16 week medication and life style modification. This reduction from phase 2 to phase 4 was 11.63 % (4.73 to 4.18 mg/dl), 11.72 % (4.18 to 3.69 mg/dl), and 10.84 % (3.69 to 3.29 mg/dl). Overall mean ± S.D. of data of phase 1, 2, 3 and 4 (figure 33) was 4.73 ± 1.04, 4.18 ± 0.75, 3.69 ± 0.59, and 3.29 ± 0.49 mg/dl.

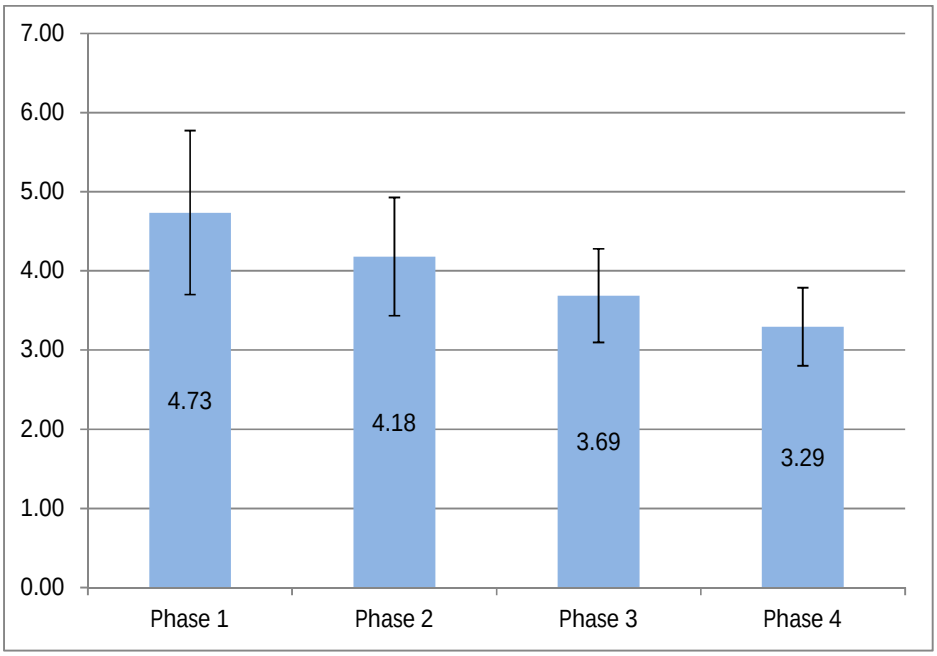

Figure 33: Mean Level of cholesterol: HDL-C ratio with S.D. in the form of error bars in all four phases in subjects of group III

If we consider about overall change in cholesterol: HDL-C ration in all three groups then group III shows more reduction 30.44% than all other two groups, followed by group II 11.88%, and group I shows least reduction of 3.87%. It shows life style modification is a more important factor in reduction cholesterol: HDL-C ration. This reduction was increased more than two times just with addition of medication in group III along with life style changes.

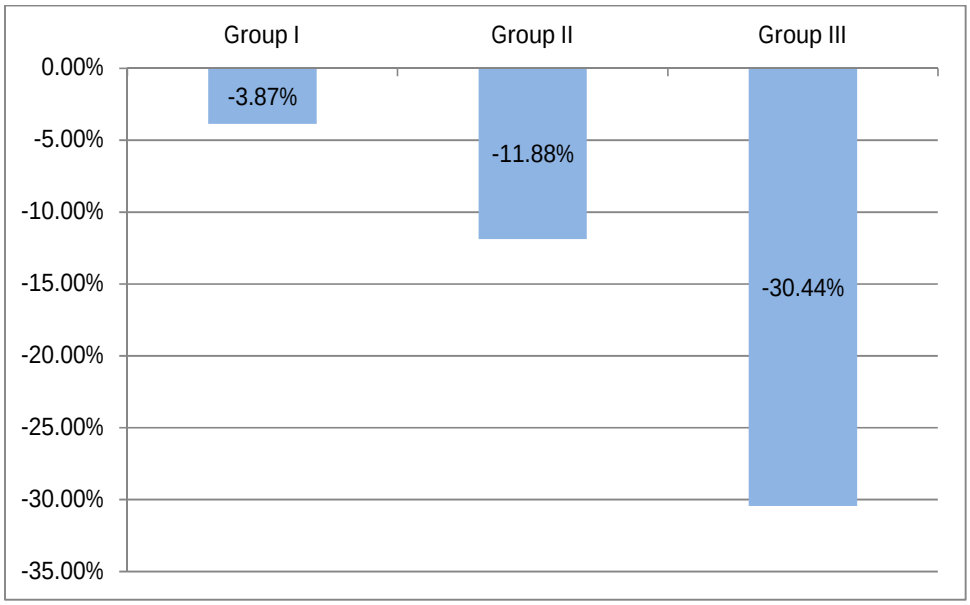

Figure 34: Total overall percentage of reduction in the level of cholesterol: HDL-C ratio in different groups

Following graph showing overall changes in the level of cholesterol: HDL-C ratio. Blue line represent group I which is almost straight horizontally because of little change in levels. Red and green lines going downward due to decrease in level of cholesterol: HDL-C ratio. Group III showing more reduction than group II which prove impact of medication along with life style modification.

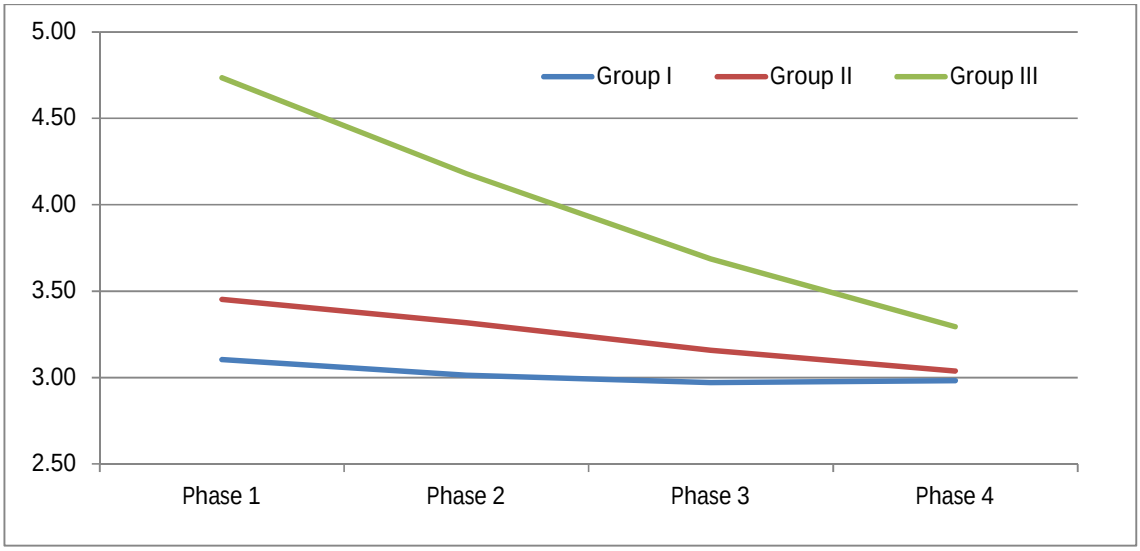

Figure 35: Pattern of change in the level of cholesterol: HDL-C ratio in 16 weeks in all three different groups

CHAPTER 6

SUMMARY AND CONCLUSION

Reduction in the level of cholesterol is very less in control group than all other groups. Second group showing more reduction in the level of cholesterol than control group which means, diet modification and daily exercise has great impact on cholesterol level in body. If we compare second group with third group then second group is lacking behind. In second group reduction was 11.74% whereas in third group it's 14.7%. The only extra thing which third group is taking is medication, which means medication along with diet modification and daily exercise has much more positive impact on cholesterol level than life style modification alone.

Similar kind of result found in triglyceride level also. Reduction in the level of TG is very less in control group than all other groups. In second group reduction was 17.21% whereas in third group it's 19.21%. it means medication along with diet modification and daily exercise has much more positive impact on triglyceride level.

In group I a rise of 0.38% in the level of LDL-C was reported which is not having any significant importance due to slight change. In group II reduction in LDL-C level was reported which is 17.13%, this shows that life style modification including changes in diet and daily exercise have significant impact on LDL-C reduction. But reduction in group III is more than group II it clearly indicates addition of medicine along with lifestyle modification speed up the rate of LDL-C level.

If we talk about all three groups then in group I change in the level of VLDL-C was 0%, whereas in group II due to life style modifications 16.95% VLDL-C was reduced. If we see the reduction in group III, it was 21.87%. it means if we add medicine along with life style modification then it can increase the rate of VLDL-C reduction.

Increase in HDL-C was 2.75% in group I, 0.82% in group II and 18.7% in group III. This data means life style modification not having significant impact on HDL-C level as there is not any significant raised values was obtained from group II, but medication shown its full impact on level of HDL-C as HDL-C level raised by 18.7% in group III which is highly significant.

If we consider about overall change in cholesterol: HDL-C ration in all three groups then group III shows more reduction 30.44% than all other two groups, followed by group II 11.88%, and group I shows least reduction of 3.87%. It shows life style modification is a more important factor in reduction cholesterol: HDL-C ration. This reduction was increased more than two times just with addition of medication in group III along with life style changes.

Data was also compared gender wise to find out difference in male and female subjects, but no significant difference was found. Only minor difference was reported in all three group which is not significant clinically.

From this study it's clear that life style modification shown its impact on reduction level of cholesterol, triglyceride, LDL-C and VLDL-C, but it fail to increase level of HDL-C. On addition of medicine along with life style modification the reduction in the level of cholesterol, triglyceride, LDL-C and VLDL-C increased sharply and it also increases level of HDL-C.

So if a person improves his/her lifestyle they can reduce the risk of CVD.

BIBLIOGRAPGY

1. Minino AM, Murphy SL, Xu J, Kochanek KD. Deaths: final data for 2008. Natl Vital Stat Rep. 2011;59:1–126.
2. Grundy, S. M., D. Bilheimer, H. Blackburn, W. V. Brown, P. 0. Kwiterovich et al; Rationale of the diet-heart statement of the American Heart Association. Cirdation. 1982:65:839A-854A.
3. Eurlings PM, van der Kallen CJ, Geurts JM, van Greevenbroek MM, de Bruin TW. Genetic dissection of familial combined hyperlipidemia. Mol Genet Metab 2001;74:98-104.
4. Ros E. Intestinal absorption of triglyceride and cholesterol. Dietary and pharmacological inhibition to reduce cardiovascular risk. Atherosclerosis. 2000 Aug;151(2):357-79.
5. Weiss SB, Kennedy EP, Kiyasu JY. The enzymatic synthesis of triglycerides. J. Biol. Chem.1960;235:40–44.
6. Lehner R, Kuksis A. Biosynthesis of triacylglycerols. Prog. Lipid Res. 1996;35(2):169–201.
7. Dircks L, Sul HS. Acyltransferases of de novo glycerophospholipid biosynthesis. Prog. Lipid Res.1999;38(56):461–479.
8. Go AS, Mozaffarian D, Roger VL, Benjamin EJ, Berry JD, Blaha MJ et al; American Heart Association Statistics Committee and Stroke Statistics Subcommittee. Heart disease and stroke statistics—2014 update: a report from the American Heart Association. Circulation. 2014;129:e28–e292
9. Kelly RB. Diet and exercise in the management of hyperlipidemia. Am Fam Physician. 2010; 81: 1097-102.
10. Tamer G, Mert M, Tamer I, Mesci B, Kilic D, Arik S. Effects of thyroid autoimmunity on abdominal obesity and hyperlipidaemia. Endokrynol Pol 2011; 62: 421-428.
11. Marshall WJ. Lipids and Lipoproteins. In: Illustrated Text Book of Clinical Chemistry, 2nd edition, Gower Medical Publishing, London. 1992: 222 – 237.
12. Bennett DR. Drug Evaluation Annual. Published by the American Medical Association 1995: 2455-2500.

13. Baron RB. Lipid Abnormalities. In: Current Medical Diagnosis and Treatment. The McGraw-Hill Company, 44th ed. 2005: 1202-1213.
14. Rosuvastatin-a new lipid-lowering drug. Med Lett Drugs Ther 2003; 45: 81-83.
15. Hasani-Ranjbar S, Nayebi N, Moradi L, Mehri A, Larijani B, Abdollahi M. The efficacy and safety of herbal medicines used in the treatment of hyperlipidemia; a systematic review. Curr Pharm Des 2010; 16: 2935-2947
16. Jones P. Comparative dose efficacy study of atorvastatin versus simvastatin, pravastatin, lovastatin and fluvastatin in patients with hypercholesterolemia (The CURVES study). Am J Cardiol 1998; 81: 582-587.
17. Davidson MH. Safety profiles for the HMG-CoA Reductase Inhibitors. Drugs 2001; 61: 197-206
18. Katcher HI, Hill AM, Lanford JL, Yoo JS, Kris-Etherton PM. Lifestyle approaches and dietary strategies to lower LDL-cholesterol and triglycerides and raise HDL-cholesterol. Endocrinol Metab Clin North Am. 2009;38(1):45–78.
19. Raine KD. Addressing poor nutrition to promote heart health: moving upstream. Can J Cardiol 2010;26:21C-24C.
20. Health Canada. Canada's Food Guide. 2011. Available online: http://www.hc-sc.gc.ca/fn-an/foodguide-aliment/index-eng.php (accessed on 28 November 2013)
21. The American Heart Association's Diet and Lifestyle Recommendations. Available online:http://www.heart.org/HEARTORG/GettingHealthy/Diet-and-Lifestyle-Recommendations_UCM_ 305855_Article.jsp (accessed on 20 November 2013).
22. Van Gaal, L.F.; Wauters, M.A.; De Leeuw, I.H. The beneficial effects of modest weight loss on cardiovascular risk factors. Int. J. Obes. Relat. Metab. Disorders 1997, 21, S5–S9.
23. Bueno, N.B.; Vieira de Melo, I.S.; Lima de Oliveira, S.L.; da Rocha Ataide, T. Very-low carbohydrate ketogenic diet v. low-fat diet for long-term weight loss: A meta-analysis of randomised controlled trials. Brit. J. Nutr. 2013;110:1178–1187.
24. Krieger, J.W.; Sitren, H.S.; Daniels, M.J.; Langkamp-Henken, B. Effects of variation in protein and carbohydrate intake on body mass and composition during restriction: A meta-regression. Am. J. Clin. Nutr. 2006;83:260–274.
25. Howard, B.V.; Van Horn, L.; Hsia, J.; Manson, J.E.; Stefanick, M.L.; Wassertheil-Smoller et al; Low-fat dietary pattern and risk of cardiovascular disease: The women's health initiative randomized controlled modification trial. J. Am. Med. Assoc. 2006;295:655–666.

26. Brehm, B.J.; Seeley, R.J.; Daniels, S.R.; D'Alessio, D.A. A randomized trial comparing a very low carbohydrate diet and a calorie-restricted low fat diet on body weight and cardiovascular risk factors in healthy women. J. Clin. Endocrinol. Metab. 2003;88:1617–1623.
27. Adrienne Forman. Foods That Lower Cholesterol. Available online: http://health.howstuffworks.com/diseases-conditions/cardiovascular/cholesterol/foods-that-lower-cholesterol2.htm
28. Durstine J.L. & W.L. Haskell. Effects of exercise training on plasma lipids and lipoproteins. Exercise and Sports Science Reviews. 1994;22:477-522.
29. Melanie Nichols, Nick Townsend, Peter Scarborough, and Mike Rayner. Cardiovascular disease in Europe, epidemiological update. European Heart Journal, 2014:10.1093
30. Prachi Bhatnagar, Kremlin Wickramasinghe, Julianne Williams, Mike Rayner, Nick Townsend. The epidemiology of cardiovascular disease in the UK 2014. Heart:10.1136
31. Friedman M, Friedland GW. Medicine's 10 greatest discoveries. New Haven (CT): Yale University Press; 1998. Available online: https://www.questia.com/library/journal/1P3-43804834/medicine-s-10-greatest-discoveries
32. Igor E. Konstantinov, MD, PhD, Nicolai Mejevoi, MD, PhD, and Nikolai M. Anichkov, MD, DMedSc. Nikolai N. Anichkov and His Theory of Atherosclerosis. Tex Heart Inst J. 2006; 33(4): 417–423.
33. Adel H. Allam, MD; Randall C. Thompson, MD; L. Samuel Wann, MD; Michael I. Miyamoto, MD, MS; Abd el-Halim Nur el-Din, PhD; Gomaa Abd el-Maksoud, PhD; Muhammad Al-Tohamy Soliman, et al; Atherosclerosis in Ancient Egyptian Mummies. J Am Coll Cardiol Img. 2011;4(4):315-327
34. Juha Pekkanen, M.D., Ph.D., Shai Linn, M.D., Dr.P.H., Gerardo Heiss, M.D., Ph.D., Chirayath M. Suchindran, Ph.D., Arthur Leon, M.D., Basil M. Rifkind, M.D., et al; Ten-Year Mortality from Cardiovascular Disease in Relation to Cholesterol Level among Men with and without Preexisting Cardiovascular Disease. N Engl J Med 1990; 322:1700-1707.

35. David J Decewicz, David M Neatrour, Amy Burke, Mary Jane Haberkorn, Heather L Patney, Marina N Vernalis et al; Effects of cardiovascular lifestyle change on lipoprotein subclass profiles defined by nuclear magnetic resonance spectroscopy, Lipids in Health and Disease2009;8:26
36. R. James Barnard, PhD. Effects of Life-style Modification on Serum Lipids, Arch Intern Med. 1991;151(7):1389-1394
37. Umamaheswari Kannan, Kavita Vasudevan, Kavita Balasubramaniam, Dhanalakshmi Yerrabelli, Karthik Shanmugavel, Nitin Ashok John. Effect of Exercise Intensity on Lipid Profile in Sedentary Obese Adults, J Clin Diagn Res. 2014; 8(7): BC08–BC10.
38. Neetu Mishra. The Role of Physical Exercise and Diet Modification on Lipid Profile and Lipid Peroxidation in Long Term Glycemic Control Type 2 Diabetics, Gen Med (Los Angel) 2014; 2:3-8
39. Hegsted, D. M.; Mcgandy, R. B.; Myers, M. L.; Stare, F. J. Quantitative effects of dietary fat on serum cholesterol in man. American Journal of Clinical Nutrition 1965 Vol. 17 pp. 281-295
40. Howard BV, Van Horn L, Hsia J, Manson JE, Stefanick ML, Wassertheil-Smoller S et al; Low-fat dietary pattern and risk of cardiovascular disease: the Women's Health Initiative Randomized Controlled Dietary Modification Trial. JAMA. 2006 Feb 8;295(6):655-666.
41. Shaomei Yu-Poth, Guixiang Zhao, Terry Etherton, Mary Naglak, Satya Jonnalagadda, and Penny M Kris-Etherton. dietary intervention programs on cardiovascular disease risk factors: a meta-analysis. Am J Clin Nutr. April 1999;69(4): 632-646
42. Artiss JD, Zak B. Measurement of cholesterol concentration. In: N Rifai, GR Warnick , MH Dominiczak (eds) Handbook of Lipoprotein Testing. AACC Press, Washington, 1997; 99-114.
43. Bachorik PS, Albers JJ: Precipitation methods for quantification of lipoproteins. In: Methods in Enzymology, Albers JJ and Segrest JP (eds), Academic Press, Orlando, 1986; Vol 129 (Part B), 78- 100
44. McGowan, M. W. Artiss, J. D. Stranberg, D. R. Zak, B. A.,: Peroxidase coupled method for the colorimetric determination of serum Triglycerides, Clin.Chem. 1983; 29: 538-542
45. Stone NJ, Robinson JG, Lichtenstein AH, Goff DC Jr, Lloyd-Jones DM, Smith SC Jr, et al. Treatment of blood cholesterol to reduce atherosclerotic cardiovascular disease risk in adults: synopsis of the 2013 American College of Cardiology/American Heart Association cholesterol guideline. Ann Intern Med. 2014 Mar 4;160(5):339-43.

46. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem. 1972;18:499-502.

47. Lemieux, I., Lamarche, B., Couillard, C., Pascot, A., Cantin, B., Bergeron, J., . . .& Despres, J. (2001). Total cholesterol/HDL cholesterol ratio vs LDL cholesterol/HDL cholesterol ratio as indices of ischemic heart disease risk in men. JAMA Internal Medicine, 161(22), 2685-2692.