"MORPHOLOGICAL CHANGES IN THE RED BLOOD CELLS AS A MARKER OF OXIDATIVE STRESS IN METABOLIC SYNDROME AND ANTIOXIDANTS AS AN ADD ON THERAPY IN THE REVERSAL OF CHANGES - A RANDOMIZED, OPEN LABEL, COMPARATIVE PILOT STUDY"

Dissertation submitted to

THE TAMILNADU

DR. M.G.R. MEDICAL UNIVERSITY

In partial fulfillment for the award of the degree of

DOCTOR OF MEDICINE

IN

PHARMACOLOGY

INSTITUTE OF PHARMACOLOGY
MADRAS MEDICAL COLLEGE
CHENNAI - 600 003
APRIL 2017

CERTIFICATE

This is to certify that the dissertation entitled, **"MORPHOLOGICAL CHANGES IN THE RED BLOOD CELLS AS A MARKER OF OXIDATIVE STRESS IN METABOLIC SYNDROME AND ANTIOXIDANTS AS AN ADD ON THERAPY IN THE REVERSAL OF CHANGES - A RANDOMIZED, OPEN LABEL, COMPARATIVE PILOT STUDY"** submitted by DR.GOWTHAMI.R, in partial fulfillment for the award of the degree of Doctor of Medicine in Pharmacology by The Tamil Nadu Dr.M.G.R.Medical University, Chennai is a bonafide record of the work done by her in the Institute of Pharmacology, Madras Medical College during the academic year 2014-17.

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CERTIFICATE OF THE GUIDE

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Place:
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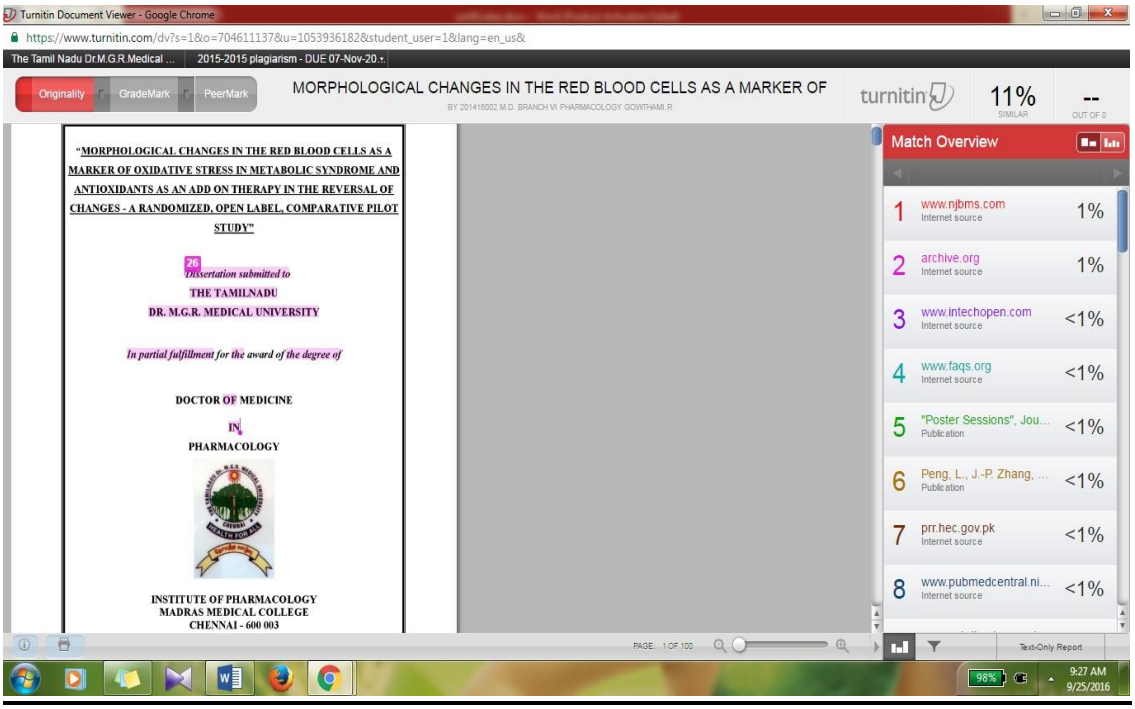
DECLARATION

I, Dr. GOWTHAMI.R solemnly declare that the dissertation titled **"MORPHOLOGICAL CHANGES IN THE RED BLOOD CELLS AS A MARKER OF OXIDATIVE STRESS IN METABOLIC SYNDROME AND ANTIOXIDANTS AS AN ADD ON THERAPY IN THE REVERSAL OF CHANGES - A RANDOMIZED, OPEN LABEL, COMPARATIVE PILOT STUDY"** been prepared by me and submitted to Tamil Nadu Dr.MGR Medical University, Chennai in partial fulfillment of the rules and regulations for the M.D degree examination in Pharmacology.

Date: DR. GOWTHAMI.R

Place:

TURNITIN ANTI-PLAGIARISM SOFTWARE – CERTIFICATE



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DEDICATION

I dedicate this book to my dad **MR. K. RAJARAM** who wanted me to be a great doctor.

"MISS YOU DAD"

CONTENTS

INTRODUCTION

INTRODUCTION

Metabolic syndrome includes a combination of any three of the following like abdominal obesity, hyperglycemia, hypertension and hypertriglyceridemia. It is often associated with increased risk of development of Type 2 Diabetes mellitus and Cardiovascular disease[1].

The coexistence of insulin resistance, hyperglycemia, hypertension and hypertriglyceridemia in many patients indicates that all these conditions are caused by same etiological factor.

The prevalence of metabolic syndrome is approximately twice in urban areas than in rural population. This may be due to unfavourable modification of lifestyle and dietary habits in a presumably genetically vulnerable population[2].

Mortality in metabolic syndrome is mainly due to coronary heart disease and stroke. Micro vascular complications like diabetic retinopathy, diabetic nephropathy and neuropathy are serious health problems in deterioration of the quality of life and premature death.

Psychological stress plays an important role in pathophysiology of metabolic syndrome due to over activity of sympathetic nervous system which leads to elevated Adrenaline followed by Glucagon, Corticosteroids and Growth hormone levels[3].

Insulin resistance is mainly due to increased secretion of Insulin and decrease in activity due to increased activity of anti-insulin hormones like Adrenaline, Glucagon and Corticosteroids.

Oxidative stress plays an important role in the development of insulin resistance, macro and micro vascular complications of diabetes. It also alters the balance between sympathetic and parasympathetic system leading to hypertension[4].

Red blood cells (RBCs) are the first cells to be exposed to reactive oxygen species. ROS can cause structural damage to the cells like cell membrane damage causing crenated edges and Heinz bodies due to damaged hemoglobin. Therefore RBC morphology can be used as a marker for oxidative stress[5].

In this study antioxidants like Alpha tocopherol and Ascorbic acid are given to the patients with metabolic syndrome to reverse insulin resistance, decrease blood pressure and to correct dyslipidemia using RBC as a biomarker for oxidative stress along with clinical improvement[6].

REVIEW OF LITERATURE

REVIEW OF LITERATURE

METABOLIC SYNDROME

DEFINIITON

Metabolic syndrome is a cluster of abnormalities that includes glucose intolerance, insulin resistance, hyperinsulinemia, dyslipidemia (low high density lipoprotein and elevated triacylglycerols) hypertension and abdominal obesity. The metabolic syndrome is also associated with chronic systemic inflammation that contributes to the pathogenesis of insulin resistance and atherosclerosis[7].

EPIDEMIOLOGY

Metabolic syndrome is considered as one of the major public health problems. For the past few years its prevalence has increased worldwide due to lifestyle changes, increasing obesity and reduced physical activity. International Diabetic Federation have estimated the prevalence as 20-25% among the adult population in the world[8]. About one-third of urban South Asians has metabolic syndrome[9]. In India study done by Deepa et al. among adult population of >20 years in an urban city have showed 18.3% prevalence. In this study 17% of males and 19.4% of females showed features of metabolic syndrome. Prevalence is higher among females and progressively increasing upto the age of 69 years[10]. People diagnosed with metabolic syndrome have three times increased risk of developing cardiovascular disease and five times greater

risk of developing type 2 diabetes mellitus. They may add to 366 million diabetic patients worldwide in 2030.

PATHOGENESIS[11]:

Metabolic syndrome develops as a result of complex interplay of genetic, environmental factors, psychological stress and chronic inflammation. Insulin resistance is the important pathogenic mechanism that leads to the development of hyperglycemia and dyslipidemia.

Insulin resistance is the failure of target tissues to respond normally to insulin. This insulin resistance results in

1. Increased gluconeogenesis in liver

2. Decreased cellular uptake of glucose and synthesis of glycogen in skeletal muscle.

3. Increased activity of enzyme hormone sensitive lipase in adipose tissue induces lipolysis and production of free fatty acid into the circulation this in turn aggravates insulin resistance[12].

4. Reduced levels of glucose transporter GLUT 4 on the cell membrane and reduction in the tyrosine phosphorylation of Insulin receptor and Insulin Receptor Substrate proteins (IRS) in peripheral tissues.

1. INFLAMMATION

Chronic inflammation plays an important role in the pathogenesis of metabolic syndrome and type 2 diabetes[7]. Presence of proinflammatory cytokines like IL-1,

TNF-α and inflammatory mediators like F2 Isoprostanes (8-Iso-PGF2α) in plasma confirms this hypothesis[4,14].

IL-1 and TNF-α are released from mononuclear cells, macrophages and adipocytes in response to tissue damage. These proinflammatory cytokines induce the synthesis of various inflammatory mediators like Platelet activating factor (PAF), Prostaglandins and Leukotriens. In addition to this they also mediate some inflammatory responses[13].

Several families of isoeicosanoids (F2 Isoprostanes) are generated at significant concentration by non-enzymatic oxidation of Arachidonic acid catalysed by free radicals[13].

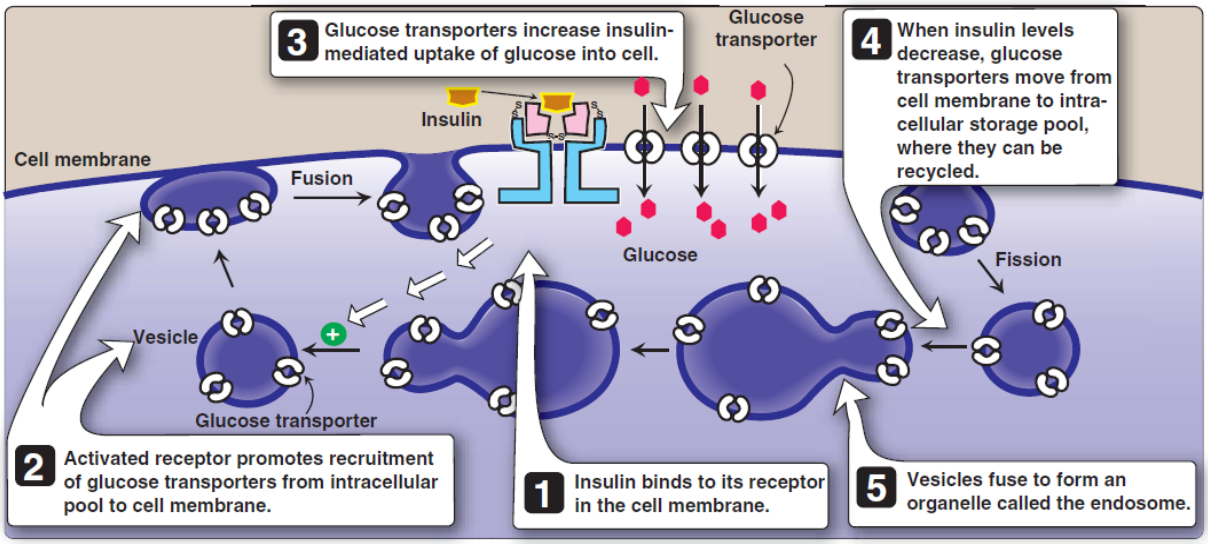
The production of these isoprostanes can be suppressed by antioxidants and not inhibited by NSAIDS. Measuring the levels of PGF2α Isomers, 8-Iso-PGF2α is considered the most accurate method to assess oxidative stress status in vivo[13].

These isoprostanes can bind to prostanoid receptors and activate inflammatory responses. They can also directly bind to cells and cause cell damage including pancreatic β cell damage.

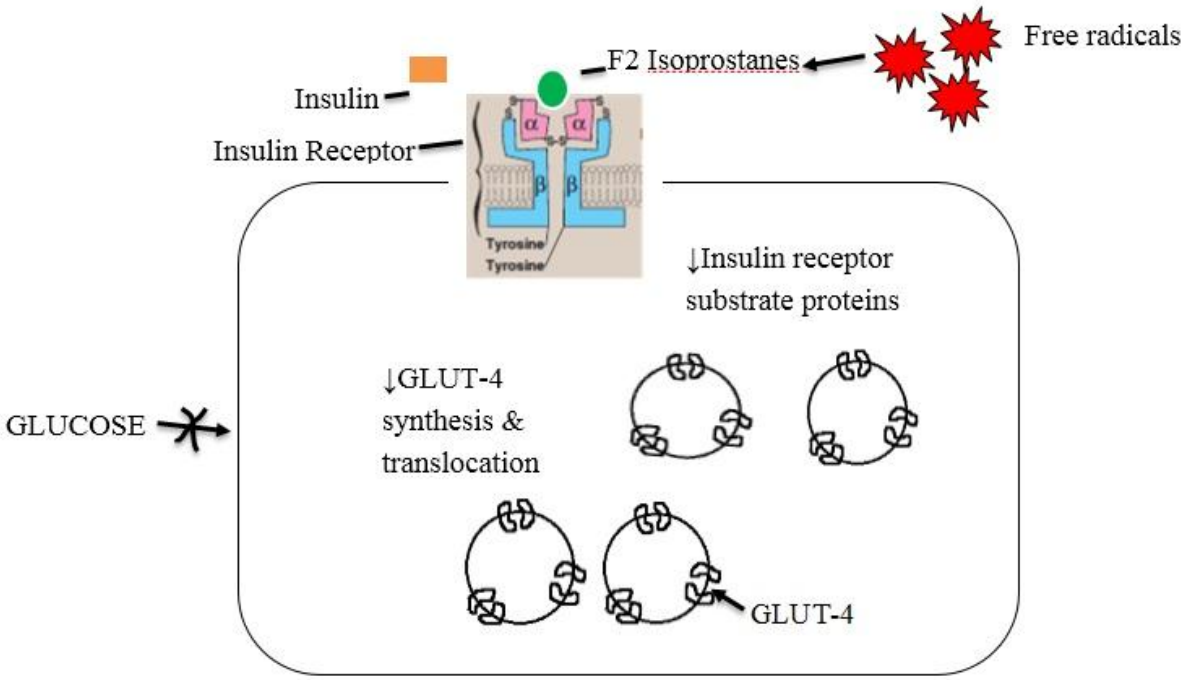
INSULIN RESISTANCE

The circulating isoprostanes binds to the insulin receptor and antagonize the effect of insulin[14, 15].This prevents the recruitment of glucose transporter-4 (GLUT-4) by Insulin from the cytoplasm to cell membrane and inhibit transport of glucose into the cell causing hyperglycemia and insulin resistance[12].

I. Recruitment of Glucose transporter GLUT – 4 from intracellular stores to the cell membrane mediated by insulin



II. Inhibition of recruitment of Glucose transporters to the cell membrane due to oxidative stress.



2. DIABETES MELLITUS

Under normal condition skeletal muscle and adipose tissue requires insulin for glucose transport. Many tissues like Hepatocytes, Erythrocytes, Central nervous system, Renal tubules, Cornea etc., have insulin insensitive system for glucose transport[12].

The binding of insulin to the receptor in the cell membrane of skeletal muscle and adipocytes promotes the recruitment of insulin-sensitive glucose transporter (GLUT-4) from a pool present in intracellular vesicles. This increases glucose transport within seconds which is an immediate response of insulin. Changes in enzymatic activity and phosphorylation of proteins induced by insulin occurs over minutes to hours. This is due to increase in gene expression through increased transcription and translation[12].

Psychological stress activates sympathetic nervous system and increases release of Adrenaline which in turn increases Glucagon secretion by stimulating α cells of pancreas (β_2 receptor action) and inhibits insulin release from beta cells (α_2 receptor action)[16].Adrenaline and Glucagon elevate blood glucose level by increasing breakdown of liver glycogen (glycogenolysis) and gluconeogenesis from fat and amino acids. They also block the action of insulin aggravating hyperglycemia[17].

GLUCONEOGENESIS[18]

Gluconeogenesis is the synthesis of glucose in body with the help of cytosolic and mitochondrial enzymes from precursors such as lactate, pyruvate, glycerol, α-ketoacids. Liver (90%) and Kidney (10%) are the major organs producing glucose.

Prolonged fasting, decreased insulin and increased stress hormones such as adrenaline and glucagon stimulate gluconeogenesis[18].

Catecholamines and Glucagon cause lipolysis in adipose tissue through activation of adenylyl cyclase. This stimulates enzyme Hormone sensitive lipase (HSL) in adipocytes and produces hydrolysis of Triacylglycerol (TAG) into Glycerol and Free fatty acids. Glycerol is used for glucose production and free fatty acids are used for synthesis of Triglycerides, VLDL and LDL in liver[19].

INSULIN RESISTANCE

Anti-insulin hormones (Adrenaline, Glucagon) along with Reactive oxygen species (Oxidative stress) and inflammatory cytokines (TNF-α, IL-6, Isoprostanes) contributes to insulin resistance in Type 2 diabetes[20].

HYPERINSULINEMIA

Normally pancreatic beta cells respond to the hyperglycemia by increasing release of more insulin into the circulation to reduce plasma glucose level resulting in **hyperinsulinemia**. Hyperinsulinemia itself causes downregulation of insulin receptor by increasing internalization and degradation of insulin receptor contributing to insulin resistance[21].Hyperglycemia (glucotoxicity) and increased free fatty acids (lipotoxicity) compromise beta cell function and suppress insulin release resulting in type 2 diabetes mellitus[21]. Diagnosis of type 2 diabetes mellitus is done when the measured fasting plasma glucose ≥ 126 mg/dL, random plasma glucose ≥ 200 mg/dL, HbA1c > 6.5%.

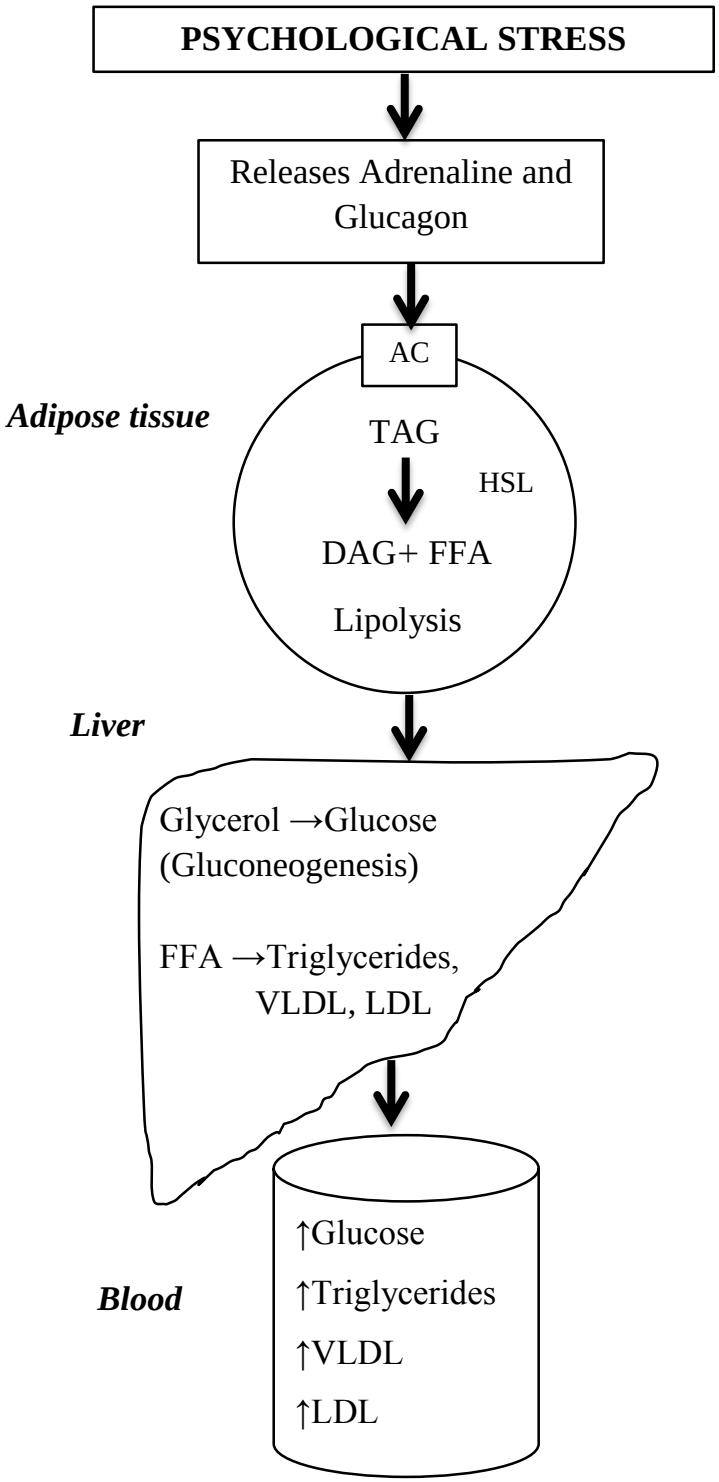


Chart 1: Pathogenesis of Diabetes mellitus.

TAG - Triacylglycerol; DAG – Diacylglycerol; AC – Adenylyl cyclase; HSL- Hormone sensitive lipase; FFA- Free fatty acid; IR- Insulin receptor; IRS- Insulin receptor substrates; GLUT4- Glucose transporter 4; LDL- Low density lipoprotein; VLDL- Very low density lipoprotein

3. HYPERTENSION [22- 24]

Hypertension is diagnosed when systolic blood pressure is ≥140 mmHg and diastolic blood pressure is ≥ 90mmHg. Essential hypertension has multifactorial origin. Environmental factors like smoking, alcohol and emotional stress stimulate sympathetic activity in a genetically prone person to develop hypertension.

Following psychological stress, Epinephrine and Norepinephrine are released which stimulate α_1 receptor and increase peripheral vascular resistance, thereby increasing systolic and diastolic blood pressure. They also stimulate Renin angiotensin system and secretion of Aldosterone which contributes to vasoconstriction and sodium and water retention respectively. Corticosteroids sensitize the blood vessels to vasoconstrictor effect of adrenaline and also cause salt and water retention[17].

Oxidative stress (ROS) reduces the bioavailability of Nitric oxide (NO) by reacting with nitric oxide to produce Peroxynitrite ($ONOO^-$) which is a reactive nitrogen species (RNS). This decreased NO level increases peripheral vascular resistance due to unopposed α_1 sympathetic action[22].

Nitric oxide regulates autonomic function in the central nervous system. Decreased availability of nitric oxide in the brain leads to imbalance in parasympathetic and sympathetic nervous system. This also contributes to sympathetic overactivity[23].

Atherosclerosis causes arterial stiffness and narrowing of the lumen. ROS oxidizes the circulating Low density lipoprotein (LDL) which is taken up by the

macrophages to form foam cells. These foam cells are deposited on the endothelium causing atheromatous plaques[24].

Increased sympathetic activity causes activation of renin angiotensin system, increases release of aldosterone and corticosteroids. This contributes to hypertension in metabolic syndrome. Hypertension and Atherosclerosis are the risk factors for cardiovascular disease (CVD) in metabolic syndrome.

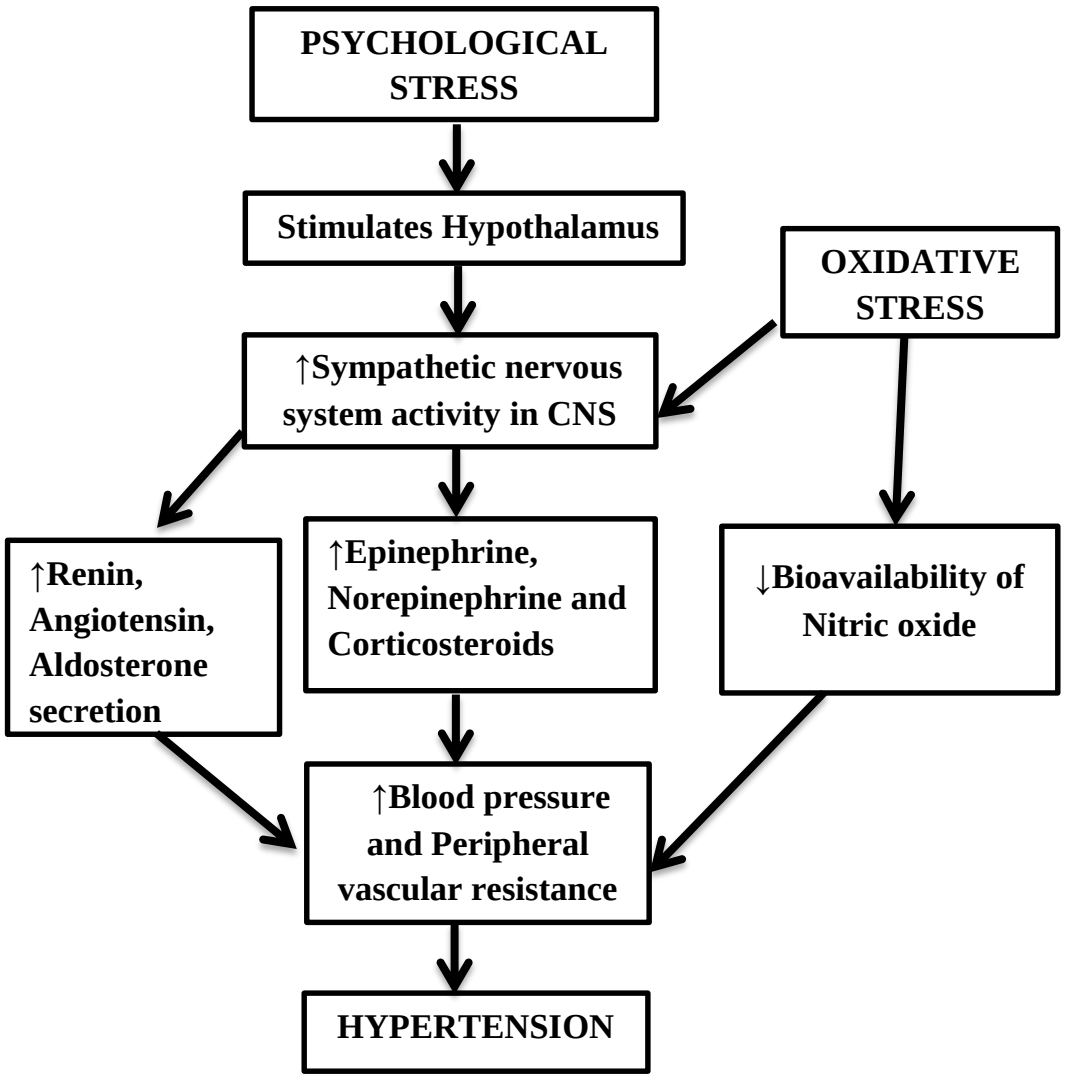


Chart 2: Pathogenesis of Hypertension.

4. DYSLIPIDEMIA[24]

Dyslipidemia is characterized by elevated total cholesterol > 200 mg/dL, triglycerides >150 mg/dL, raised very low density lipoprotein (VLDL) >30 mg/dL, raised low density lipoprotein (LDL) >100 mg/dL and decreased High density lipoprotein (HDL) < 40 mg/dL in males and < 50 mg/dL in females.

Hypertriglyceridemia is the most common lipid abnormality in patients with insulin resistance. This is due to an increase in synthesis of triglyceride rich VLDL in liver from fatty acids that are released due to lipolysis by Adrenaline and Glucagon.

Adrenaline and Glucagon activates enzyme Hormone sensitive lipase (HSL) in adipocytes and produces hydrolysis of Triacylglycerol (TAG) into Glycerol and Free fatty acids. Glycerol is used for glucose production and free fatty acids are used for synthesis of triglycerides, VLDL and LDL in liver[19].

VLDL is secreted into the blood by the liver. Triacylglycerol in VLDL is degraded by lipoprotein lipase converting VLDL to LDL in the plasma. The primary function of LDL particles that are rich in cholesterol and cholesteryl esters, is to provide cholesterol to the peripheral tissues. LDL bind to the LDL receptor present on cell membrane. The LDL receptor complex is internalized by endocytosis.

The vesicle containing LDL is transferred to lysosome where it is degraded by lysosomal acid hydrolases releasing free cholesterol, amino acids, fatty acids and phospholipids. These fatty acids are converted to cholesterol by enzyme HMG CoA reductase.

In oxidative stress the ROS oxidize the circulating LDL thus preventing the LDL to bind with LDL receptor present on the cell membrane to deliver the cholesterol. This increases the level of LDL in plasma. Oxidized LDL is taken up by the macrophages to form foam cells. These foam cells are deposited on the endothelium causing atheromatous plaques.

HDL is a heterogenous lipoprotein that contains apolipoprotein (Apo A-I) synthesized by the liver and acquires lipid in the blood. HDL particles serve as a reservoir of Apo C-II (Apolipoprotein) which is an activator of lipoprotein lipase. It takes up cholesterol from non-hepatic (peripheral) tissues and returns it to liver as cholesteryl esters. This process is called Reverse cholesterol transport. The cholesterol is used for the bile acid synthesis which is excreted via bile and to the steroidogenic cells for synthesis of hormones. Hence they are designated as "Good cholesterol carrier". Nascent HDL are disc shaped particles containing phospholipids and apolipoproteins A, C and E.

In reverse cholesterol transport cholesteryl ester rich HDL (HDL_2) delivers esterified cholesterol to liver and steroidogenic cells. The lipid depleted HDL (HDL_3) is released back into blood. This is mediated by scavenger receptor in the liver.

The oxidized LDL cannot bind to LDL receptor and deliver cholesterol to the cells. This depletes the cholesterol storage in cells. As a result HDL cannot take up cholesterol from peripheral tissues. The lipid depleted HDL (HDL_3) is degraded due to lipid poor apoprotein (Apo A- I). This decreases the plasma level of HDL in oxidative stress.

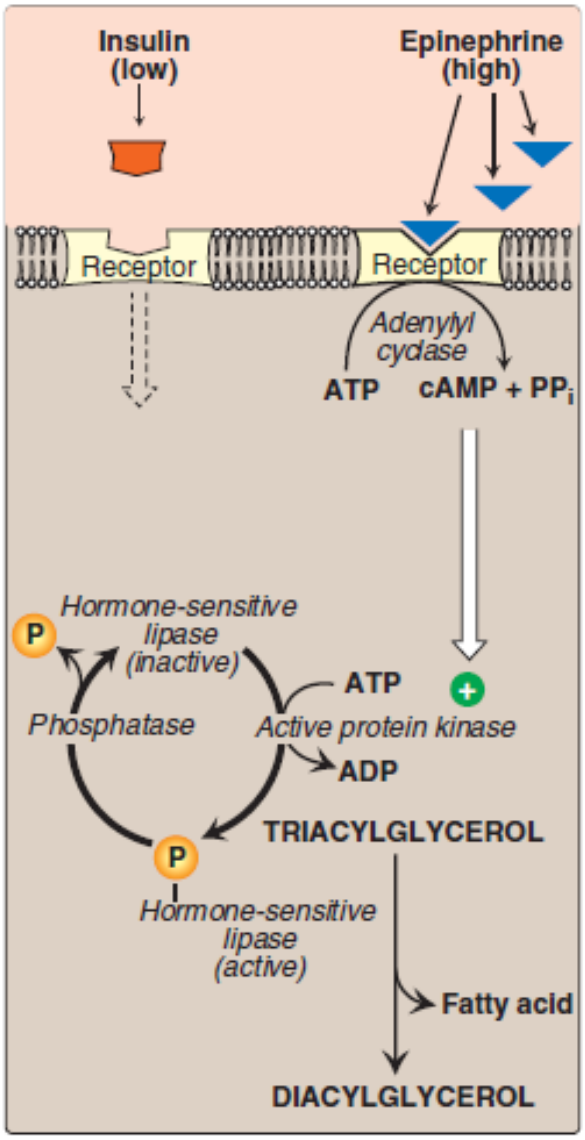


Chart 3: Hormonal regulation of triacylglycerol degradation in the adipocyte.

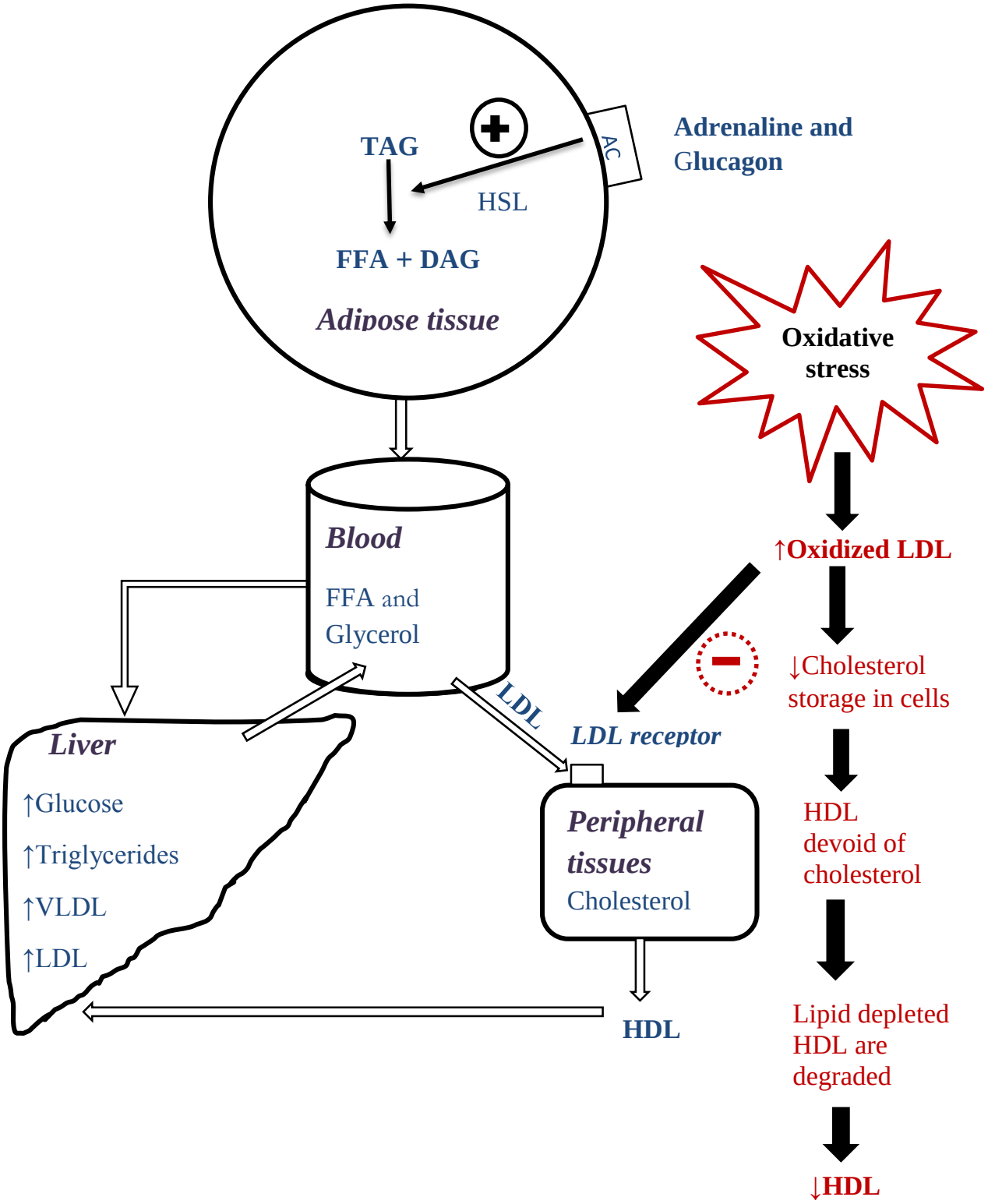


Chart 4: Pathogenesis of Dyslipidemia

[TAG - Triacylglycerol; DAG- Diacylglycerol; HSL- Hormone sensitive lipase; AC - Adenylyl cyclase; FFA- Free fatty acid]. Glycerol is used for synthesis of glucose (gluconeogenesis).Free fatty acids are used for synthesis of Triglycerides, VLDL and LDL. Lipid depleted HDL undergoes degradation.

5. OBESITY[7, 11]

Obesity is defined as a state of excessive adipose tissue mass and statistically as a weight that is 20% or more above the average weight per height. More than 80% individuals in type 2 diabetes are obese and incidence of type 2 diabetes increases proportionately with obesity[11]. Body mass index calculation gives the measurement of classes of body weight.

Class of body weight	**Body mass index (BMI)**
Underweight	< 18.5
Normal	18.5 to 24.9
Overweight	25 to 29.9
Obese	≥ 30

Adipose tissue not only acts a store house of fat it also has endocrine function that releases cytokines in response to changes in metabolic status. The variety of proteins secreted by adipocytes into the systemic circulation and are collectively called as adipokines or adipose cytokines. They are Adiponectin, Leptin, Interleukin-6 (IL-6) and Tumour necrosis factor – α (TNF-α). TNF-α and IL-6 increases lipolysis in adipocytes and decreases insulin sensitivity[11].

Adiponectin, an adipocyte cytokine is released during lipolysis by anti-insulin hormones. Adiponectin increases insulin release, decreases inflammation and increases food intake by stimulating feeding centre in hypothalamus. In obesity the level of adiponectin is low and contributes to insulin resistance[7].

Leptin, an adipocyte cytokine, the secretion increases in proportionate to fat stores following a full meal. It prevents overconsumption of calories by inhibiting feeding centre in the hypothalamus. When the body fat decreases, Leptin level decreases and Adiponectin level increases[7].

RISK FACTORS [25]:

1. ENVIRONMENTAL FACTORS:

Lifestyle changes, reduced physical activity and dietary habits such as consumption of high fat and low fibres increases the risk of development of insulin resistance and type 2 diabetes mellitus.

2. GENETIC FACTORS:

The high prevalence of insulin resistance in Nauru Islanders of the Pacific, the Pima Indians in Arizona, and the urban Wanigela people in Papua New Guinea suggests a strong association between genetic factors and insulin resistance. Any mutations in the insulin receptors genes, the nuclear receptor peroxisome proliferator activator receptor γ (PPAR γ), polymorphism of genes relevance to insulin action leads to insulin resistance.

3. AGING

Metabolic syndrome is more common in elderly population due to age associated reduction in the functions of mitochondria contributes to insulin resistance.

4. OBESITY

As the adiposity increases, the risk of developing associated diseases such as diabetes, hypertension and cardiovascular disease increases[7].

CLINICAL PRESENTATION [26, 27.]

Patients with metabolic syndrome are mostly asymptomatic. Some may present with raise in waist circumference and Acanthosis nigricans, a velvety pigmented hyperkeratotic skin lesion of flexures and neck. If they develop diabetes they may present with common symptoms like Polyuria, Polydipsia, Polyphagia and Acute Weight loss. Fatigue, Blurring of vision, Pruritis vulvae or Balanitis due to Candida, Staphylococcal skin infections can also occur. In case of Hypertension patient may complain of Headache, Somnolence, Nausea, Vomiting, Confusion and Visual Disturbance. Presence of Xanthoma, Xanthelasma and Corneal Arcus before 40 years points to dyslipidemia.

PHYSICAL EXAMINATION [26, 27, 28]

- Weight and height measurement (Body Mass Index)
- Waist circumference
- Measurement of Blood pressure
- Visual acuity testing
- Fundoscopic examination
- Checking peripheral pulses and sensations of foot
- Screening of skin and dental infections.
- Acanthosis nigricans

COMPLICATIONS:

- Retinopathy
- Nephropathy
- Neuropathy
- Ischemic or hemorrhagic Stroke
- Coronary artery disease
- Peripheral vascular disease
- Malignant hypertension
- Heart failure
- Pancreatitis
- Retinal vein thrombosis

INVESTIGATIONS:

- Complete blood count
- Fasting and Post Prandial Blood sugar level
- HbA1C
- HOMA-IR for insulin resistance
- Serum Urea and Creatinine
- Serum lipid profile
- Urine for protein, sugar
- Serum electrolytes
- Liver function test
- Fundus examination
- ECG, Echocardiogram
- Doppler and Nerve conduction studies

DIAGNOSTIC CRITERIA[29]:

According to National Cholesterol Education Program (NCEP) Adult Treatment Panel III in 2001, diagnosis of metabolic syndrome requires the presence of at least three of the following five criteria:

- Central obesity: Waist circumference > 102cm in men, >88cm in women.

- Hypertriglyceridemia : Triglycerides ≥150 mg/dL or specific medication
- Low high density lipoprotein (HDL) cholesterol: < 40 mg/dL in men, < 50 mg/dL in women or specific medication.
- Hypertension: Blood pressure ≥130/85 mmHg or specific medication.
- Fasting plasma glucose ≥100 mg/dL or specific medication or previously diagnosed type 2 diabetes.

MANAGEMENT

1. Non pharmacological management
   - Diet
   - Physical activity
   - Stress reduction
2. Treatment of associated condition.
   - Insulin resistance
   - Diabetes mellitus
   - Hypertension
   - Dyslipidemia
3. Management of complications
   - Retinopathy
   - Nephropathy
   - Neuropathy
   - Cardiovascular diseases

NON PHARMACOLOGICAL MANAGEMENT[30]:

1. Diet

- Diet should be rich in fresh fruits, vegetables, whole grains, lean poultry and fish.

- Fat consumption should be kept at approximately 30% of total calories intake in which saturated fat should be < 7% and trans fat should be < 1%
- Reducing 500 kcal intake per day leads to weight loss of one pound per week.

2. Physical activity

- Increase in physical activity improves insulin sensitivity, decreases blood pressure, lowers lipid abnormalities and reduces obesity.
- At least 30 – 60 minutes /day of moderate intensity aerobic exercises such as walking, swimming, jogging, cycling and climbing should be carried out for ≥3 times /week.
- Patients of > 35 years of age with sedentary life style should undergo cardiac evaluation before initiation of an exercise program.

3. Stress reduction

- Yoga, Meditation, Relaxation therapy.
- Breathing exercises.

MEDICAL MANAGEMENT[31-33]:

A. INSULIN RESISTANCE[31]:

1. METFORMIN

- It is a biguanide, increases insulin sensitivity by activation of AMP dependent protein kinase. Reduces liver gluconeogenesis and increases glucose uptake.
- Adverse Effects: Abdomen pain, nausea, metallic taste, vitamin B12 deficiency.

2. THIAZOLIDINEDIONES:

- Selective agonist for nuclear receptor Peroxisome proliferator activated receptor γ (PPAR γ) that enhances transcription of genes responsible for insulin action, fatty acid metabolism and increases GLUT 4 expression.
- Adverse Effects: Plasma expansion, edema, weight gain, hepatic dysfunction.

B. <u>DIABETES MELLITUS</u>[31]

1. SULFONYLUREAS:

- Blocks K^+ ATP channel in pancreatic beta cell and increases insulin release.
- Adverse Effects: Hypoglycemia, weight gain due to fluid retention, rash

2. MEGLITINIDES/D-PHENYLALANINE ANALOGUES:

- Blocks K_{ATP} channel in pancreatic beta cell and increases release of insulin
- Adverse Effects: hypoglycaemia, dyspepsia, headache

3. GLUCAGON-LIKE PEPTIDE 1 (GLP-1) RECEPTOR AGONISTS

- GLP-1 is an incretin released from the intestine after meals in response to glucose. GLP-1 induces release of insulin, suppresses release of glucagon, delays gastric emptying and decreases appetite.
- GLP-1 analogues are long acting resists degradation by Dipeptidyl peptidase-4.

4. DIPEPTIDYL PEPTIDASE-4 (DPP) INHIBITORS:

- Insulin secretagogue acts indirectly by inhibiting enzyme DPP 4 that causes rapid degradation of endogenous GLP-1.
- Adverse Effects: Nasopharyngitis, cough and loose stools.

5. α-GLUCOSIDASE INHIBITORS:

- By inhibiting α- glucosidase enzyme it prevents degradation of polysaccharides in small intestine. Absorption of carbohydrates are reduced.
- Adverse Effects: Flatulence, Diarrhoea, GI disturbances.

6. AMYLIN ANALOGUES:

- Amylin, a polypeptide released from beta cells of pancreas, decreases glucagon secretion by acting in brain. It delays gastric emptying, promotes satiety.
- Adverse Effects: Nausea, hypoglycaemia

7. BROMOCRIPTINE (DOPAMINE D_2 AGONIST):

- Acts on the dopaminergic D2 receptor in hypothalamus to control circadian rhythm of growth hormone, prolactin, ACTH and reduces insulin resistance.

8. SGLT-2 INHIBITORS:

- Decreases re-absorption of glucose filtered in the proximal tubules of kidney and causes glucose loss in urine.
- Adverse Effects: Urinary tract infections, electrolyte abnormalities.

9. INSULIN: Increases liver glycogen synthesis and peripheral glucose uptake.

C. <u>HYPERTENSION</u>[32]

1. THIAZIDE DIURETICS:

- Inhibits Na^+-Cl^-symport in distal tubule and cortical diluting segment in nephrons leads to diuresis. This reduces volume of plasma, cardiac output, arterial stiffness and total peripheral resistance.
- Adverse effects: hypokalaemia, glucose intolerance, alteration of lipid profile.

2. ANGIOTENSIN CONVERTING ENZYME (ACE) INHIBITORS:

- By inhibiting ACE it decreases production of Angiotensin II, a powerful vasoconstrictor. ACE inhibitors reduce peripheral resistance, lower blood pressure and inhibit sodium retention by aldosterone.
- Adverse effects: dry cough, angioedema, hypotension, hyperkalaemia.

3. ANGIOTENSIN RECEPTOR BLOCKERS:

- Inhibits angiotensin II receptor (AT_1 receptor) and blocks effects of angiotensin II such as vasoconstriction, increased central sympathetic outflow, release of aldosterone and renal sodium absorption.
- Adverse effect: hypotension, increased serum potassium levels, headache, upper gastrointestinal disturbance.

4. BETA ADRENERGIC RECEPTOR BLOCKERS:

- By inhibiting β_1 receptors reduces heart rate, renin secretion, central sympathetic outflow , norepinephrine release
- Adverse effects: altered lipid profile and blood glucose, rebound hypertension.

5. CALCIUM CHANNEL BLOCKERS:

- Acts by blocking voltage dependent L-type Calcium channel in vascular smooth muscle to decreases heart rate, conduction and contractility and peripheral vascular resistance, relaxes larger vessels and coronaries.
- Adverse effects: headache, ankle edema, flushing, gingival hyperplasia.

D. DYSLIPIDEMIA [33]

1. HMG –COA REDUCTASE INHIBITORS (STATINS):

- They are competitive inhibitors of HMG-CoA reductase. Statins Inhibits cholesterol synthesis, increases LDL receptor expression, uptake and breakdown of LDL.
- Adverse effects: Gastrointestinal symptoms, headache, rise in serum transaminase, myopathy.

2. **BILE ACID SEQUESTRANTS:**

- These are ion exchange resins binds to bile acids in the intestine and prevents its enterohepatic circulation. Increases excretion of bile salts, cholesterol and clearance of IDL, LDL and VLDL in plasma.
- Adverse effects: unpalatable, flatulence, GIT symptoms.

3. LIPOPROTEIN LIPASE ACTIVATORS (FIBRATES):

- Fibrates causes activation peroxisome proliferator-activated receptor α resulting in increased synthesis of lipoprotein lipase enzyme and fatty acid oxidation.
- Adverse effects: Epigastric pain, diarrhoea, skin rashes, eosinophilia.

4. LIPOLYSIS AND TRIGLYCERIDE SYNTHESIS INHIBITORS:

- Nicotinic acid inhibits triglyceride synthesis in liver and reduces production of VLDL, IDL and LDL. It increases lipoprotein lipase activity.
- Adverse effects: marked cutaneous flushing and itching, dyspepsia.

5. STEROL ABSORPTION INHIBITOR

- Ezetimibe inhibits absorption of cholesterol and phytosterols from intestine by blocking cholesterol transport protein NPC1L1 in the intestinal mucosa.
- Adverse effects: reversible liver dysfunction and myositis.

STRESS

DEFINITION[34]:

Stress is defined as a circumstance that disturbs the normal physiological or psychological functioning of a person through the stimulation of sympathetic nervous system.

STRESS RESPONSE[16, 35]:

Normally human body tries to counteract the stress by producing series of changes to protect from serious damage. Acute stress prepares body for fight-or-flight response. Chronic stress depletes body's resources and causes damage to cells.

HORMONES RELEASED DURING STRESS

1. Adrenal medulla: Epinephrine and Norepinephrine.
2. Adrenal cortex: Glucocorticoids, Aldosterone.
3. Anterior pituitary gland: Growth hormone, ACTH.
4. Pancreas: Glucagon.

STRESS RELATED DISEASES [16]:

- Coronary artery disease
- Hypertension, Dyslipidemia
- Rheumatoid arthritis
- Diabetes mellitus
- Asthma, Eczema, Acne.
- Peptic ulcer
- Irritable bowel syndrome
- Ulcerative colitis
- Hypothyroidism
- Stroke, Insomnia, Depression

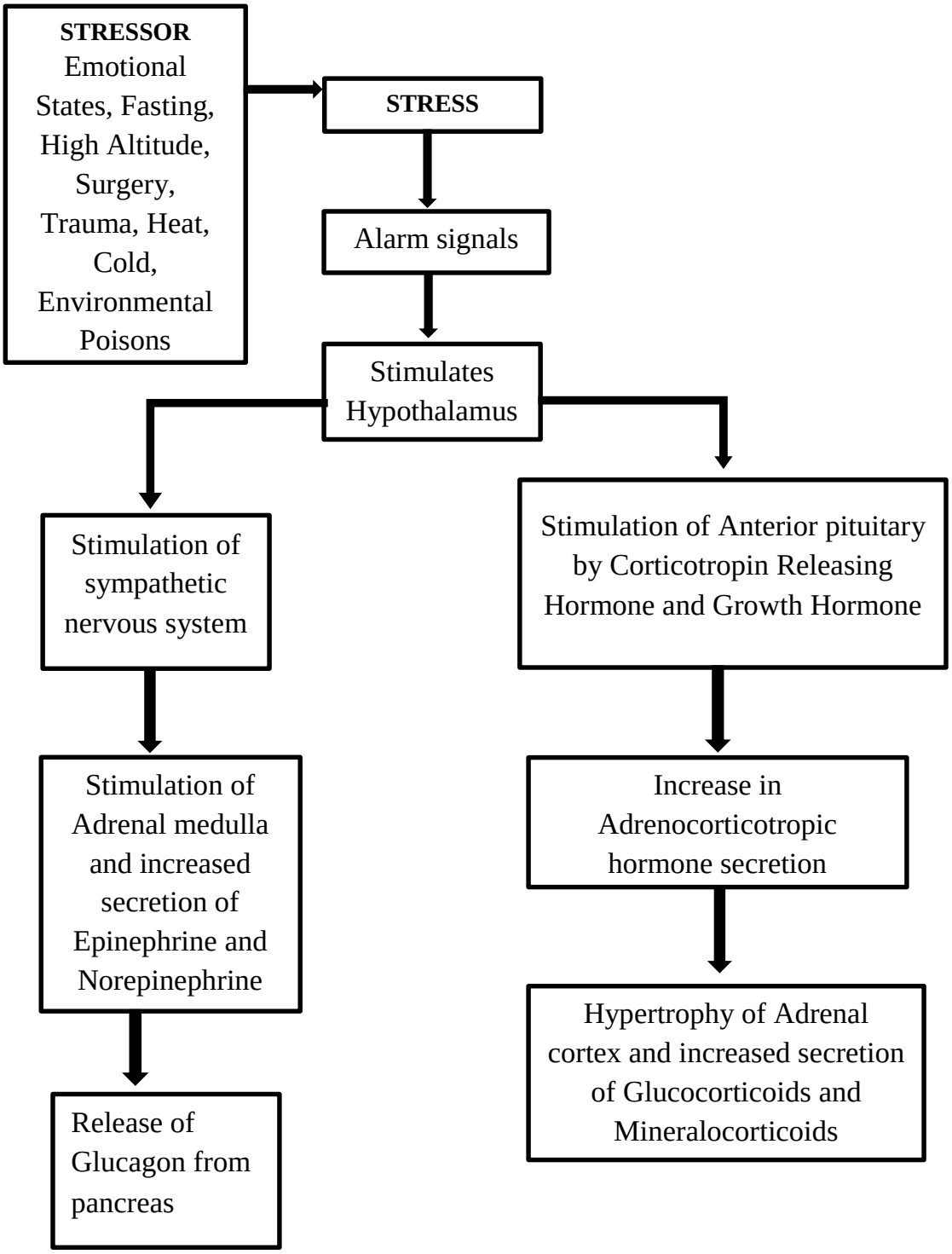


Chart 4: Stress Response

STRESS AND DIABETES MELLITUS[36]

Catecholamines: ➢ Decreases glycogen synthesis ➢ Increases glycogenolysis in liver and muscle ➢ Increases gluconeogenesis ➢ Reduces insulin secretion from beta cells of pancreas.	**Glucocorticoids** ➢ Increases gluconeogenesis from acids in liver. ➢ Decreases peripheral utilization of glucose except in brain and heart. ➢ Decreases insulin sensitivity.
Glucagon ➢ Increases hepatic breakdown of glycogen. ➢ Increase hepatic glucose synthesis from amino acids. ➢ Inhibits utilization of glucose in peripheral tissues by inhibiting enzyme pyruvate kinase.	**Growth hormone** ➢ Increases gluconeogenesis. ➢ Increases glucose release from liver ➢ Decreases glucose uptake and utilization in muscle

STRESS AND HYPERTENSION[16]

Catecholamines ➢ Raises blood pressure by causing vasoconstriction and increase in peripheral vascular resistance through α_1 receptor in blood vessels. ➢ Increases heart rate, force of contraction and cardiac output through β_1 receptors	**Glucocorticoids** ➢ Raises blood pressure by sensitizing blood vessels to vasoconstrictor effect of catecholamines. ➢ Increases cardiac output through direct stimulant effect on heart.

➢ Increases Angiotensin II production through renin release. Angiotensin II, a powerful vasoconstrictor, raises blood pressure and aldosterone release. ➢ Aldosterone increases blood volume by causing sodium and water reabsorption.	

STRESS AND DYSLIPIDEMIA[36]

Catecholamines	**Glucocorticoids**
➢ Causes adipose tissue lipolysis and increase plasma free fatty acid through β action. ➢ Opposes antilipolytic effect of insulin by preventing insulin release from pancreas ➢ Promotes glucagon mediated lipolysis	➢ Promotes lipolysis in peripheral adipose tissue by its permissive action. ➢ Causes redistribution of fat by mobilizing fat from peripheral sites and stores in the central areas like abdomen, face, neck and shoulder.
Glucagon	**Growth hormone**
➢ Promotes lipolysis and utilizes free fatty acid for ketone production.	➢ Induces lipolysis in adipose tissue and ketone body production.

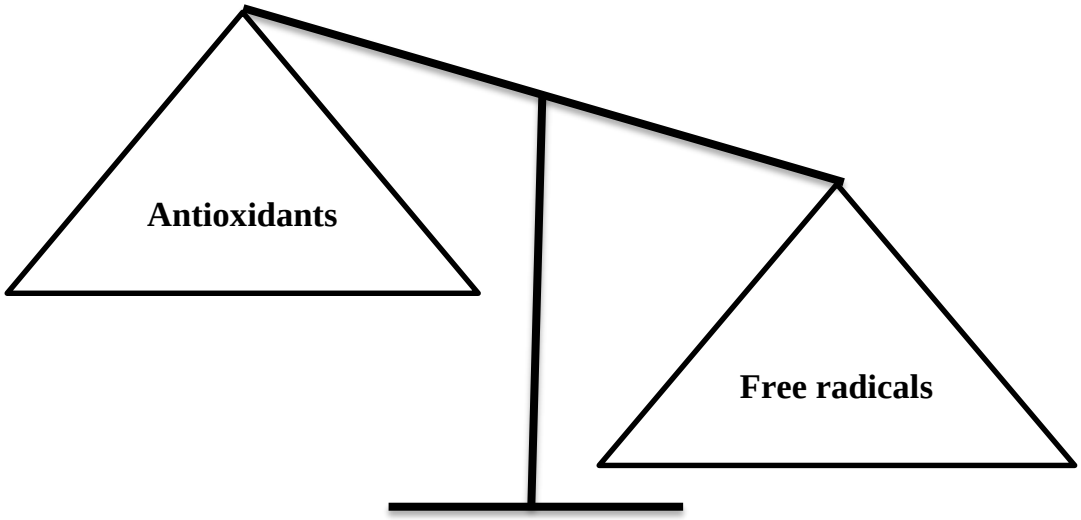
OXIDATIVE STRESS

FREE RADICALS[37]:

Free radicals are chemical species that have single unpaired electron in an outer orbit. These unpaired electrons are highly reactive and attack adjacent molecules like proteins, carbohydrates, lipids, nucleic acids and convert it into free radicals, thus propagating the chain of damage.

OXIDATIVE STRESS[38]:

Under physiological conditions many free radicals are produced during cellular metabolism. They are maintained at steady state levels by antioxidants (endogenous or exogenous) which act as free radical scavengers. Oxidative stress, an imbalance occurs when the production of free radicals exceeds the detoxification capacity of cellular antioxidant system resulting in biological damage.



TYPES OF FREE RADICALS [38]:

Reactive oxygen species

- Superoxide anion (O_2.-)
- Hydroxyl (.OH)
- Hydrogen peroxide (H_2O_2)
- Hypochlorous acid (HOCl)
- Peroxy radical (ROO)

Reactive nitrogen species

- Peroxynitrite ($OONO^-$)
- Nitric oxide (NO.)
- Nitrogen dioxide (NO_2.-)

GENERATION OF FREE RADICALS[37, 39]:

ENDOGENOUS SOURCES

- The oxidation-reduction reactions occurring during normal metabolism.
- Enzymes xanthine oxidase, Nitric oxide synthase, NADPH oxidase
- Inflammation and phagocytosis
- Transition metals (iron, copper)
- Ischemia reperfusion injury

EXOGENOUS SOURCES

- Absorption of radiant energy (ultraviolet rays, x rays)
- Metabolism of exogenous drugs and chemical by enzymes
- Environmental pollution and toxins
- Smoking

PATHOLOGICAL EFFECTS[37]:

- Lipid peroxidation and loss of integrity of plasma membrane and organelles can result in chronic inflammation.
- In DNA single or double strand breaks, cross linking, adduct formation & malignant transformation result in cancer.
- Covalent cross links, structural change and degradation of proteins can result in auto immune diseases.

RED BLOOD CELLS

Red blood cells or Erythrocytes are biconcave, disc shaped non nucleated cells of size 7-8μm filled with hemoglobin[40].

Hemoglobin is a red coloured protein contains iron helps in transport of respiratory gases such as oxygen and carbon dioxide in the blood. The process by which erythrocytes are produced in bone marrow from pluripotent hematopoietic stem cells is called as Erythropoiesis[40].

STRUCTURE OF RBC [41, 42]

RBC membrane is made up of proteins (50%), lipids (40%) and carbohydrates (10%). It contains two layers of lipid (phospholipids, cholesterol and glycolipids), transmembrane proteins (Glycophorin A, Anion channel and Rh proteins) and a network of cytoskeletal proteins (α- and β -Spectrin, Actin, Ankyrin, Protein 4.1).

Interconnection of these proteins with cell membrane gives RBC a biconcave shape and deformability so that they can pass through tiny capillaries of 4µm diameter without any damage.

RBCs lacks nucleus, DNA, mitochondria, ribosomes, endoplasmic reticulum and cannot synthesize protein. Hemoglobin constitutes 95% of protein in cytoplasm and 5% are enzymes necessary for production of energy and maintenance of hemoglobin in functional (reduced) state such as Carbonic anhydrase, Pyruvate kinase, Glucose -6-phosphate dehydrogenase, Glutathione reductase.

METABOLISM OF RBC [41, 42]

- The only source of energy for RBCs is glucose that enters cells by facilitated diffusion without the help of insulin.
- Energy that is required for maintenance of cell shape, water content of cell, functioning of sodium potassium pump is obtained through anaerobic glycolytic pathway (Emden Meyerhof pathway).
- Glucose enters Hexose monophosphate shunt to generate NADPH that is used for reducing oxidized intracellular antioxidant glutathione.

HEMOLYTIC ANEMIA IN METABOLIC SYNDROME [43, 44]:

Red blood cells are the first cells to be damaged in oxidative stress. They are constantly exposed to free radicals as they have high cellular oxygen and hemoglobin concentration.

Normally RBCs protects themselves from oxidative stress induced hemolysis with the help of intracellular antioxidants Glutathione (GSH). The intracellular reduced glutathione pool is maintained by NADPH. RBCs are completely dependent on Glucose-6- phosphate dehydrogenase enzyme for NADPH production.

In metabolic syndrome, there is insulin resistance leading to reduced expression of G6PD enzyme and subsequent inhibition of pentose phosphate pathway. This results in reduced production of NADPH. Reduction in NADPH levels decreases intracellular concentration of reduced glutathione. In conditions of severe oxidative stress, Glutathione (GSH) gets oxidized to Glutathione disulfide (GSSG) which leaks through the damaged cell membrane. As the RBCs lacks nucleus and mitochondria it cannot synthesize GSH and enzymes and became vulnerable to free radicals.

Free radicals cause damage to spectrin and lipid bilayer of RBC alters discoid shape and reduces membrane stability and flexibility of cells. ROS increases oxidation of hemoglobin to methemoglobin which has poor oxygen carrying capacity. Formation of disulfide cross linkages in hemoglobin by ROS alters its primary protein structure resulting in precipitation of oxidized hemoglobin as **Heinz bodies.** When these RBCs pass through spleen, reticuloendotelial cells removes Heinz bodies and forms **Bite cells.** This reduces lifespan of RBCs ultimately resulting in **Hemolysis and Anemia**. Excessive hemolysis increases erythropoiesis and release of premature RBCs called **Reticulocytes** into the circulation.

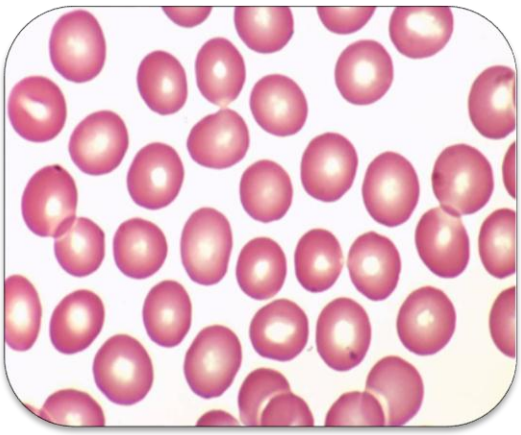
Free radicals increase production of Eicosanoid isomer (8isoPGF2α) through non enzymatic oxidation of arachidonic acid that can activate prostanoid receptor and contributes to inflammation[13]. It is also a potent mediator of oxidative stress causes damage to the RBC producing irregularly contracted **crenated RBCs[5].**

Measuring these isoprostanes level in plasma gives accurate measure of oxidative stress occurring in vivo[13].

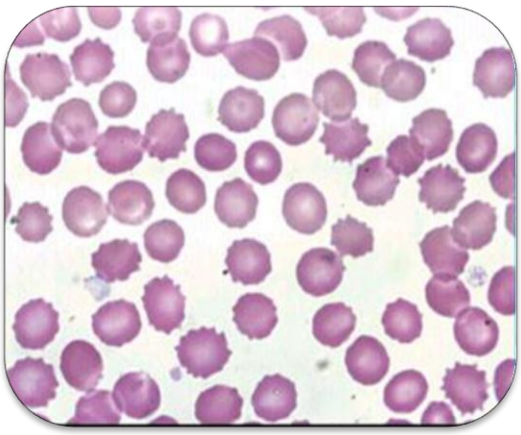
Increased glucagon synthesis during psychological stress inhibits the glycolytic enzyme pyruvate kinase. This decreases ATP production which is required for metabolism and for maintaining the flexible biconcave shape. Alterations in the shape and poor red cell deformability leads to **hemolytic anemia.**

STRUCTURAL CHANGES IN RBC DUE TO FREE REDICALS

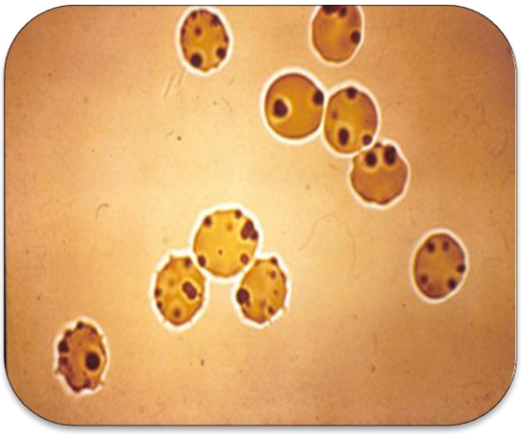
- Irregularly contracted & Crenated cells – due to alteration in the membrane integrity[5].
- Heinz bodies– aggregate of denatured hemoglobin due oxidation[44.]
- Bite cells– remaining cells after Heinz bodies are removed by spleen[45].
- Spherocytes– small, dense spherical structure cells without central pallor[46].
- Reticulocytes – immediate precursor of RBC containing reticulum (remnant of RNA)[46]



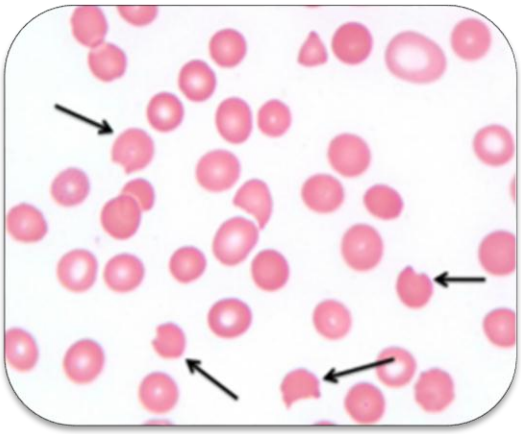
NORMAL RBC



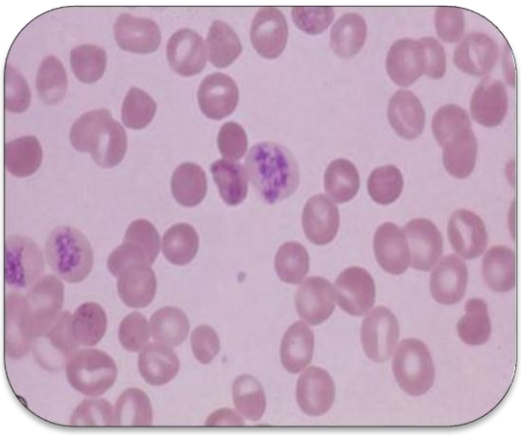
CRENATED RBC



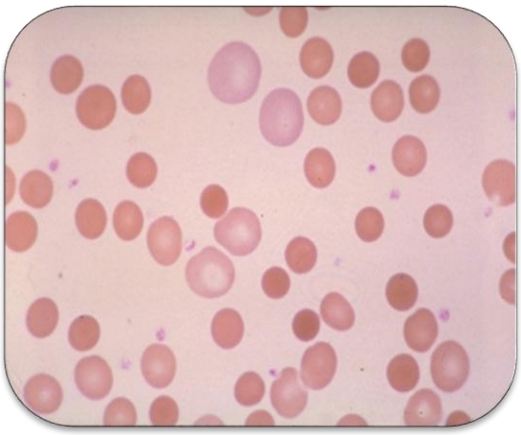
HEINZ BODIES



BITE CELLS



RETICULOCYTES



SPHEROCYTES

Chart 5: Structural changes in RBC due to free radicals

NITRIC OXIDE

- Nitric oxide (NO) is a free radical and acts as a signalling molecule.
- Synthesised in the vascular endothelium from L arginine and oxygen catalysed by enzyme Nitric oxide synthase (NOS) in the presence of Heme, Tetrahydrobiopterin (BH_4), Reduced Nicotinamide adenine dinucleotide phosphate (NADPH) and Flavin adenine dinucleotide (FAD).

PHYSIOLOGICAL ROLE[47, 48]

- NO inhibits calcium induced smooth muscle contraction through activation of Guanylyl cyclase and Protein kinase G enzymes resulting in vasodilatation and decrease in blood pressure.
- Dilates pulmonary vessels and reduces pulmonary arterial hypertension.
- Inhibits platelet aggregation, monocyte adhesion and migration, smooth muscle and fibroblast proliferation, decreases permeability of endothelium to lipoproteins and prevents atherosclerosis.
- Acts as a non-adrenergic non cholinergic neurotransmitter in gastrointestinal tract causes gastric emptying and in penile tissues causes penile erection.
- Enhances neurotransmitter release, long term potentiation and regulates synaptic plasticity in learning and memory.
- Protects against numerous pathogens and tumour cells by its cytotoxic action.

PATHOLOGICAL ROLE [49, 50]

Excess production:

- Sepsis – induction of NOS by bacterial lipopolysaccharides, macrophages produces severe vasodilatation leads to multi organ failure.
- Excitotocity – due to increased NMDA receptor activation causes neuronal damage resulting in dementia and neurodegenerative diseases.
- Methemoglobinemia – due to increased nitrate production and heme oxidation.
- In asthma – nitration of proteins in airway epithelium causes steroid resistance.

Reduced production

- Atherosclerosis – increased platelet aggregation, monocyte adhesion and activation, smooth muscle and fibroblast proliferation.
- Hypertension – due to decreased bioavailability causing impaired vascular relaxation.
- Eclampsia – failure of normal vasodilatation due to reduced synthesis in pregnancy.
- Infantile Hypertropic pyloric stenosis – due to reduced sphincter relaxation due to deficiency of NO producing neurons.
- Erectile dysfunction – blunted relaxation of corpora cavernosa due to decreased NO synthesis.

NITRIC OXIDE IN HYPERTENSION[47, 50]

In **oxidative stress** states the following mechanism leads to hypertension.

- Excess production of free radicals oxidizes LDL cholesterol in circulation. These oxidised LDL displaces nitric oxide synthase (eNOS) from its binding site in endothelial cells and disrupts its function.
- eNOS undergoes structural damage leading to uncoupling of electron transfer between substrate, cofactor enzymes and product resulting in increased production of superoxide anion (O_2^-) instead of NO.
- Superoxide radicals reacts with available nitric oxide in circulation and converts it into Peroxynitrite (OONO−) radical thus reducing NO bioavailability.
- Peroxynitrite (OONO−) radical causes endothelial dysfunction, lipid peroxidation, cell injury, and cell death

ANTIOXIDANTS

DEFINITION[52]:

A biological antioxidant is defined as a substance that significantly delays or inhibits oxidation of a substrate. Oxidation is a chemical reaction that transfers electrons from a substrate to an oxidizing agent. This leads to production of free radicals, which starts chain reactions and damage cells. Antioxidants are called as scavengers of free radicals.

CLASSIFICATION[38]:

Enzymatic antioxidants

- Superoxide dismutase
- Catalase
- Glutathione peroxidase
- Glutathione reductase
- Glucose 6-phosphate dehydrogenase
- NADPH

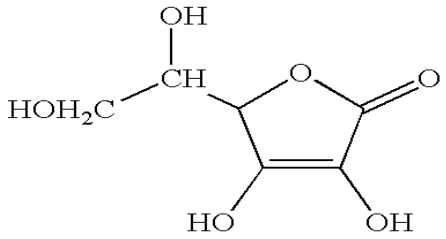
Non enzymatic antioxidants

- Vitamins C
- Vitamin E
- α Lipoic Acid
- Carotenoids
- Polyphenols
- Glutathione
- Coenzyme Q
- Minerals (Copper, Selenium, Zinc and Manganese).

VITAMIN C (ASCORBIC ACID)

- Vitamin C is a water soluble vitamin and a potent reducing agent[53].

STRUCTURE:



Ascorbic Acid

Recommended dietary allowance[54]

Children: 45mg/day

Adults: 75mg/day.

Pregnancy: 100mg/day

ACTIVE FORM:

- L-Ascorbic acid
- Dehydroascorbic acid

DIETARY SOURCES:

Lemon, Orange, Indian goose berry, Guava, Black currant, Tomato, Green chilly, Cabbage.

BIOSYNTHESIS:

- Vitamin C is an essential nutrient cannot be synthesized by humans due to lack of an enzyme L-Gulonolactone oxidase and has to be supplied in diet[55].
- It is highly concentrated in adrenal cortex.

PHARMACOKINETICS:

- Vitamin C is rapidly absorbed from intestine through simple diffusion and active transport.

- Excreted in urine as its metabolites diketogulonic acid and oxalic acid.

FUNCTIONS:

1. ANTIOXIDANT[53]

- Ascorbic acid, a strongest radical scavenger, reacts with free radicals by undergoing a single-electron oxidation yields a non-reactive intermediate.
- It acts as a first line of defence against free radicals in plasma, interstitial fluids and soluble phases of cells.
- Prevents oxidative damage to DNA, Lipids, Proteins and Nitric oxide.
- Regenerates the metabolically active reduced form (tocopherol) of vitamin E – hence combination with vitamin E produces **synergistic action[54].**
- By scavenging oxidants it protects reduced form of Glutathione.

METABOLIC SYNDROME[53]

- Reduces insulin resistance by inhibiting free radical induced production of isoprostanes (8isoPGF2α) from arachidonic acid.
- Prevents complications due to diabetes by reducing glycosylation of plasma proteins and accumulation of sorbitol.
- Increases bioavailability of NO and promotes vasodilatation.
- Improves arterial stiffness in diabetes patients by protecting ions pumps from free radical damage.

- Decreases blood pressure by modulating the autonomic nervous system that is it restores sympathovagal balance and improves spontaneous baroreceptor sensitivity[23].
- Acts as a coenzyme in bile acid synthesis from cholesterol catalysed by enzyme 7 α hydroxylase. Thus excess cholesterol can be excreted in bile[54].
- It reduces oxidation of LDL cholesterol and decreases atherogenesis[24].
- Increases LDL receptors in the liver and increases uptake and degradation of LDL.
- Helps in regeneration of other antioxidants and maintains antioxidant pool.

2. CO-FACTOR FOR ENZYMES[55]

- Gives structural integrity to collagen by hydroxylation of prolyl and lysyl residues
- Acts as co-factor of enzymes involved in carnitine synthesis
- Serves as an electron donor for enzymes involved in the metabolism of steroids, drugs, lipids and neurotransmitters.

3. BIOAVAILABILITY OF IRON

- Reduces the ferric form (Fe^{3+}) non-heme iron to the ferrous form (Fe^{2+}) and increases its absorption in small intestine[54].
- Promotes the utilization of heme iron by enhancing its incorporation into ferritin and increasing the iron-stimulated translation of ferritin mRNA[53].

4. IMMUNITY

- Modulates Phagocytic activity, Lymphocytes and cytokine production, Interferon production, T cell gene expression and synthesis of antibodies[53].

5. ANTI INFLAMMATORY ACTION[53]

- Reduces biomarkers of inflammation (c-reactive protein) and endothelial dysfunction (tissue plasminogen activator).

6. CATARACT

- Protects lens from Ultraviolet rays and sorbitol accumulation and prevents cataract[55].

7. CANCER

- Prevents polycyclic aromatic carcinogens and nitrosamine-induced carcinogenesis[53].

8. MISCELLANEOUS

- Improves Bone health, Promotes wound healing[54]
- Improves memory and cognition in dementia and other neurodegenerative disorder[54].

DEFICIENCY[54]

- Scurvy
- Hemorrhages of skin and mucous membrane
- Impaired wound healing
- Weakening of bones
- Decreased body's iron stores
- Dental malformations
- Swollen and painful joints

ADVERSE EFFECTS

High dose >2g/day can cause Mild GI upset, Headache, Sleep disturbances, Oxalate stones in kidney[54].

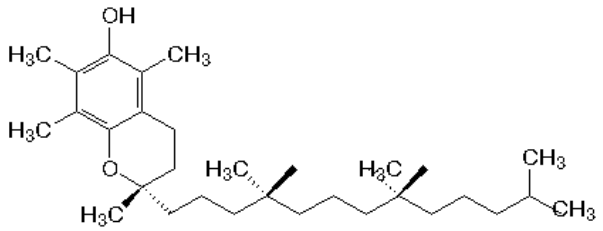
USES

- Prevention and treatment of Scurvy
- Anemia
- Diabetes mellitus
- Hypertension
- Common cold
- Osteoarthritis and rheumatoid arthritis
- Non healing leg ulcer and Bed sores
- Methemoglobinemia- as a reducing agent
- Acidification of urine in urinary tract infections.

VITAMIN E (α-TOCOPHEROL)

- Vitamin E is a fat soluble vitamin available in eight forms: Tocopherol -4 ,tocotrienols -4 and obtained only in diet[56.]
- α-tocopherol is the most active form.

CHEMICAL STRUCTURE



Vitamin E (α-tocopherol)

Recommended dietary allowance[57]
Children 5mg/day
Adult 10mg/day
Pregnancy 10mg/day
Lactation 12mg/day

SOURCES[57]

Wheat germ oil, Sunflower oil, Safflower oil, Rice-bran oil, Soybean oil, Green leafy vegetables, Nuts, Cereals, Fish and Egg yolk.

PHARMACOKINETICS[58]

- Vitamin E is absorbed along with fat in small intestine in alcohol form.
- Carried by chylomicrons and incorporated into VLDL and LDL.
- Stored in adipose tissue, muscle and liver and localized in cell membranes.

FUNCTIONAL ROLE

1. ANTIOXIDANT[56]

Vitamin E is a membrane antioxidant gets incorporated into the phospholipid bilayer. It prevents conversion polyunsaturated fatty acids (PUFA) of the cellular membranes into lipid peroxides by free radicals and has **membrane stabilizing action[5].** After scavenging the free radicals, it gets converted to nonreactive tocopheroxyl radical which is regenerated to its active form by Vitamin C.

METABOLIC SYNDROME

- Reduces insulin resistance by suppressing free radical induced production of isoprostanes (8isoPGF2α) from arachidonic acid.
- Prevents atheroma formation in intima by reduction of oxidized LDL.
- Reduces platelet adhesion, aggregation and increases NO bioavailbility.
- Produces synergistic action when combined with vitamin C.

2. BLOOD

- Maintains integrity of the RBC membrane, reduces fragility, protects RBC from hemolysis by oxidants and prevents anemia[56].
- Increases heme synthesis by increasing the activity of enzymes Aminolevulinic acid synthase and dehydratase[57].

3. IMMUNITY[56]

- Modulates T cell function, stimulates production of lymphocytes and antibodies. Promotes Phagocytosis.

4. ANTIINFLAMMATORY ACTION[56]

- Inhibits Phospholipase A2, Cyclooxygenase and 5-Lipoxygenase activity and production of Prostaglandins and Leukotrienes.
- Inhibit production of proinflammatory cytokines from activated macrophages

5. CANCER PREVENTION[56]

- Selectively stimulate apoptosis of neoplastic cells and reduces carcinogenesis in skin due to ultraviolet rays.

6. CATARACTS[57]

- Prevents cataract formation by maintaining lens glutathione in reduced state.

7. OTHERS[57]

- Helps in nucleic acid synthesis, protects structure of mitochondria, sulphur containing enzymes, proteins and prevents oxidation of vitamin A and carotene.
- Reduces central pain processing by suppression of nitric oxide.
- Required for normal reproductive function.

DEFICIENCY SYMPTOMS[56, 57]:

- Myopathies
- Peripheral neuropathy
- Retinopathy
- Hemolysis of RBC, Anemia
- Spinocerebellar Ataxia
- Decreased immunity

ADVERSE DRUG REACTIONS

High dose (>2000 mg/day) can produce Headache, GI upset, nausea, Bleeding tendency[57]

USES

- Diabetes mellitus
- Chronic hemolysis in G6PD deficiency
- Intermittent claudication
- Benign breast tumor
- Nocturnal leg cramps
- Atherosclerosis
- Rheumatoid arthritis
- Osteoarthritis
- Parkinson's disease
- Alzheimer's disease

AIM AND

OBJECTIVES

AIM AND OBJECTIVE

AIM:

To study the efficacy of Vitamin E and Vitamin C in metabolic syndrome.

OBJECTIVES:

PRIMARY OBJECTIVE:

To study the morphological changes of red blood cell as a marker of oxidative stress and the efficacy of Antioxidants such as Vitamin C and E as an add on therapy to standard treatment in metabolic syndrome.

SECONDARY OBJECTIVE:

Improvement in blood glucose, blood pressure and lipid profile.

METHODOLOGY

METHODOLOGY

STUDY DESIGN:

Randomized, Open label, Comparative Pilot study

STUDY POPULATION:

Adult patients diagnosed with metabolic syndrome and on treatment for Hypertension, Diabetes Mellitus and Dyslipidemia.

STUDY CENTER:

Institute of Internal Medicine, Madras medical college & Rajiv Gandhi Government General Hospital, Chennai.

STUDY PERIOD:

September 2015 – April 2016

SAMPLE SIZE:

60 patients (30 patients in control group and 30 patients in test group)

STUDY DURATION:

8 weeks study period and post treatment follow up period for 4 weeks per patient.

ELIGIBILITY CRITERIA

INCLUSION CRITERIA:

- Age: 40-70 years
- Sex-both genders
- Patients diagnosed with metabolic syndrome and on treatment for Hypertension, Diabetes and Dyslipidemia for the duration of 1‑2 years.
- Patients willing to participate and give written informed consent.

EXCLUSION CRITERIA:

- Patients with fasting blood glucose > 250 mg/dL, total cholesterol >300 mg/dL, triglycerides >200 mg/dL, blood pressure > 160/ 100mmHg.
- Patients with other secondary causes of hypertension, hyperglycemia and dyslipidemia such as hepatic, renal, thyroid and other endocrine diseases.
- Pregnant and lactating women.
- Patients with hematological disorders.
- Patients with history of smoking and alcohol intake.
- Patient enrolled in any other study.

STUDY PROCEDURE:

The study was conducted in accordance with Declaration of Helsinki and Good Clinical Practice (GCP) guidelines after obtaining approval from the Institutional Ethics Committee, Madras Medical College. Patients diagnosed with metabolic syndrome and on treatment for Hypertension, Diabetes and Dyslipidemia for the duration of 1-2 year were recruited from the outpatient department of the Institute of Internal Medicine, Madras Medical College & Rajiv Gandhi Government General Hospital. They were explained about the purpose, procedures and benefits of the study. Written informed consent was obtained from the patients who were willing to participate in the trial in the prescribed format in regional language prior to performance of any study related procedure.

The demographic details were obtained and recorded. Patients were screened by History, General examination, Systemic examinations and Lab investigations. Those who fulfilled inclusion and exclusion criteria were enrolled and randomized to either control group or test group.

- The following **lab investigations** were performed during screening.

- Fasting blood sugar (FBS)
- Lipid profile
- Complete blood count (CBC)
- Liver function tests (LFT)
- Renal function tests (RFT)
- Urine analysis

RECRUITMENT:

- 121 patients were screened and 60 patients who fulfilled the inclusion and exclusion criteria were recruited into the study as 30 for the control group and 30 for the test group.

- No drop-out of patients occurred in both groups

RANDOMIZATION:

The enrolled patients were randomized by simple randomization into control and test group and received the study drug or standard therapy respectively.

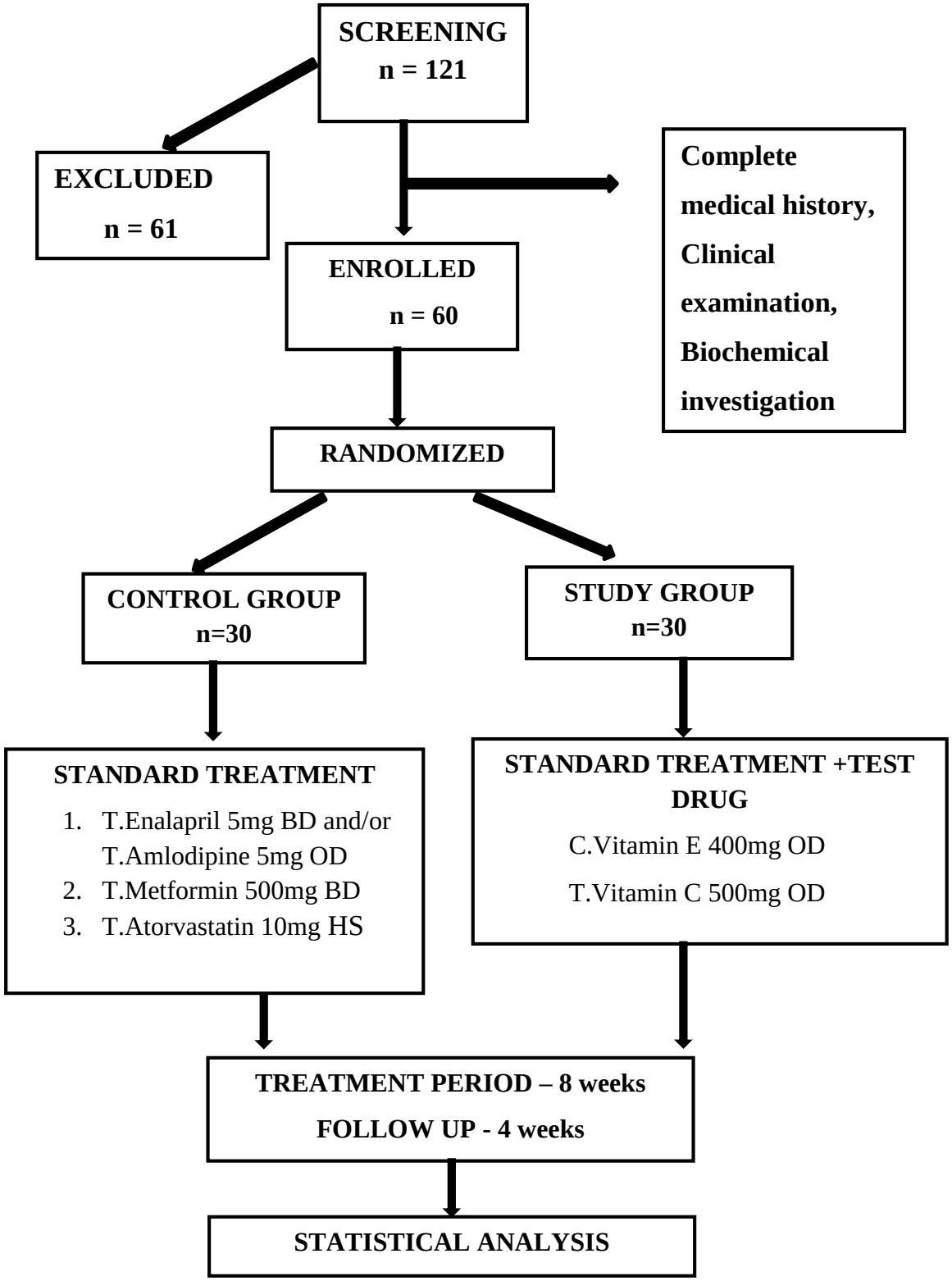
TREATMENT PLAN:

GROUP A - Control group (n=30): patients received standard treatment for hypertension, diabetes and dylipidemia.

GROUP B - Study group (n=30): patients received standard treatment plus one tablet of Vitamin C -500mg and one capsule of Vitamin E -400mg once daily.

Study medication were issued for 4 weeks at a time. Patients were asked to review at the end of 4 weeks with the empty blister packs to check for compliance and medications were issued for subsequent 4 weeks.

STUDY FLOW CHART:



SCHEDULED VISITS

SCREENING:

- Written Informed consent obtained
- Demographic details obtained
- Medical history obtained
- General & systemic examination done
- Blood and urine samples taken for baseline Laboratory investigations (CBC, FBS, RFT, Lipid profile, LFT, RBC morphology and Urine analysis).
- Enrolment done.

Visit 1

- Randomization done
- Clinical examination done and vital signs recorded
- Study medications issued for 4 weeks
- Instructed to return empty strips during subsequent visit
- Asked to report adverse event, if any occurs.

Visit 2 (end of 4 weeks)

- Clinical examination done and Vital signs recorded
- Empty strips collected and compliance assessed
- Adverse events ,if any recorded
- RBC morphology studied
- Fasting blood sugar and Lipid profile measured
- Study medications issued for another 4 weeks

Visit 3 (end of 8 weeks)

- Clinical examination done and Vital signs recorded.
- RBC morphology studied
- Complete blood count, Fasting blood sugar, Lipid profile, Renal function test, Liver function tests and urine analysis are done.
- Adverse events, if any recorded

Visit 4 (end of 12 weeks)

- Clinical examination done and Vital signs recorded
- Adverse events, if any recorded
- RBC morphology studied.
- Fasting blood sugar and Lipid profile measured

ASSESSMENT

I**. Morphological changes in RBCs**: 1ml of blood collected from patient was centrifuged at 3000 rpm for 5 minutes. The supernatant was discarded and the packed cells are diluted with equal volume of 0.9% normal saline and centrifuged again. The supernatant was discarded again and the packed cells are reconstituted as 10% v/v suspension with 0.9% normal saline. A drop of this suspension was put on a glass slide under a cover slip and studied under high power microscope.

Under the high power microscope, a central field was selected. 100 RBCs were analysed and the number of Crenated RBCs with Heinz bodies were counted for all patients in both groups at each visit and recorded as a percentage.

II. **Blood pressure measurement, Fasting blood sugar and Lipid profile –** were done at each visit.

INSTRUCTIONS TO PATIENTS:

The patients were instructed to take medicines regularly. They were advised to pay visit for assessment, collection of drugs and to report if any adverse reactions occur.

FOLLOW UP:

The patients were followed up for period of 4 weeks post treatment and assessment of vitals, fasting blood sugar, lipid profile and RBC morphology were done. After the completion of 12 weeks of study period, they were provided appropriate medical care at Institute of Internal medicine, Rajiv Gandhi Government General Hospital, Chennai.

ADVERSE EVENTS:

Adverse events reported during the study period by the patient were recorded. Seriousness of the event, causality assessment done and appropriate treatment was given.

WITHDRAWAL:

During the trial patient was allowed to withdraw his/her voluntary consent and opt out of study at any time and assured of continued good quality medical care. If any serious adverse event observed by the physician or reported by the patient the investigator was powered to withdraw him/her from the study.

STATISTICAL ANALYSIS:

The data obtained were analysed statistically using SPSS vs. 21. P value <0.05 was considered to be statistically significant.

STATISTICAL TEST	PARAMETER
➢ Pearson chi- square test	-Sex distribution
➢ Student's paired t-test	-Differences within the groups in Blood pressure, Fasting blood sugar, Lipid profile and RBC morphology.
➢ Independent t-test	-Difference between the control and test groups
➢ Student's paired t- test	-Differences in other biochemical investigations performed at 0 week and at the end of 8weeks

RESULTS

RESULTS

TABLE 1: MEAN AGE DISTRIBUTION

GROUPS	n (No. of patients)	MEANAGE (in years)	SD	p VALUE
CONTROL	30	51.13	3.46	0. 67
STUDY	30	50.73	3.60	

Table 1 – shows mean age distribution of patients among control & study groups.

The mean age of patients in control group was 51.13 and in the study group was 50.73 (p=**0.67**), this showed there was no significant age difference between two groups.

FIGURE 1: MEAN AGE DISTRIBUTION

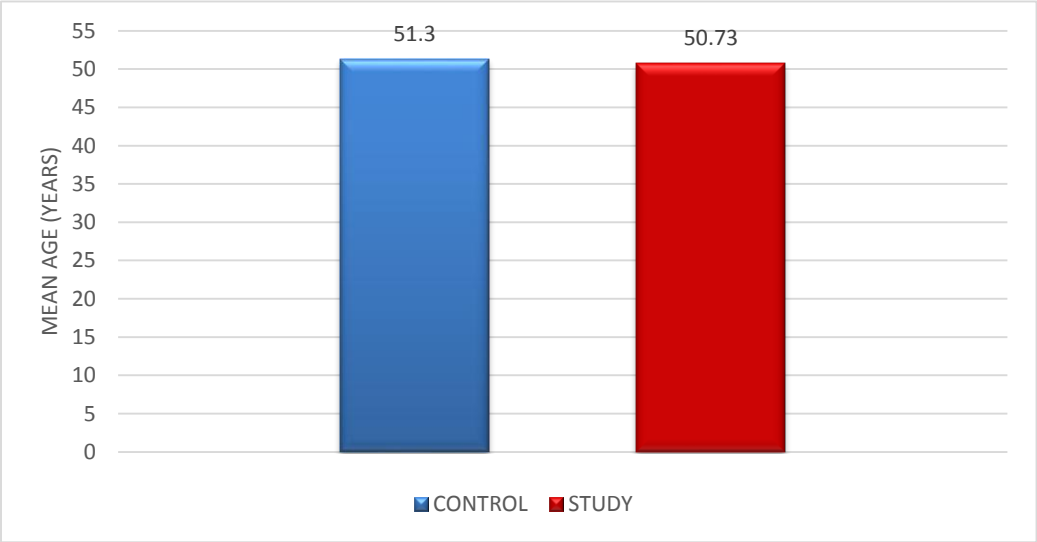


Figure 1 is a graphical representation of Table 1.

TABLE 2: GENDER DISTRIBUTION

SEX DISTRIBUTION	GROUPS			
	CONTROL		STUDY	
	n	%	n	%
MALE	18	60%	20	66.7%
FEMALE	12	40%	10	33.3%
TOTAL NO. OF PATIENTS	30	100%	30	100%

Table 2 - shows sex distribution among two groups. In control group, male were 18 (**60%**) and female were 12 (**40%**). In study group, 20 were male (**66.7%**) and 10 were female (**33.3%**).

FIGURE 2: GENDER DISTRIBUTION

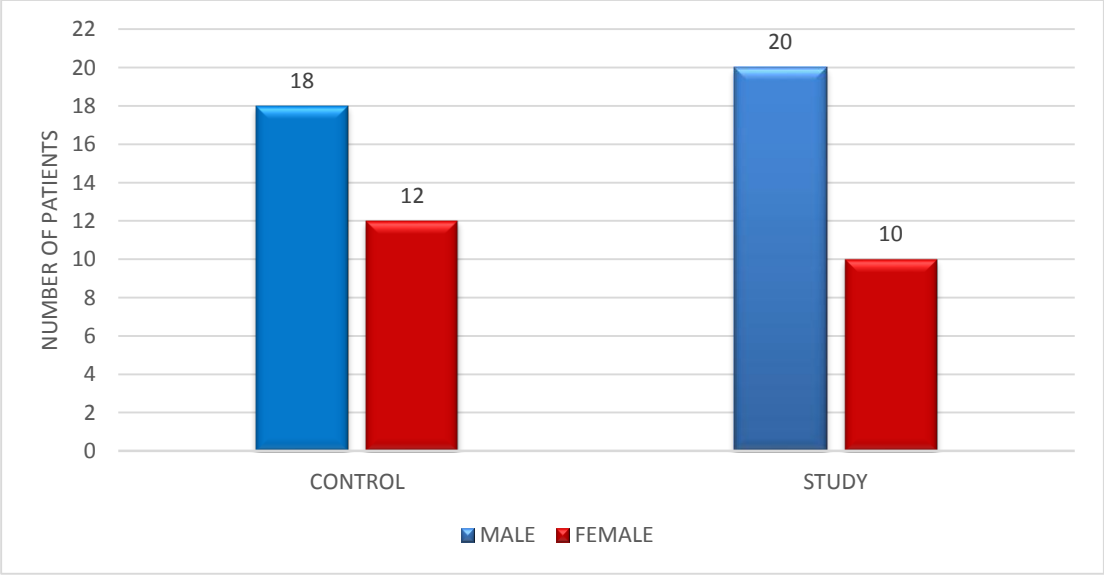


Figure 2 is a graphical representation of Table 2

TABLE 3: MEAN FASTING BLOOD GLUCOSE (mg/dl)

GROUPS	0 WEEK		AT THE END OF 8 WEEKS		p value
	MEAN (mg/dl)	SD	MEAN (mg/dl)	SD	
CONTROL	148.37	45.15	132.30	36.07	0.024
STUDY	154.47	40.01	112.93	26.92	<0.001
p value	0.58		0.023		

Table 3- shows the mean fasting blood glucose levels in both groups.

- At 0 week, the mean fasting blood glucose levels in control group and study group was 148.37 mg/dl and 154.47 mg/dl respectively. Both groups were comparable at baseline (p = 0. 58).
- At the end of 8 weeks mean fasting blood glucose level was reduced to 132.30 mg/dl (p=0.024) in control group and 112.93 mg/dl (p<0.001) in study group.
- Comparing both groups, study group showed a statistically significant reduction than control group (p=0.023) at the end of 8 weeks.

FIGURE 3: MEAN FASTING BLOOD GLUCOSE (mg/dl)

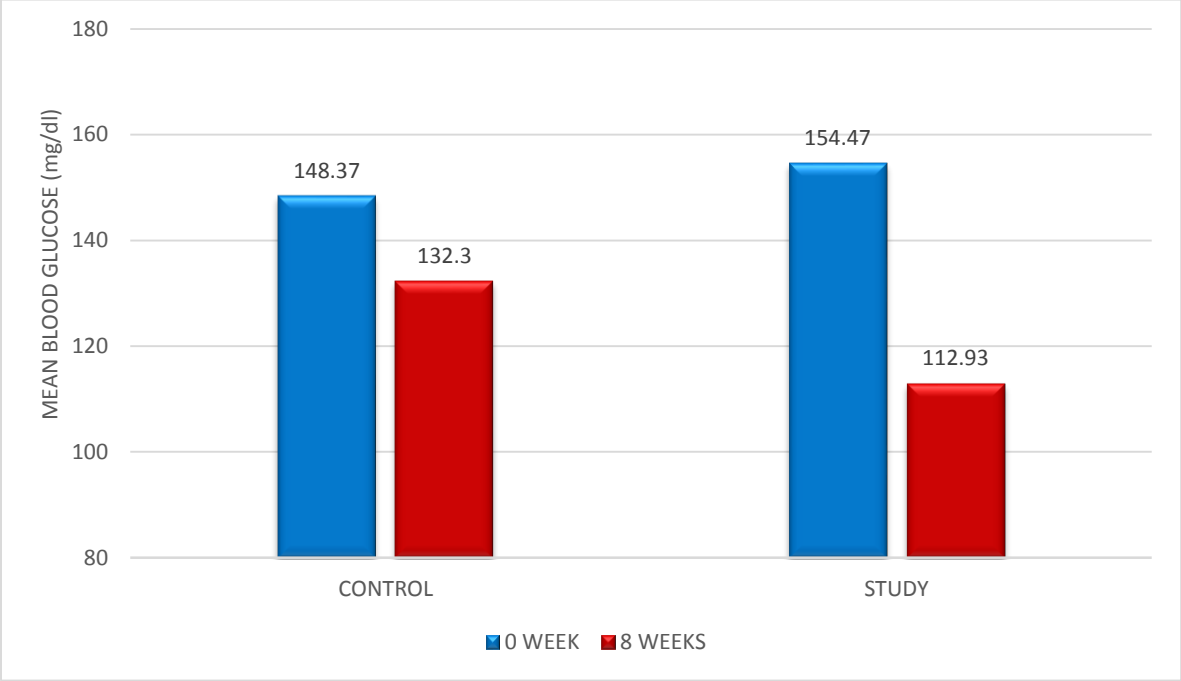


Figure 3 is a graphical representation of Table 3

TABLE 4: MEAN PERCENTAGE OF CRENATED RBCs WITH HEINZ BODIES (%)

GROUPS	0 WEEK		AT THE END OF 8 WEEKS		p value
	MEAN (%)	SD	MEAN (%)	SD	
CONTROL	84.03	10.63	81.83	9.76	0.072
STUDY	87.73	7.86	5.90	1.97	< 0.001
p value	0.13		< 0.001		

Table 4 - shows the mean percentage of crenated red blood cells with Heinz bodies in control and study groups

- The percentage of crenated RBCs with Heinz bodies at baseline in control group and study group was 84.03% and 87.73% respectively. There was no significant difference between two groups (p =0.13).
- At the end of 8 weeks the percentage of crenated RBCs with Heinz bodies was significantly decreased in study group (5.90%, p<0.001) but not in control group (81.83% ,p =0.072).
- Comparing both the groups at 8 weeks, study group showed statistically significant reduction compared to the control group (p<0.001).

FIGURE 4: MEAN PERCENTAGE OF CRENATED RBCs WITH HEINZ BODIES (%)

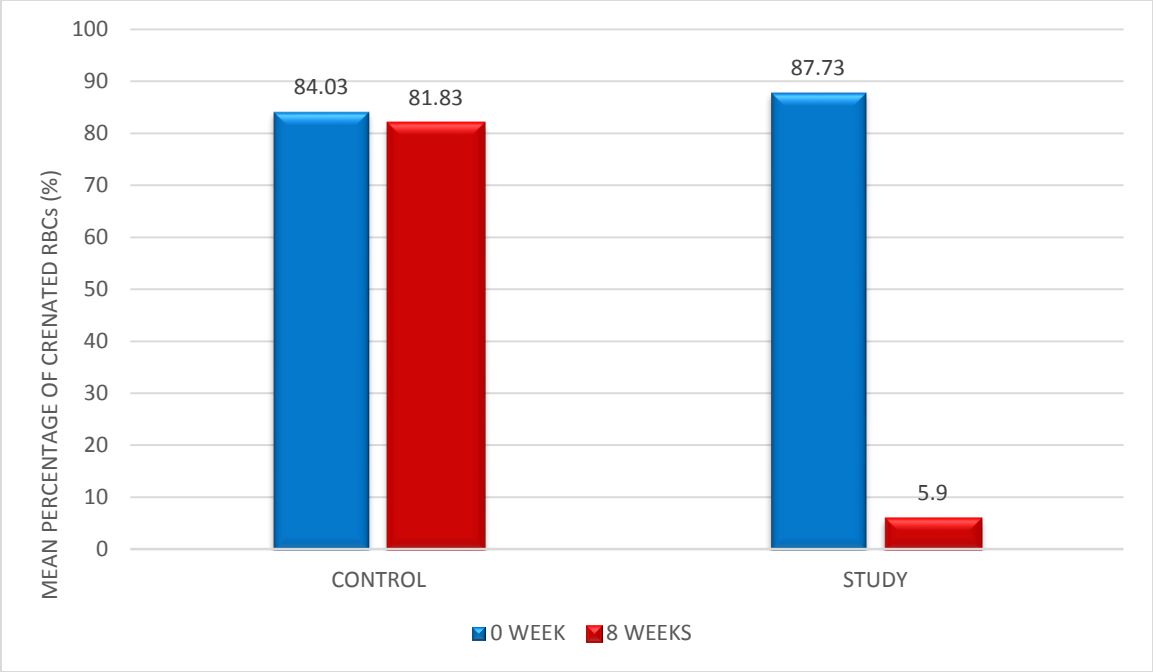
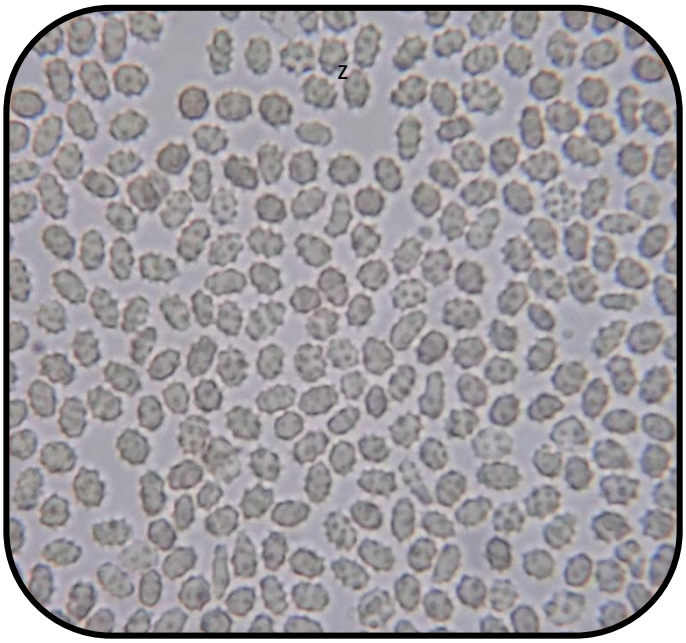


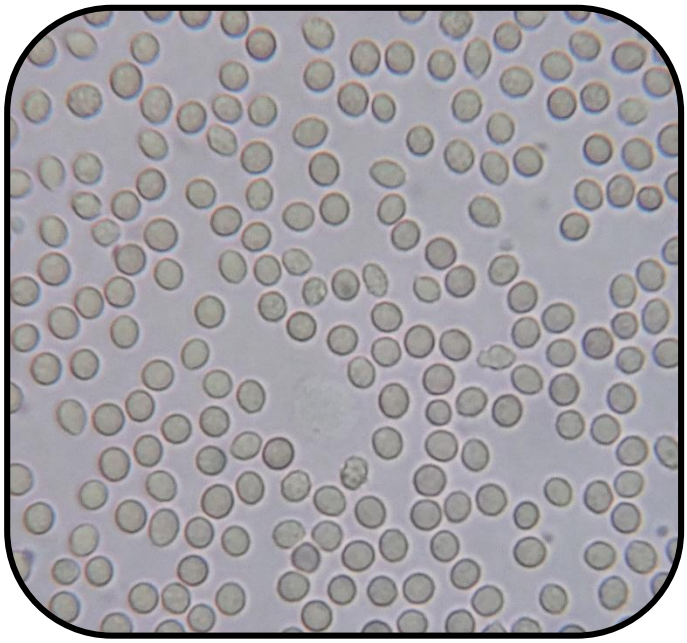
Figure 4 is a graphical representation of Table 4

MORPHOLOGY OF RBCs

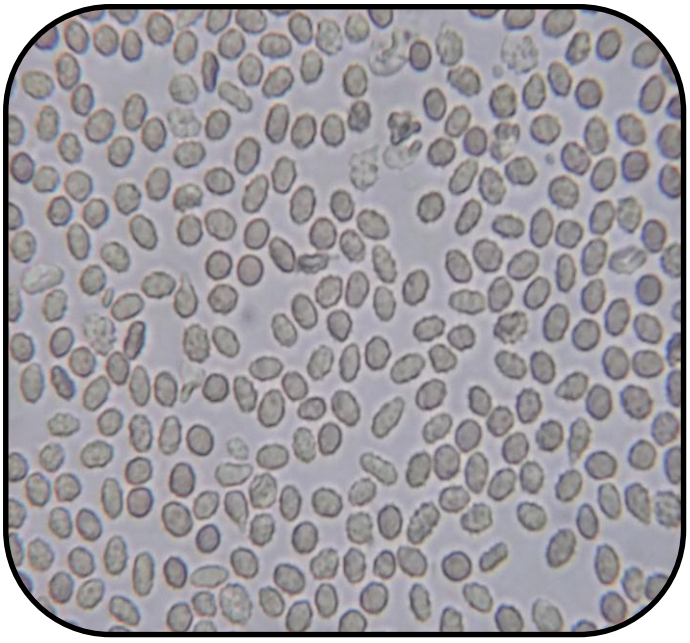
Patient 1: Before treatment



Patient 1: After treatment



Patient 2: Before treatment



Patient 2: After treatment

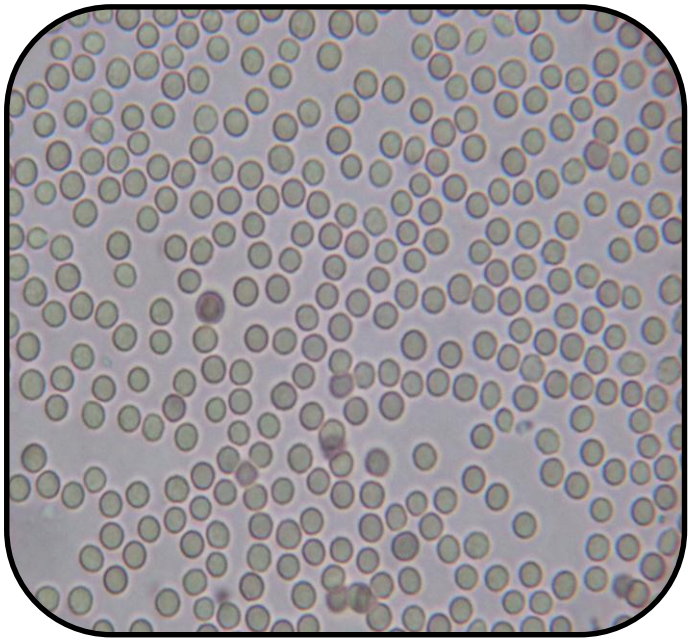


TABLE 5: MEAN HEMOGLOBIN (g %)

GROUPS	0 WEEK		AT THE END OF 8 WEEKS		p value
	MEAN (g %)	SD	MEAN (g %)	SD	
CONTROL	10.46	1.37	10.42	1.34	0.42
STUDY	10.23	1.56	11.49	1.49	< 0.001
p value	0.54		0.005		

Table 5 - shows mean hemoglobin values of patients in control and study group.

- Within the control group there was no significant change (p =0.42) in mean hemoglobin value (10.46 g % at 0 week to 10.42g % at 8 week).
- Within study group there was significant increase (p <0.001) in mean hemoglobin value (10.23 g % at 0 week to 11.49g % at 8 week).
- Between two groups there was no significant difference (p =0.54) in mean hemoglobin value at 0 week but at 8th week study group showed statistically significant increase (p =0.005) in mean hemoglobin value than control group.

FIGURE 5: MEAN HEMOGLOBIN (g %)

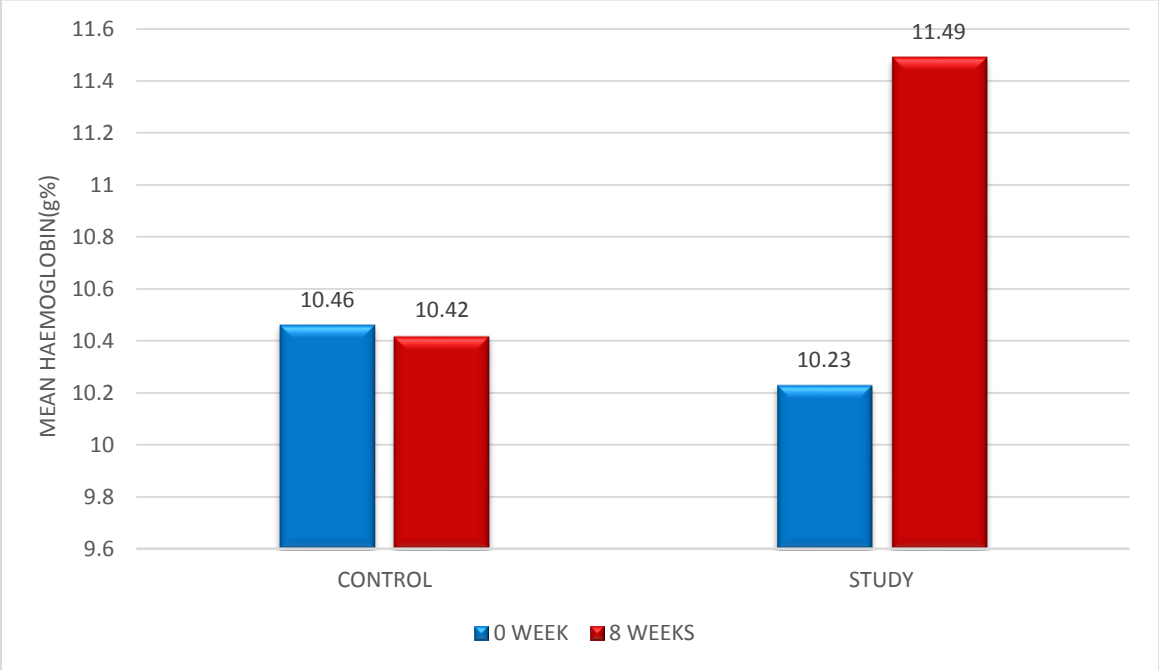


Figure 5 is a graphical representation of Table 5

TABLE 6: MEAN RED BLOOD CELL (RBC) COUNT (millions/µL)

GROUPS	0 WEEK		AT THE END OF 8 WEEKS		p value
	MEAN (millions/µL)	SD	MEAN (millions/µL)	SD	
CONTROL	3.59	0.53	3.55	0.48	0.19
STUDY	3.53	0.60	3.95	0.60	< 0.001
p value	0.72		0.006		

Table 6 - shows the mean Red blood cell (RBC) count of patients in control and study groups

- There was no statistically significant difference (p =0.19) in mean Red blood cell count within control group at 0 week (3.59 millions/µL) and at the end of 8 weeks (3.55 millions/µL).
- In study group there was a significant increase (p<0.001) in RBC count from 3.53 millions/µL at 0 week to 3.95 millions/µL at the end of 8 weeks.
- There was no difference in RBC count between two groups at 0 week (p = 0.72) but at the end of 8 weeks study group showed statistically significant increase (p=0.006) in mean Red blood cell (RBC) count compared to control group.

FIGURE 6: MEAN RED BLOOD CELL COUNT (millions/µL)

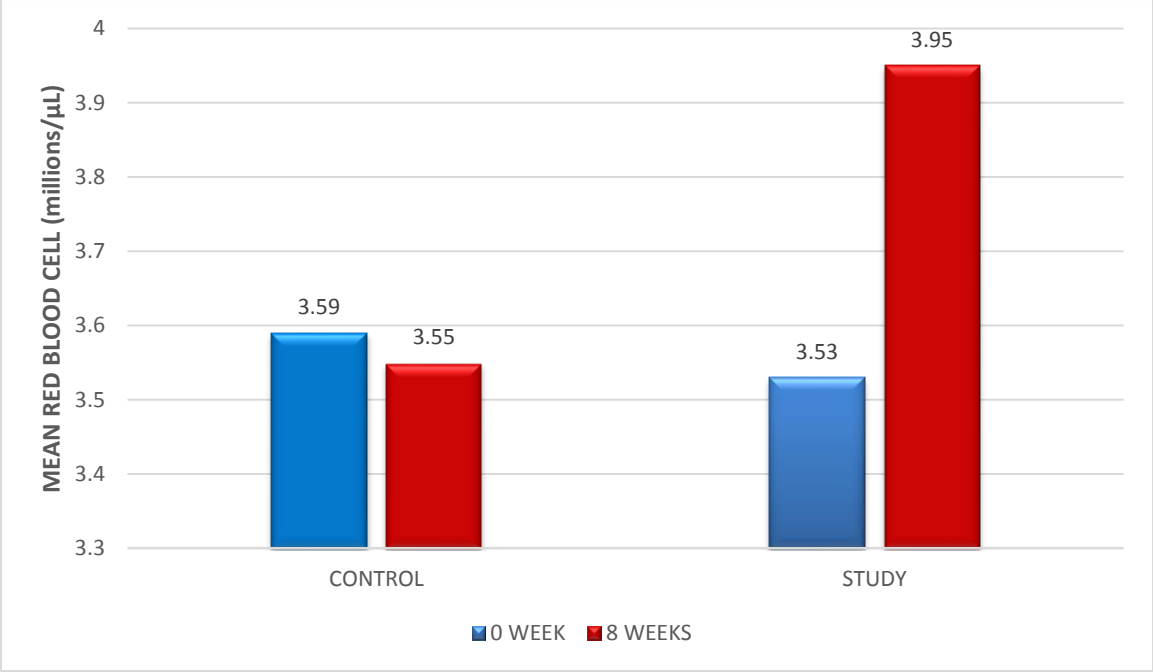


Figure 6 is a graphical representation of Table 6

TABLE 7: MEAN TOTAL CHOLESTROL (mg/dL)

GROUPS	0 WEEK		AT THE END OF 8 WEEKS		p value
	MEAN (mg/dl)	SD	MEAN (mg/dl)	SD	
CONTROL	240.20	41.90	228.80	32.62	0.017
STUDY	243.20	36.25	191.93	27.42	<0.001
p value	0.76		<0.001		

Table 7 - shows the mean total cholesterol in control and study groups

- In Control group the mean total cholesterol was decreased from 240.20 mg/dl at 0 week to 228.80 mg/dl at 8 weeks (p =0.017).
- In study group the mean total cholesterol was significantly decreased (p<0.001) from 243.20 mg/dl at 0 week to 191.93 mg/dl at 8th week.
- At baseline intergroup comparison showed no difference (p = 0.76). But at the end of 8 weeks study group showed statistically significant reduction (p<0.001) in total cholesterol values compared to control group.

FIGURE 7: MEAN TOTAL CHOLESTROL (mg/dL)

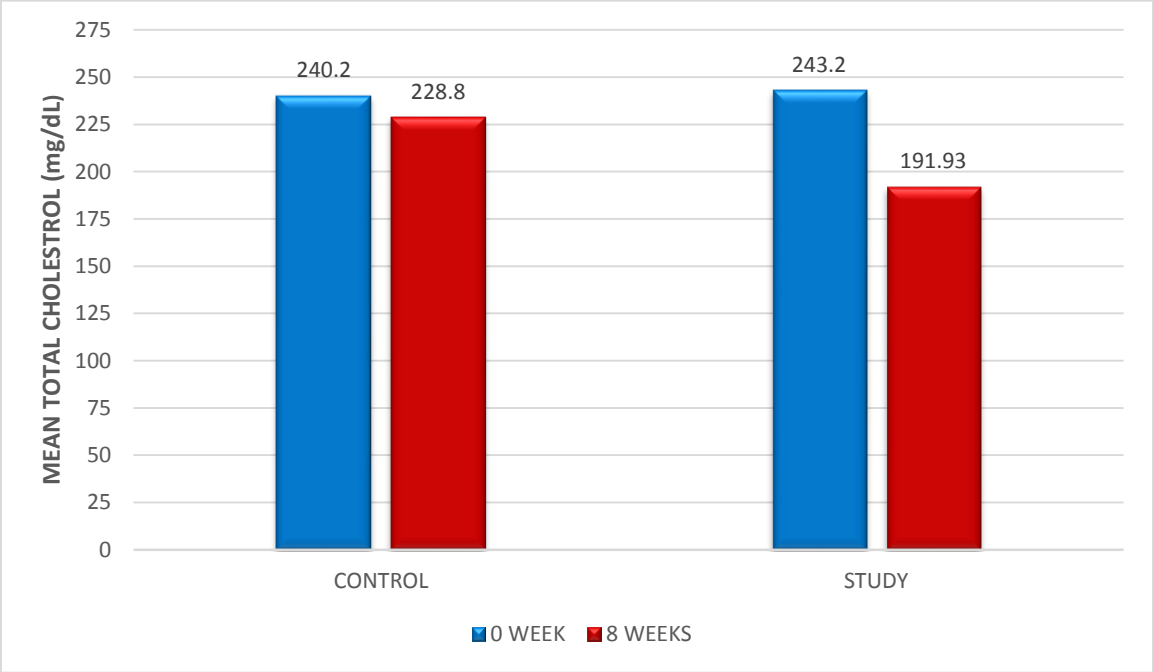


Figure 7 is a graphical representation of Table 7

TABLE 8: MEAN LDL CHOLESTROL (mg/dL)

GROUPS	0 WEEK		AT THE END OF 8 WEEKS		p value
	MEAN (mg/dl)	SD	MEAN (mg/dl)	SD	
CONTROL	163.01	44.09	152.68	32.36	0.030
STUDY	159.98	33.82	126.51	27.45	<0.001
p value	0.76		0.001		

Table 8 depicts mean LDL cholesterol levels in both groups at 0 week and at the end of 8 weeks.

- At 0 week the mean LDL Cholesterol was 163.01 mg/dl in control group and 159.98mg/dl in study group
- At the end 8 weeks the mean LDL Cholesterol was found to be 152.68mg/dl (p=0.030) in control group and 126.51mg/dl (p<0.001) in study group.
- Both groups were comparable at 0 week (p=0.76). By the end of 8 weeks the study group showed more decrease in LDL cholesterol level than control group and this was found to be statistically significant (p=0.001).

FIGURE 8: MEAN LDL CHOLESTROL (mg/dL)

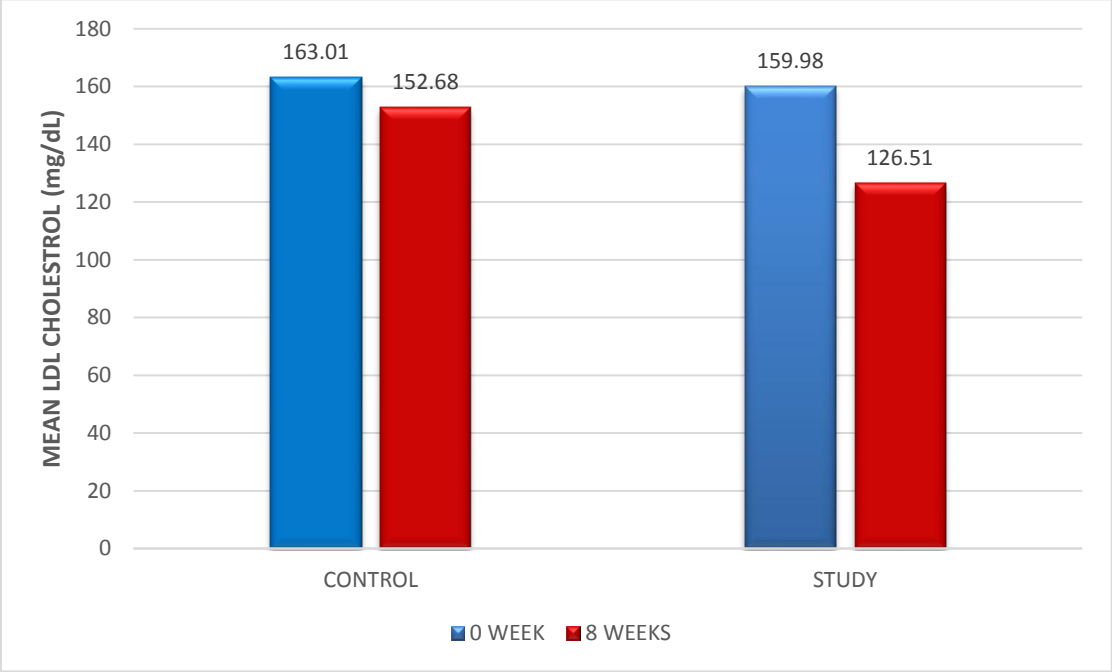


Figure 8 is a graphical representation of Table 8

TABLE 9: MEAN TRIGLYCERIDES (mg/dL)

GROUPS	0 WEEK		AT THE END OF 8 WEEKS		p value
	MEAN (mg/dl)	SD	MEAN (mg/dl)	SD	
CONTROL	172.80	24.32	161.93	15.75	0.011
STUDY	174.03	23.01	157.67	12.59	0.002
p value	0.84		0.25		

Table 9 shows mean triglycerides levels in both groups at 0 week and at the end of 8 weeks.

- At 0 week mean triglycerides levels in control group was 172.80 mg/dl and study group was 174.03 mg/dl.
- At the end of 8 weeks triglycerides levels in control group was 161.93 mg/dl (p=0.011) and in study group was 157.67 mg/dl (p=0.002).
- Comparing both groups, there was no statistically significant difference at 0 week (p= 0.84) and at the end of 8 weeks(p=0.25)

FIGURE 9: MEAN TRIGLYCERIDES (mg/dL)

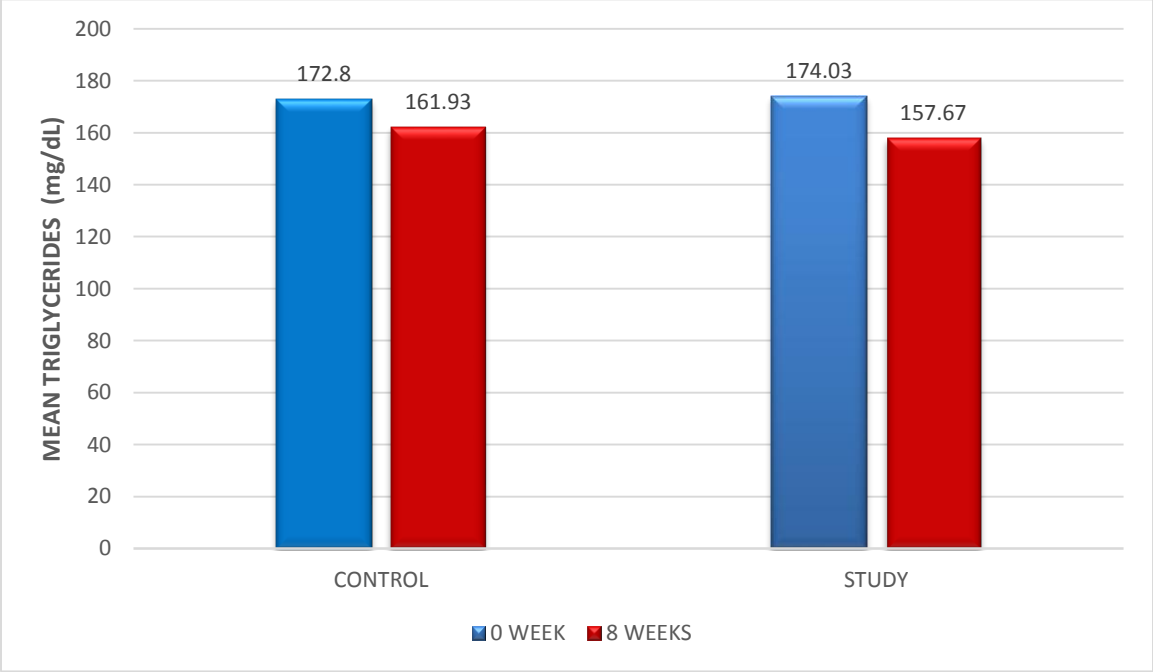


Figure 9 is a graphical representation of Table 9

TABLE 10: MEAN VLDL CHOLESTROL (mg/dL)

GROUPS	0 WEEK		AT THE END OF 8 WEEKS		p value
	MEAN (mg/dl)	SD	MEAN (mg/dl)	SD	
CONTROL	34.56	4.86	32.38	3.15	0.011
STUDY	34.29	4.48	31.63	2.55	0.008
p value	0.82		0.31		

Table 10 shows mean VLDL cholesterol levels in both groups at 0 week and at the end of 8 weeks.

- The mean VLDL cholesterol levels at 0 week in control group was 34.56 mg/dl and in study group was 34.29 mg/dl.
- At the end of 8 weeks the mean VLDL cholesterol levels in control group was 32.38mg/dl (p=0.011) and in study group 31.63 mg/dl, (p =0.008)
- Comparing both groups there was no statistically significant difference at 0 week (p =0. 82) and at end of 8 weeks (p=0.31)

FIGURE 10: MEAN VLDL CHOLESTROL (mg/dL)

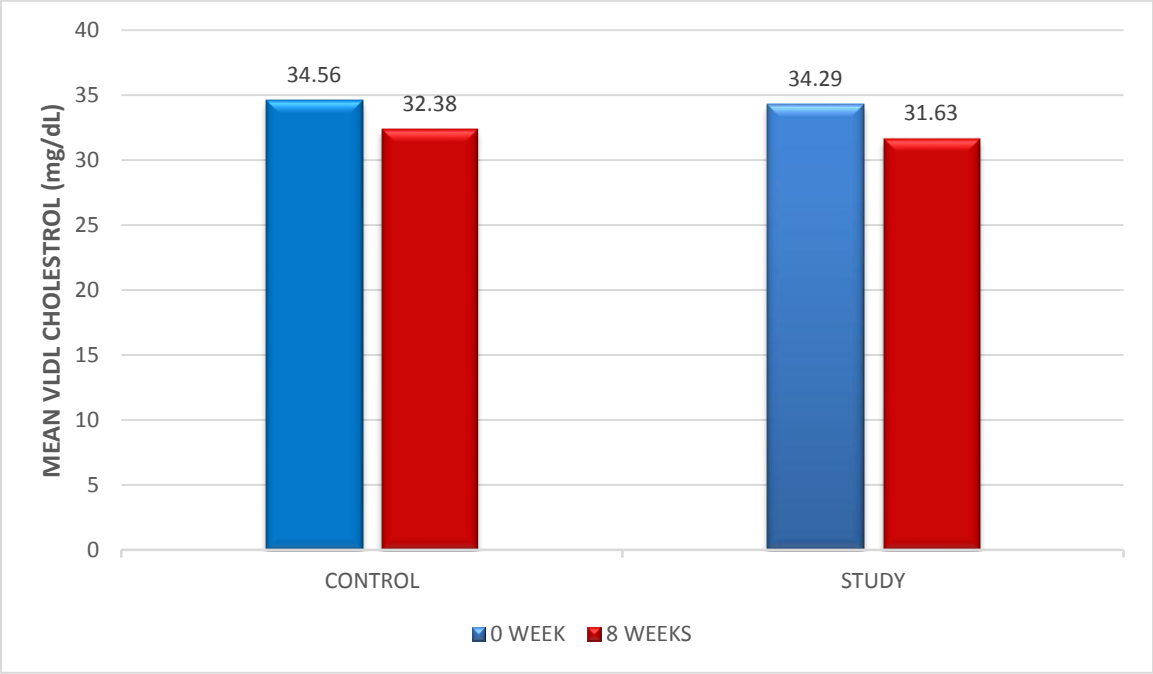


Figure 10 is a graphical representation of Table 10

TABLE 11: MEAN HDL CHOLESTROL (mg/dL)

GROUPS	0 WEEK		AT THE END OF 8 WEEKS		p value
	MEAN (mg/dl)	SD	MEAN (mg/dl)	SD	
CONTROL	39.00	1.53	39.97	2.29	0.053
STUDY	39.26	2.25	41.23	2.12	0.001
p value	0.60		0.03		

Table 9 depicts mean HDL cholesterol levels in both groups at 0 week and at the end of 8 weeks.

- Comparison of mean HDL cholesterol levels at 0 week and at the end of 8 weeks showed no difference in control group (39.00 mg/dl to 39.97 mg/dl, p=0.053) but study group showed significant increase in HDL levels (39.26 mg/dl to 41.23mg/dl, p=0.001).
- Comparing control and study group there was no significant difference in mean HDL cholesterol levels at 0 week (p=0. 60) but at the end of 8 weeks study group showed statistically significant increase at 8 week (p=0.03).

FIGURE 11: MEAN HDL CHOLESTROL (mg/dL)

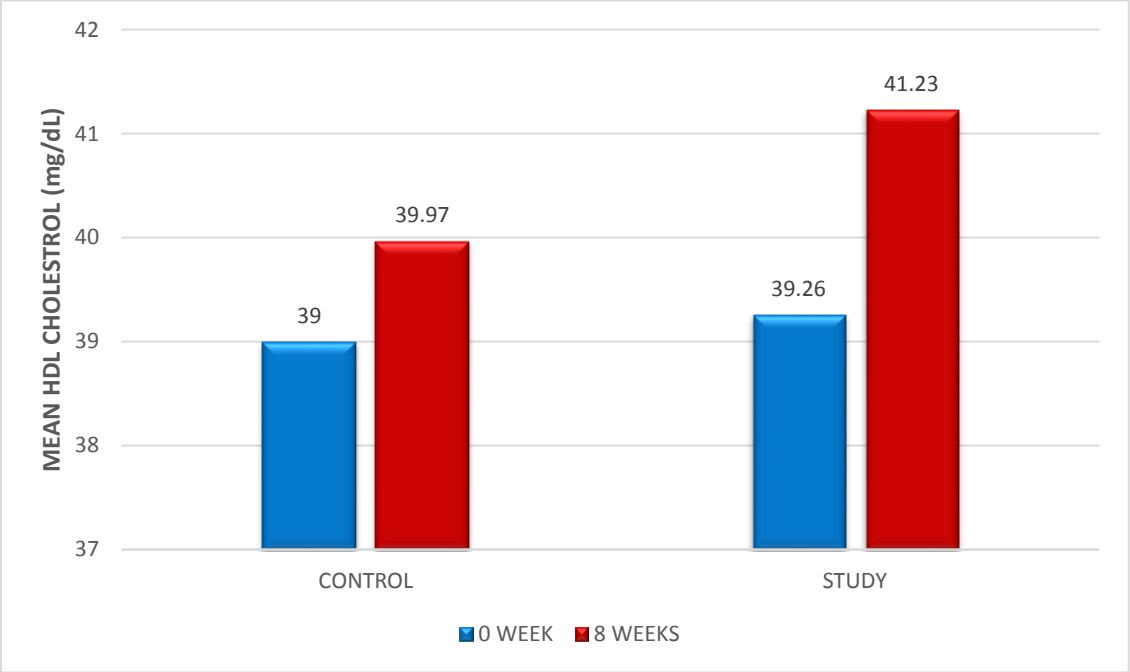


Figure 11 is a graphical representation of Table 11

TABLE 12: MEAN SYSTOLIC BLOOD PRESSURE (mmHg)

GROUPS	0 WEEK		AT THE END OF 8 WEEKS		p value
	MEAN (mm Hg)	SD	MEAN (mm Hg)	SD	
CONTROL	139.27	8.92	137.73	8.25	0.023
STUDY	141.53	8.87	133.13	7.02	<0.001
p value	0.33		0.024		

Table 12 - shows the mean systolic blood pressure in control and study groups

- In Control group the mean systolic blood pressure was decreased from 139.27 mm Hg at 0 week to 137.73 mm Hg at the end of 8 weeks (p =0.023).
- In study group the mean systolic blood pressure was significantly decreased (p<0.001) from 141.53 mm Hg at 0 week to 133.13 mm Hg at the end of 8 weeks.
- At baseline intergroup comparison showed no difference (p = 0.33). But at the end of 8 weeks there was statistically significant reduction (p = 0.024) of mean systolic blood pressure in study group compared to control group.

FIGURE 12: MEAN SYSTOLIC BLOOD PRESSURE (mmHg)

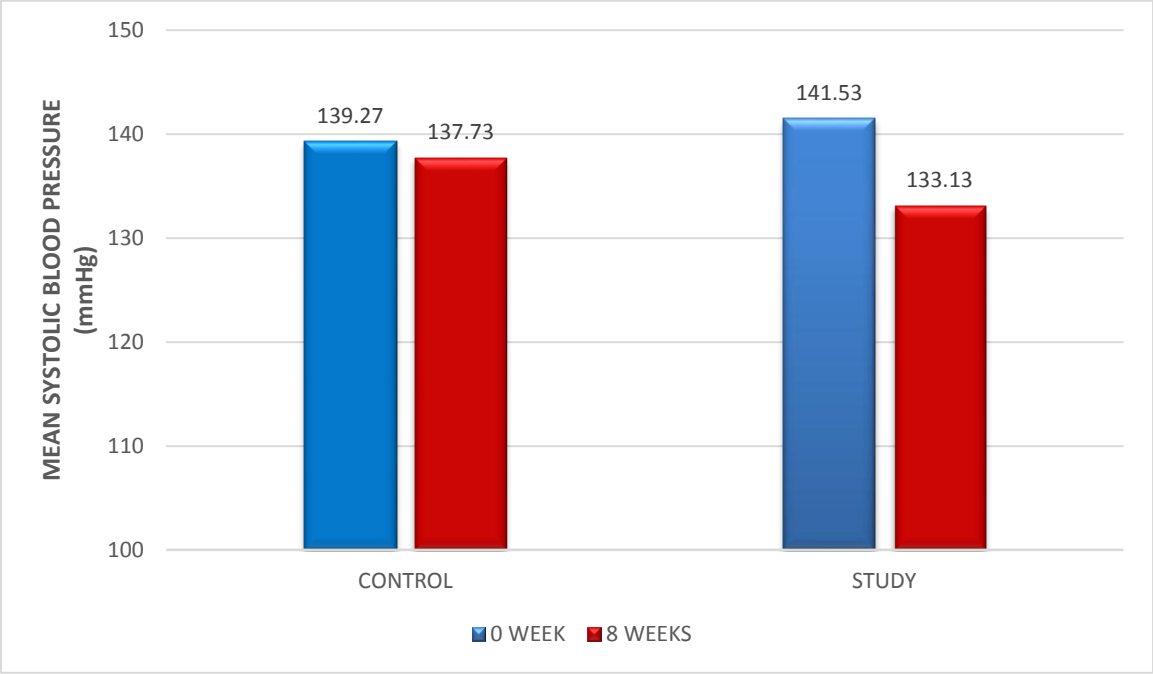


Figure 12 is a graphical representation of Table 12

TABLE 13: MEAN DIASTOLIC BLOOD PRESSURE (mmHg)

GROUPS	0 WEEK		AT THE END OF 8 WEEKS		p value
	MEAN (mm Hg)	SD	MEAN (mm Hg)	SD	
CONTROL	84.13	8.95	82.60	8.38	0.005
STUDY	82.13	8.62	76.93	6.55	<0.001
p value	0.38		0.005		

Table 13 - shows the mean diastolic blood pressure in control and study groups

- The mean diastolic pressure in control group was 84.13 mmHg and in study group was 82.13 mmHg at 0 week. At the end of 8 weeks the mean diastolic blood pressure was 82.60 mmHg in control group and 76.93 mmHg in study group.
- There was a statistically significant reduction in mean diastolic blood pressure within the study group ($p<0.001$) at the end of 8 weeks.
- There was no significant difference between the control and study group at 0 week ($p = 0.38$) but at the end of 8 weeks there was statistically significant reduction ($p=0.005$) in diastolic blood pressure in study group compared to control group.

FIGURE 13: MEAN DIASTOLIC BLOOD PRESSURE (mmHg)

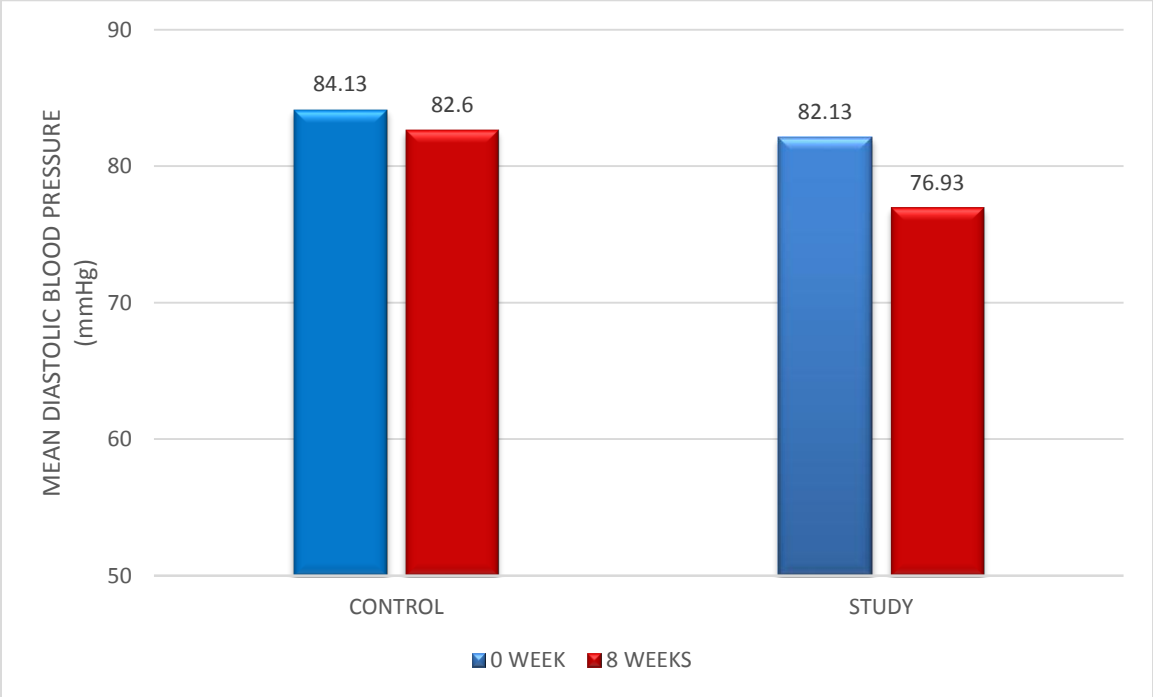


Figure 13 is a graphical representation of Table 13

TABLE 14: DISTRIBUTION OF BODY MASS INDEX (kg/m²)

BODY MASS INDEX	GROUPS			
	CONTROL		STUDY	
	n	%	n	%
<18.5 kg/m²	0	0%	0	0%
18.5 – 24.9 kg/m²	17	56.7%	19	63.3%
25– 29.9 kg/m²	11	36.7%	10	33.3%
≥30 kg/m²	2	6.6%	1	3.3%
TOTAL NO. OF PATIENTS	30	100%	30	100%

Table 14– shows distribution of body mass index of patients among control and study groups. Most of the patients in both groups had BMI within normal limits 18.5 – 24.9 kg/m². 36.7% patients in control group and 33.3% in study group were overweight (25– 29.9 kg/m²). Only **6.6%** in control group **3.3%** in study group were obese (≥30 kg/m²).

FIGURE 14: DISTRIBUTION OF BODY MASS INDEX (kg/m²)

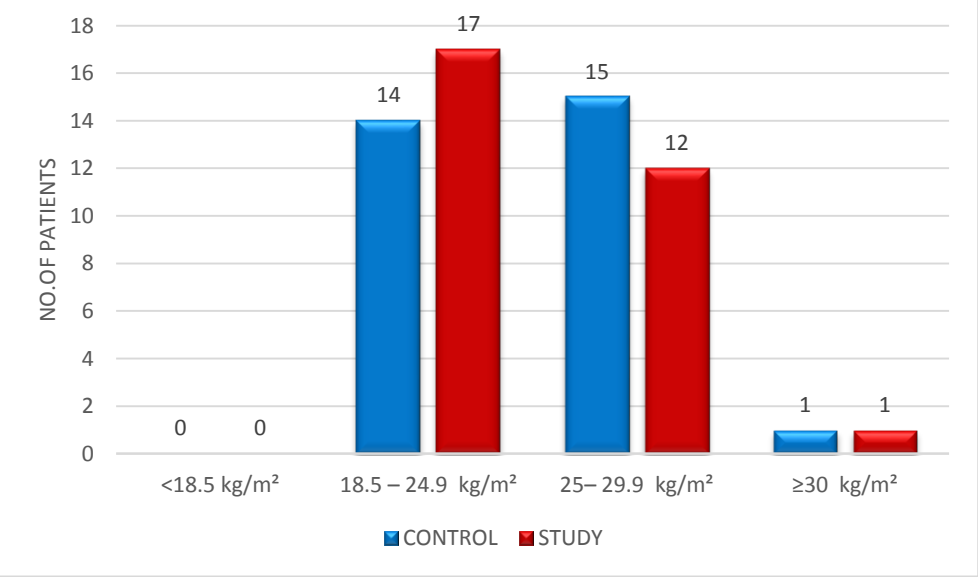


TABLE 15: OTHER BIOCHEMICAL INVESTIGATIONS

PARAMETER	CONTROL			STUDY		
	0 WEEK (MEAN)	AT THE END OF 8WEEKS (MEAN)	P VALUE	0 WEEK (MEAN)	AT THE END OF 8WEEKS (MEAN)	P VALUE
SGOT	30	29.93	0.90	30.50	30.30	0.71
SGPT	31.73	31.67	0.78	31.90	31.77	0.71
BILIRUBIN	0.83	0.85	0.56	0.83	0.81	0.53
SERUM UREA	25.70	25.07	0.45	26.87	25.90	0.18
SERUM CREATININE	0.77	0.72	0.70	0.72	0.67	0.17

Table 15- shows the biochemical investigations at 0 week and at the end of 8 weeks in control and study groups. There was no significant change in lab parameters before and after study in both the groups.

TABLE 16: ADVERSE EVENT PROFILE

ADVERSE EVENT	CONTROL GROUP	STUDY GROUP
NAUSEA	1	1
ABDOMINAL PAIN	2	1
DIARRHEA	1	1
HYPOGLYCEMIA	2	2
HEADACHE	1	0
MYALGIA	3	1
INSOMNIA	1	0

FIGURE 16: ADVERSE EVENT PROFILE

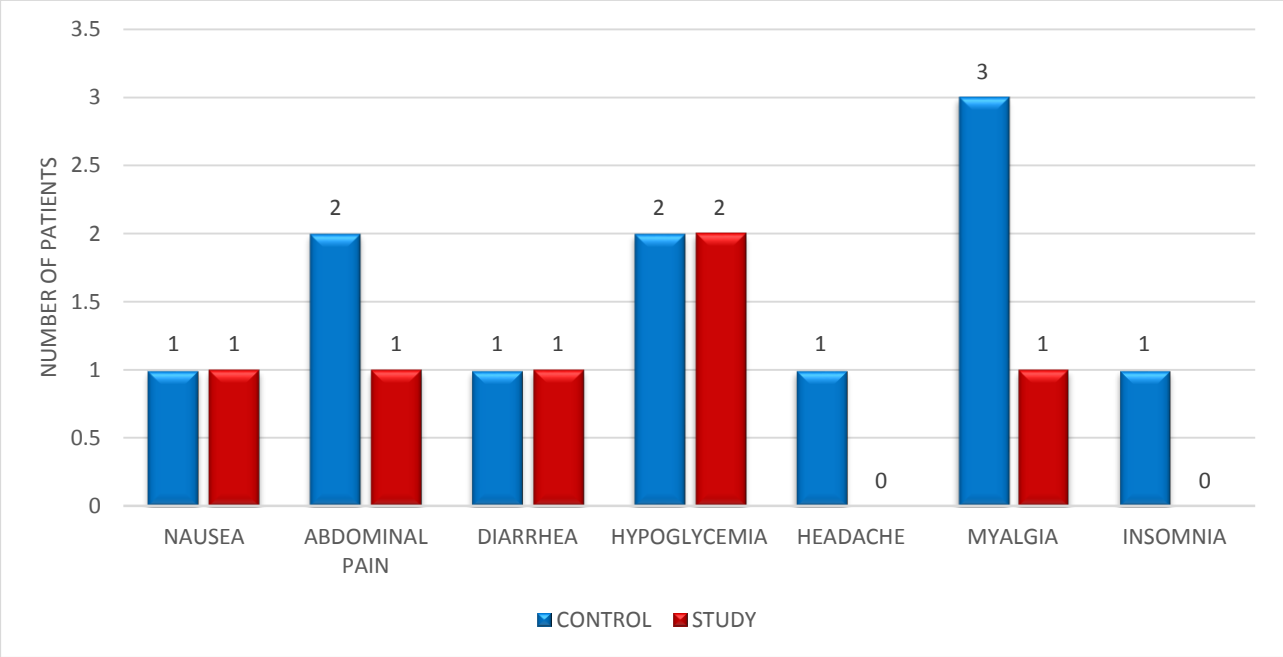


TABLE 17: INCIDENCE OF ADR

	CONTROL GROUP (n=30)	STUDY GROUP (n=30)
NO. OF ADR's	**11 (36.7%)**	**6 (20%)**

Table 17 shows the incidence of ADR's among patients in control and study groups. ADRs were 11 (36.7%) in the control group and 6 (20%) in the study groups.

FIGURE 17: INCIDENCE OF ADR

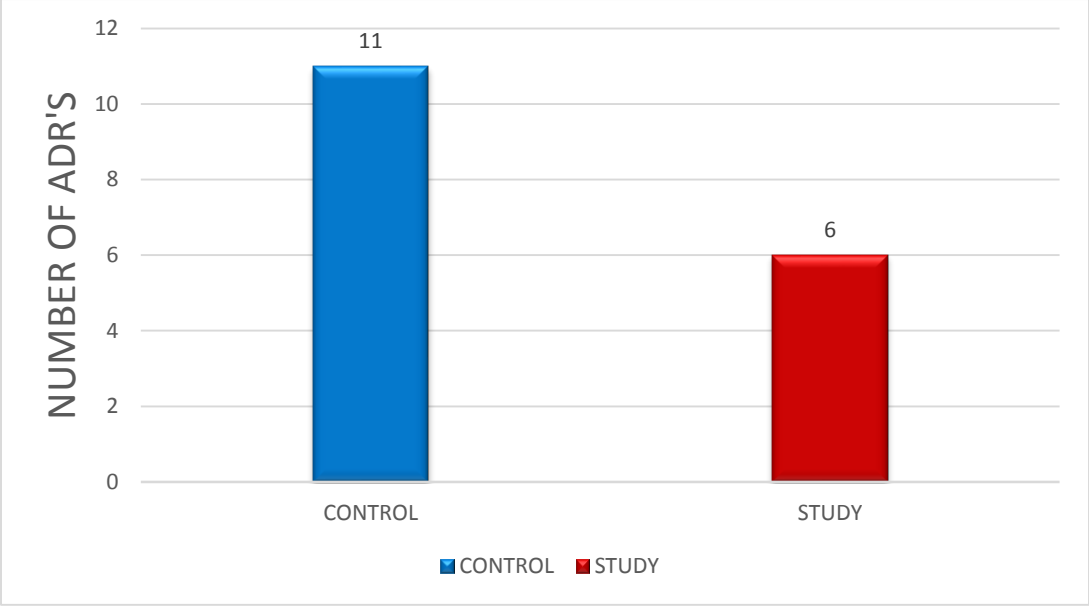
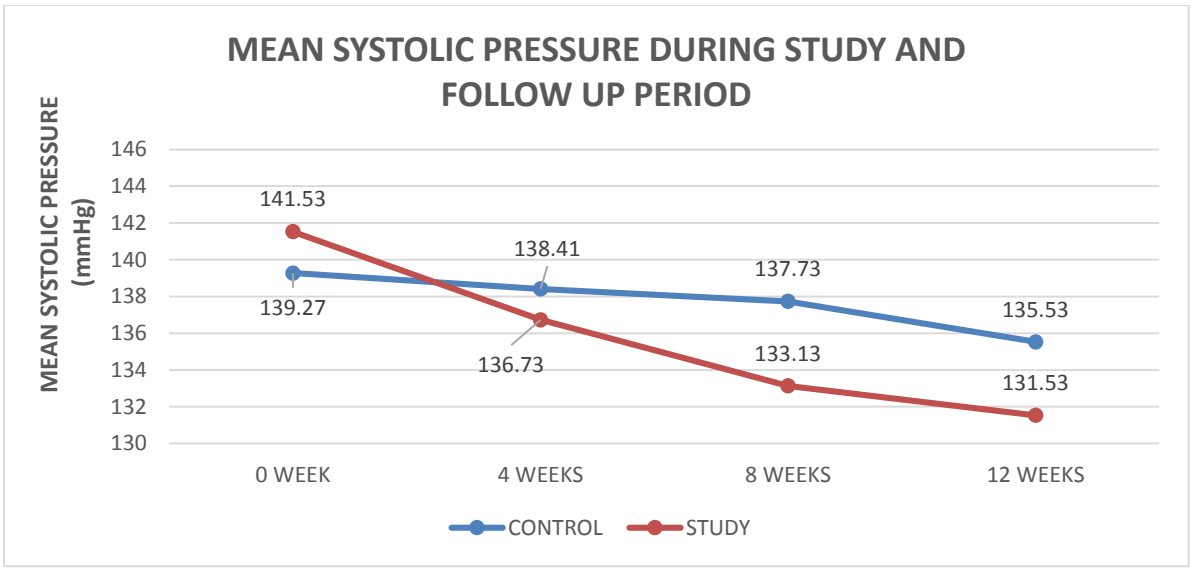
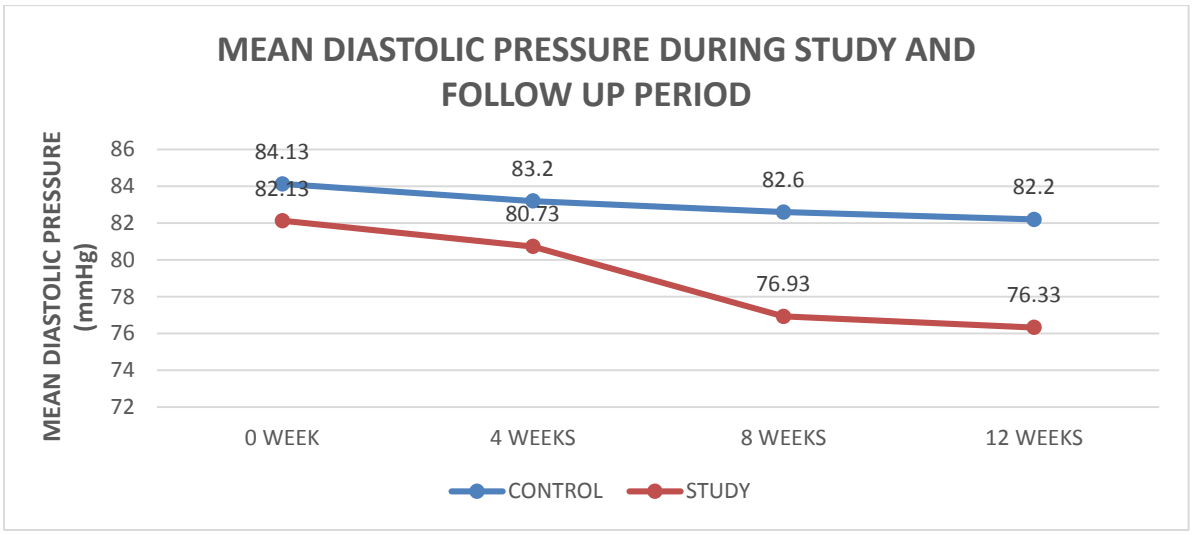
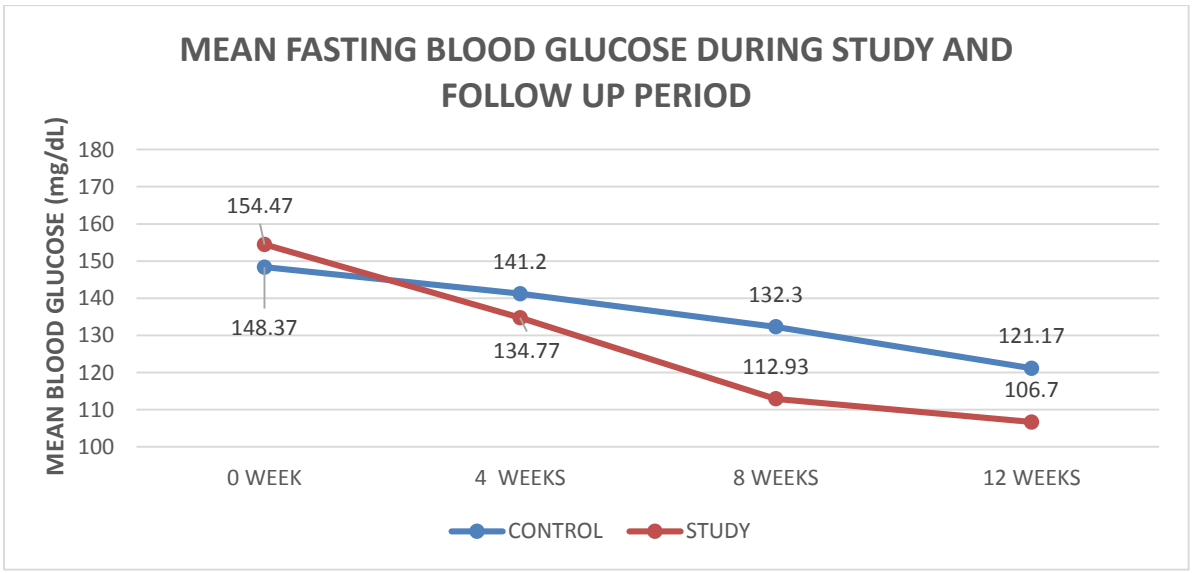
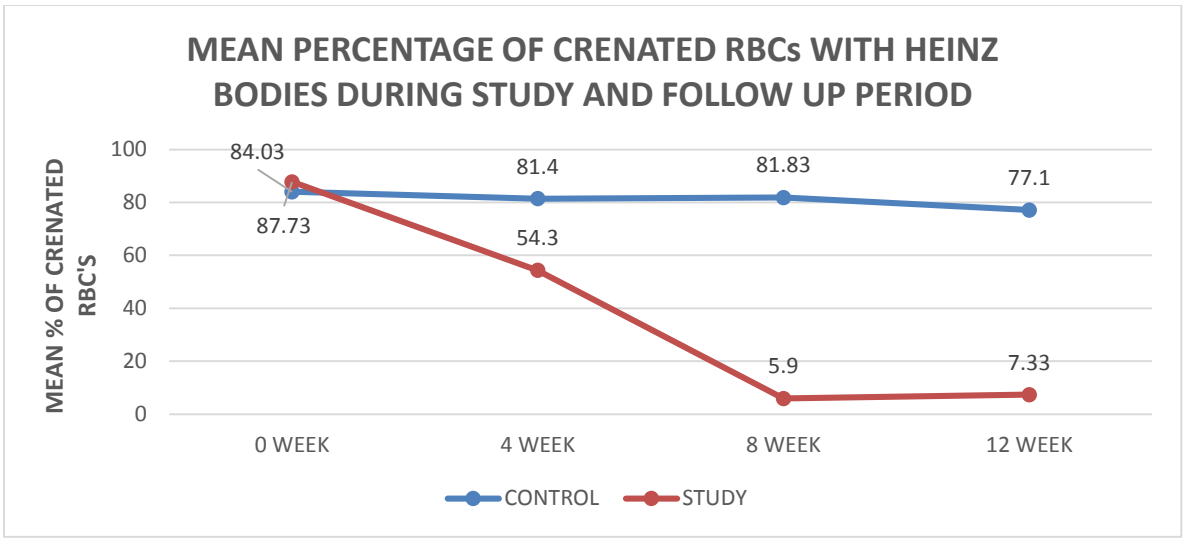


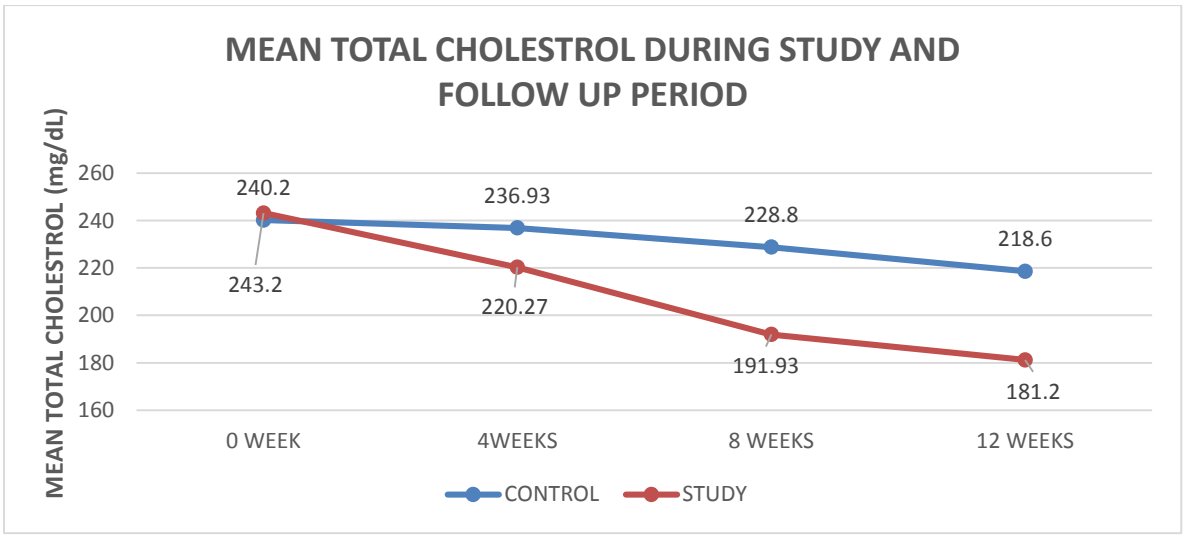
Figure 17 is a graphical representation of Table 17

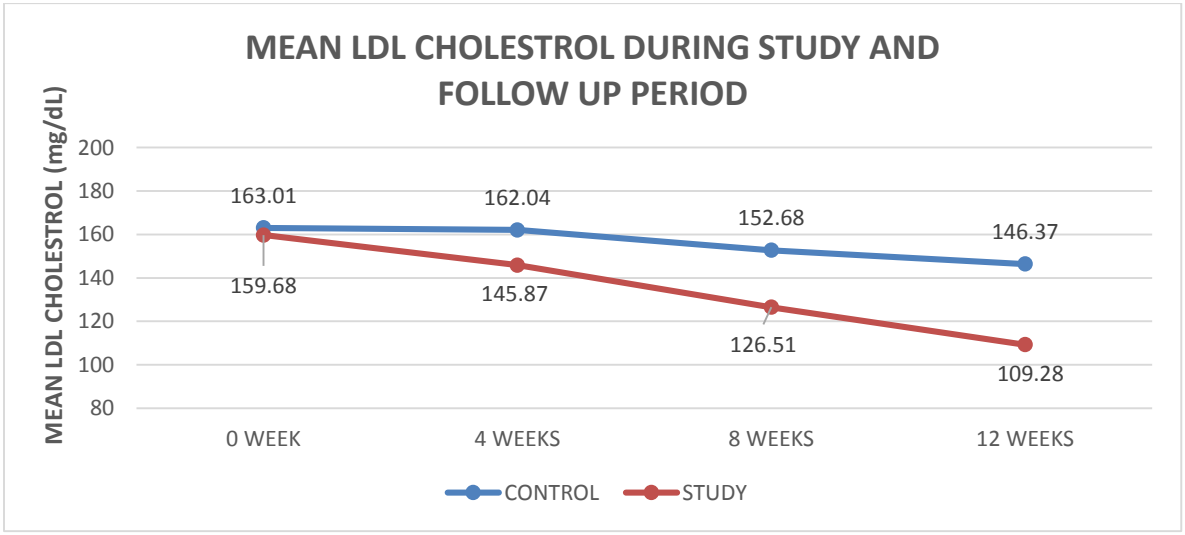

MEAN SYSTOLIC PRESSURE DURING STUDY AND FOLLOW UP PERIOD
MEAN SYSTOLIC PRESSURE (mmHg)
146
144
142
140
138
136
134
132
130
141.53
139.27
138.41
136.73
137.73
133.13
135.53
131.53
0 WEEK
4 WEEKS
8 WEEKS
12 WEEKS
CONTROL
STUDY

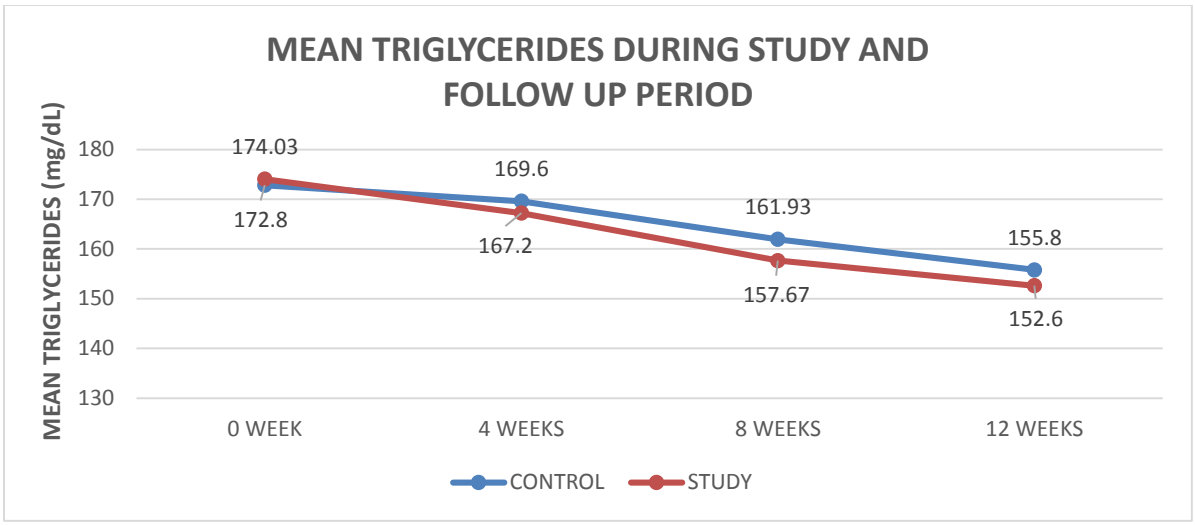

MEAN DIASTOLIC PRESSURE DURING STUDY AND FOLLOW UP PERIOD
MEAN DIASTOLIC PRESSURE (mmHg)
86
84
82
80
78
76
74
72
84.13
82.13
83.2
80.73
82.6
76.93
82.2
76.33
0 WEEK
4 WEEKS
8 WEEKS
12 WEEKS
CONTROL
STUDY

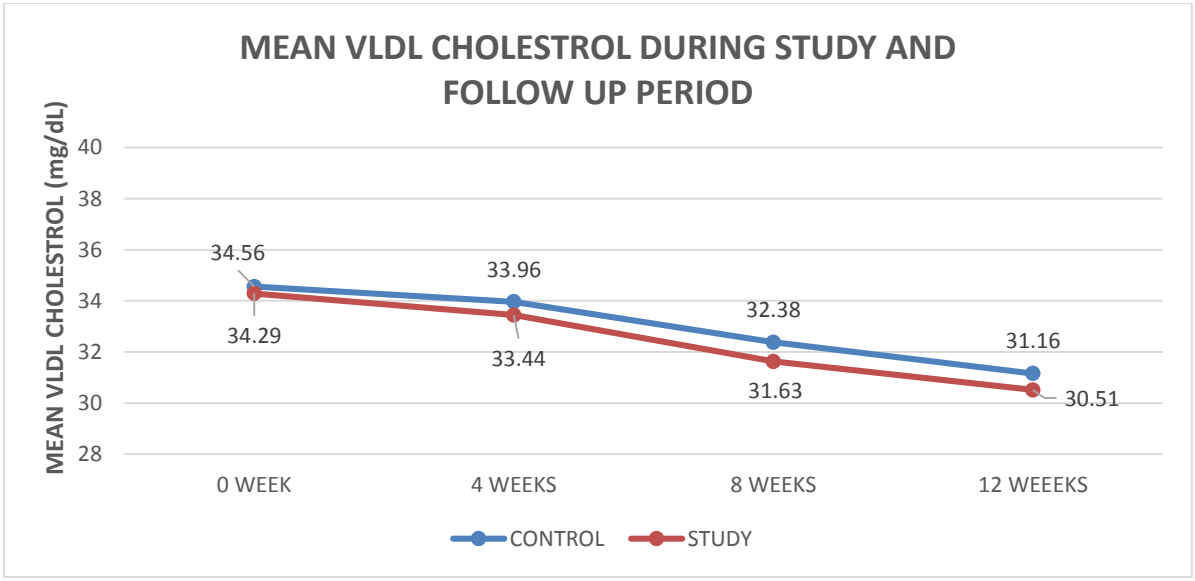

MEAN FASTING BLOOD GLUCOSE DURING STUDY AND FOLLOW UP PERIOD
MEAN BLOOD GLUCOSE (mg/dL)
180
170
160
150
140
130
120
110
100
154.47
148.37
141.2
134.77
132.3
112.93
121.17
106.7
0 WEEK
4 WEEKS
8 WEEKS
12 WEEKS
CONTROL
STUDY

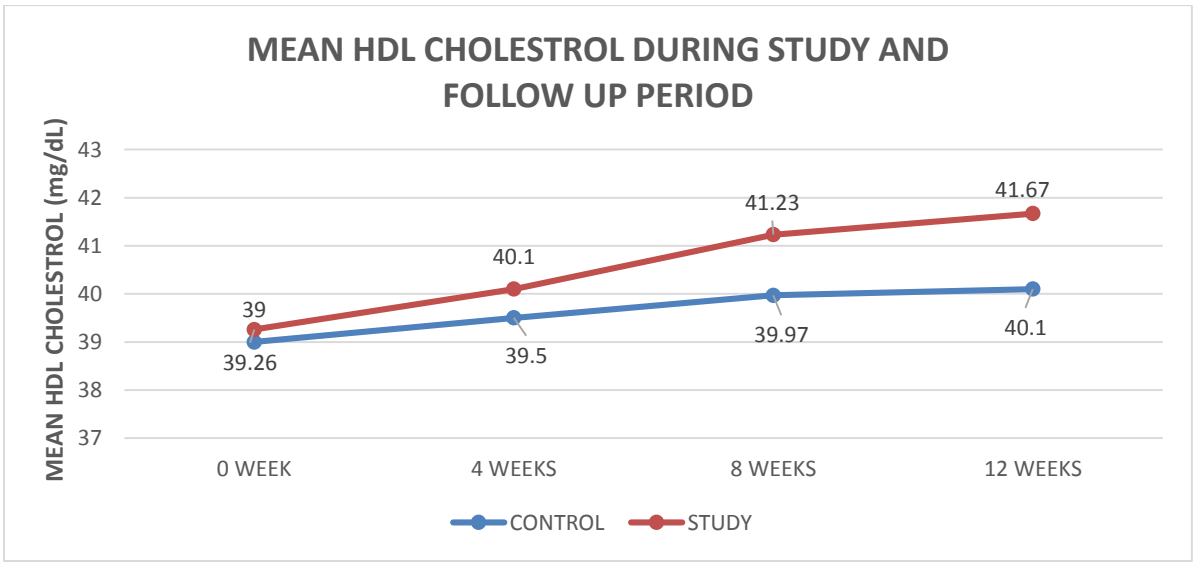

MEAN PERCENTAGE OF CRENATED RBCs WITH HEINZ BODIES DURING STUDY AND FOLLOW UP PERIOD
MEAN % OF CRENATED RBC'S
100
80
60
40
20
0
84.03
87.73
81.4
54.3
81.83
5.9
77.1
7.33
0 WEEK
4 WEEK
8 WEEK
12 WEEK
CONTROL
STUDY


MEAN TOTAL CHOLESTROL DURING STUDY AND FOLLOW UP PERIOD
MEAN TOTAL CHOLESTROL (mg/dL)
260
240
220
200
180
160
240.2
243.2
236.93
220.27
228.8
191.93
218.6
181.2
0 WEEK
4WEEKS
8 WEEKS
12 WEEKS
CONTROL
STUDY


MEAN LDL CHOLESTROL DURING STUDY AND FOLLOW UP PERIOD
MEAN LDL CHOLESTROL (mg/dL)
200
180
160
140
120
100
80
163.01
159.68
162.04
145.87
152.68
126.51
146.37
109.28
0 WEEK
4 WEEKS
8 WEEKS
12 WEEKS
CONTROL
STUDY


MEAN TRIGLYCERIDES DURING STUDY AND FOLLOW UP PERIOD
MEAN TRIGLYCERIDES (mg/dL)
180
170
160
150
140
130
174.03
172.8
169.6
167.2
161.93
157.67
155.8
152.6
0 WEEK
4 WEEKS
8 WEEKS
12 WEEKS
CONTROL
STUDY


MEAN VLDL CHOLESTROL DURING STUDY AND FOLLOW UP PERIOD
MEAN VLDL CHOLESTROL (mg/dL)
40
38
36
34
32
30
28
34.56
34.29
33.96
33.44
32.38
31.63
31.16
30.51
0 WEEK
4 WEEKS
8 WEEKS
12 WEEEKS
CONTROL
STUDY


MEAN HDL CHOLESTROL DURING STUDY AND FOLLOW UP PERIOD
MEAN HDL CHOLESTROL (mg/dL)
43
42
41
40
39
38
37
39
39.26
40.1
39.5
41.23
39.97
41.67
40.1
0 WEEK
4 WEEKS
8 WEEKS
12 WEEKS
CONTROL
STUDY

DISCUSSION

DISCUSSION

Type 2 diabetes mellitus is mostly associated with hypertension, dyslipidemia, central obesity and insulin resistance. Development of cardiovascular diseases is high in these patients[7]. The coexistence of these factors in a person proves the existence of common etiological factor.

Psychological stress is one of the most important cause for metabolic syndrome which triggers the release of stress hormones through hypothalamus. These stress hormones (Adrenaline, Glucagon, Corticosteroids etc.) is responsible for hypertension, hyperglycemia and dyslipidemia[16, 34].

Oxidative stress which follows psychological stress is responsible for the development of the following like insulin resistance by releasing inflammatory mediators, oxidation of LDL which forms atherosclerosis [24] and hypertension by decreasing nitric oxide bioavailability in the blood vessels[47].

Oxidative stress causes RBC damage which alters the morphology of RBCs[5]. In our study RBC morphology was used as a biomarker for oxidative stress.

Antioxidants like α-tocopherol and Ascorbic acid were given to the patients with metabolic syndrome, to prove that free radical injury is responsible for type 2 diabetes, hypertension and dyslipidemia.

In this study, 121 patients diagnosed with Hypertension, Type 2 diabetes and Dyslipidemia on treatment for 1-2 years duration were screened, 61 patients who did

not match study criteria were excluded. 60 patients were randomized by simple randomization into control and study groups of 30 patients each. Patients in the control group were on standard treatment with Tab.Metformin, Tab.Atorvastatin and Tab.Enalapril and/or Tab.Amlodipine. In the study group, patients received once daily dosage of Cap. Vitamin E 400mg and Tab. Vitamin C 500mg along with standard therapy for 8 weeks duration. After completion of 8 weeks treatment both groups were followed up for 4 weeks.

The percentage of crenated RBCs with Heinz bodies in patient's blood sample was used for the assessment of oxidative stress status. Parameters such as Fasting blood glucose, Systolic and Diastolic blood pressure, Lipid profile, Hemoglobin, RBC count were also assessed.

Among the 60 patients who completed the study, the mean age of patient in the control group was 51.13 years and in the study group was 50.73 years. This showed that most of the patients were middle aged. There were a higher proportion of male patients in both the control (60%) and study (66.7%) groups. The mean age did not show significant difference between control and study groups.

Most of the patients in both groups had BMI within normal limits (18.5 – 24.9 kg/m^2). 36.7% patients in control group and 33.3% in study group were overweight (25– 29.9 kg/m^2). Only 6.6% in control group 3.3% in study group were obese ($\geq$30 kg/m^2). This showed that there was lesser prevalence of obesity among diabetics in our study population.

At 0 week, the mean fasting blood glucose was 148.37mg/dl in control group and 154.47 mg/dl in study group. By the end of the study period, the mean fasting blood glucose was found to be 132.30mg/dl, ($p=0.024$) in control group and 112.93mg/dl, ($p<0.001$) in study group. Intergroup comparison showed statistically significant reduction ($p=0.023$) in mean fasting blood glucose in study group at the end of 8 weeks.

Increased presence of inflammatory mediators like isoprostanes (PGF2α) in type 2 diabetes causes insulin resistance which is hypothetically due to binding of isoprostanes to the insulin receptor and prevents the binding of insulin to the receptor causing hyperinsulinemia[14, 15].

Following antioxidant treatment (Vitamin E & C) the blood glucose level was significantly reduced in the study group. This proves the concept that isoprostanes induced by free radicals is the cause for insulin resistance in type 2 diabetes mellitus.

At the beginning of the study both control and study group showed 84.03% and 87.73% of crenated RBCs with Heinz bodies respectively. This indicates free radical induced damage to RBCs in patients with metabolic syndrome. At the end of 8 weeks there was no significant difference in the percentage of crenated RBCs with Heinz bodies in control group (81.83%, $p=0.072$) but the study group showed a statistically significant decrease to 5.90% ($p<0.001$). This clearly demonstrates that treatment with antioxidants improves integrity of RBC membrane, reduces fragility and hemolysis by preventing free radical induced oxidative damage[5].

At 0 week the mean hemoglobin was 10.46 gm/dl in the control group and 10.23 gm/dl in the study group. After 8 weeks of treatment with antioxidants, the study group showed a statistically significant increase ($p<0.001$) in hemoglobin (11.49 gm/dl) compared to the control group hemoglobin (10.42gm/dl).

The mean total RBC count was significantly increased ($p<0.001$) in study group from 3.53 million /μL at 0 week to 3.95 million /μL at end of 8 weeks while there was no significant difference in the control group (0 week–3.59 million /μL and 8 weeks-3.55 million /μL, $p=0.19$). There was a statistically significant intergroup difference ($p=0.006$) at 8 weeks. This proves that type of anemia in metabolic syndrome is hemolytic anemia caused by free radicals[5].

Lipid profile values measured at 0 week and at the end of 8 weeks were compared in both groups. In control group, the total cholesterol value which was 240.20 mg/dl at 0 week had decreased to 228.80mg/dl ($p=0.017$) at 8 weeks. In study group total cholesterol values was reduced from 243.20mg/dl at 0 week to 191.93mg/dl ($p<0.001$) at 8 week. Comparing both groups there was significant reduction ($p < 0.001$) in study group at 8 weeks.

LDL Cholesterol level of control group and study group was 163.01mg/dl and 159.98mg/dl respectively at 0 weeks. After completion of 8 weeks treatment period, LDL cholesterol was reduced to 152.68mg/dl, $p=0.030$ and 126.51 mg/dl $p<0.001$ in control and study group respectively. Study group showed significant reduction ($p=0.001$) in LDL cholesterol than control group at 8 weeks.

HDL value was significantly raised (p=0.03) in study group compared to control group at the end of 8 weeks. In control group HDL value was 39 mg/dl at 0 week and 39.97mg/dl at 8th week (p=0.053). There was no statistically significant difference in control group. In study group HDL value 39.26mg/dl at 0 week was raised to 41.23mg/dl at the end 8 weeks which was statistically significant (p=0.001).

The mean triglyceride level in control group was 172.80mg/dl at 0 week and 161.93 at 8 weeks (p = 0.011). The mean triglycerides level in study group was 174.03 mg/dl at 0 week and 157.67 mg/dl at 8 weeks (p =0.002). Intergroup analysis at the end of 8 weeks showed no difference in triglyceride levels (p = 0.25).

The VLDL cholesterol was 34.56mg/dl at 0 week and 32.38mg/dl (p = 0.011) at the end of 8 weeks in control group. In study group the VLDL cholesterol which was 34.29 mg/dl at 0 week had reduced to 31.63mg/dl (p = 0.008). Intergroup analysis showed no significant difference (p =0.31) at the end of 8 weeks.

This shows that total cholesterol and LDL are decreased in patients who took antioxidants. This proves the action of vitamin C and Vitamin E suppressed the free radical mediated oxidation of LDL, increased binding of LDL to its receptor, cellular uptake and degradation of LDL, increases bile acid synthesis and decreases total cholesterol and LDL levels in blood. The cholesterol level in peripheral cells is replenished which is taken up by HDL and are transported to the liver. The level of HDL increases because there is no degradation of HDL[24].

The mean blood pressure was slightly above average in both the groups at baseline. At 0 week the mean systolic blood pressure in control group was 139.27 mmHg and study group had a mean systolic blood pressure of 141.53mmHg. At the end of 8 weeks there was a statistically significant reduction (p=0.024) in systolic blood pressure (133.13 mmHg) in study group compared to control group (137.73 mmHg).

The mean diastolic blood pressure at 0 week in control and study group was 84.13 mmHg and 82.13mmHg respectively. After 8 weeks of treatment with antioxidants, there was a significant reduction (p=0.005) in diastolic blood pressure in study group (76.93mmHg) compared to control group (82.60mmHg).

The reduction in systolic and diastolic blood pressure in study group proves the fact that antioxidants increases the bioavailability of nitric oxide, improves endothelial dysfunction and restores the balance between sympathetic and parasympathetic nervous system in CNS[23]. This reduces the release of Adrenaline and Glucagon and the disease process is arrested.

There was no change in other biochemical parameters like Blood urea, Creatinine, Bilirubin, Aspartate transaminase and Alanine transaminase in both groups at the end of study period.

Mild adverse effects like Nausea, Vomiting, Abdominal Pain and Hypoglycemia were noted in both control (n=11) and study groups (n=6) with reduced severity of adverse effects in study group.

All the above mentioned effects were sustained in the study group at the end of the 4 week follow up period indicating that the beneficial effects of antioxidants persist for sometime even after its withdrawal.

Decrease in blood glucose level, correction of dyslipidemia and control of blood pressure by antioxidants like Vitamin E and C proves the hypothesis of psychological stress and oxidative stress in the pathogenesis of metabolic syndrome.

CONCLUSION

CONCLUSION

Chronic psychological stress increases the release of stress hormones like Adrenaline, Glucagon, and Corticosteroids etc. These hormones cause hyperglycaemia, hypertension, dyslipidaemia and oxidative stress. Thus contributing to the pathogenesis of metabolic syndrome.

Following treatment with antioxidants like Vitamin E and C there was decrease in insulin resistance, blood pressure and improvement in lipid profile. The Hemolytic anemia improved significantly following antioxidant treatment proves the fact that oxidative stress and chronic inflammation causes damage to RBCs.

Obesity was linked with the pathogenesis of metabolic syndrome but in our study most of the patients were having normal BMI.

Therefore this study proves the fact that psychological stress and oxidative stress are the most important etiological factors in the pathogenesis of metabolic syndrome.

Proper stress management and treatment with antioxidants like vitamin E and C can have a disease modifying action in metabolic syndrome.

Assessment of morphological changes in RBC is the cost effective biomarker for diagnosing oxidative stress.

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APPENDICES

APPENDIX I: ABBREVIATIONS

RBC - Red Blood Cell

IRS - Insulin Receptor Substrate Proteins

GLUT - Glucose Transporter

TNF α - Tumor Necrosis Factor Alpha

IL-1 - Interleukin 1

PG F2α - Prostaglandin F2α

NSAID - Non Steroidal Anti-Inflammatory Drugs

TAG - Tri Acyl Glycerol

DAG - Di Acyl Glycerol

HSL - Hormone Sensitive Lipase

LDL - Low Density Lipoprotein

IDL - Intermediate Density Lipoprotein

VLDL - Very Low Density Lipoprotein

HDL - High Density Lipoprotein

APO A-I -Apoprotein A-I

FFA - Free Fatty Acid

ROS - Reactive Oxygen Species

RNS - Reactive Nitrogen Species

CNS - Central Nervous System

DM - Diabetes Mellitus

CVD - Cardiovascular Disease

WHO - World Health Organization

PPARα - Peroxisome Proliferator Activated Receptor Alpha

BMI - Body Mass Index

AMPK -Adenine Monophosphate-Activated Protein Kinase

GLP1 - Glucagon like Peptide 1

GIP - Gastric Inhibiting Polypeptide.

DPP 4 - Dipeptidyl Peptidase 4

SGLT 2 - Sodium Glucose Co-Transporter 2

ACTH -Adreno Corticotropic Hormone

NADPH - Nicotinamide Adenine Dinucleotide Phosphate

FAD -Flavin Adenine Dinucleotide

GSH - Glutathione

GSSG - Glutathione Disulfide

PUFA - Poly Unsaturated Fatty Acids ATP

NOS - Nitric Oxide Synthase

NO - Nitric Oxide

iNOS - Inducible Nitric Oxide Synthase

eNOS - Endothelial Nitric Oxide Synthase

NMDA - N-Methyl-D-Aspartate

ECG -Electro Cardiography

DNA - Deoxy Ribonucleic Acid

RNA - Ribonucleic acid

ATP - Adenosine Triphosphate

PEP - PhosphoEnol Pyruvate

ANOVA - Analysis Of Variance

APPENDIX II: CASE REPORT FORM

Morphological changes in the Red blood cells as a marker of Oxidative stress in Metabolic Syndrome and Antioxidants as an add on therapy in the reversal of changes – A Randomized, Open label, Comparative Pilot study.

PATIENT DEMOGRAPHY:

NAME: AGE/SEX: OP No:

OCCUPATION: ADDRESS: CONTACT No:

DIAGNOSIS:

S.No.	Inclusion criteria	Yes	No	Exclusion criteria	Yes	No
1.	Age: 40-70 years			Patients with Fasting blood glucose >250mg/dl, Total cholesterol >300mg/dl, Triglycerides > 200mg/dl, Blood pressure >160/>100mm Hg.		
2.	Patients diagnosed with Metabolic Syndrome and on treatment for Hypertension, Diabetes mellitus and Dyslipidemia for the duration of 1-2years.			Presence of other secondary causes of hypertension, dyslipidemia or hyperglycemia such as hepatic, renal, thyroid, or other endocrine diseases.		
3.	Patients willing to participate and give written informed consent.			Patients with Haematological disorders.		
4.				Pregnant and lactating women.		
5.				Patients with history of smoking and alcohol intake		
6.				Patient enrolled in any other study		

SUBJECT: INCLUDED ☐ EXCLUDED ☐

RANDOMISATION: CONTROL GROUP ☐ TEST GROUP ☐

REASON IF EXCLUDED:

SIGNATURE OF THE PRINCIPAL INVESTIGATOR:

MEDICAL HISTORY:

VITAL SIGNS:

VISITS	SCREENING	VISIT 1	VISIT 2 4^{TH} WEEK	VISIT 3 8^{TH} WEEK	VISIT 4 12^{TH} WEEK
PULSE RATE					
BLOOD PRESSURE					
TEMPERATURE					
GENERAL EXAMINATION					
SYSTEMIC EXAMINATION					

INVESTIGATIONS:

	SCREENING	8 WEEKS
COMPLETE BLOOD COUNT		
BLOOD UREA, CREATININE		
LIVER FUNCTION TEST		
URINE ANALYSIS		

	SCREENING	4 WEEKS	8 WEEKS	12 WEEKS
LIPID PROFILE TOTAL CHOLESTROL				
HDL				
TRIGLYCREIDES				
LDL				
VLDL				
FASTING BLOOD SUGAR				
RBC MORPHOLOGY				

ADVERSE EFFECTS:

S.No.	ADVERSE EVENTS	START DATE	STOP DATE	TREATMENT GIVEN

APPENDIX III: INFORMED CONSENT FORM

MORPHOLOGICAL CHANGES IN THE RED BLOOD CELLS AS A MARKER OF OXIDATIVE STRESS IN METABOLIC SYNDROME AND ANTIOXIDANTS AS AN ADD ON THERAPY IN THE REVERSAL OF CHANGES - A RANDOMIZED, OPEN LABEL, COMPARATIVE PILOT STUDY.

Name of the Participant:

I, _____ have read the information in this form (or it has been read to me). I was free to ask any questions and they have been answered. I am over 18 years of age and, exercising my free power of choice, hereby give my consent to be included as a participant in this study.

1. I have read and understood this consent form and the information provided to me. ☐
2. I have had the consent document explained to me. ☐
3. I have been explained about the nature of the study. ☐
4. I have been explained about my rights and responsibilities by the investigator. ☐
5. I am aware of the fact that I can opt out of the study at any time without having to give any reason and this will not affect my future treatment in this hospital. ☐
6. I hereby give permission to the investigators to release the information obtained from me as result of participation in this study to the sponsors, regulatory authorities, Govt. agencies, and IEC.I understand that they are publicly presented. ☐
7. I have understand that my identity will be kept confidential if my data are publicly presented ☐
8. I have had my questions answered to my satisfaction. ☐
9. I have decided to be in the research study. ☐

I am aware that if I have any question during this study, I should contact the investigator. By signing this consent form I attest that the information given in this document has been clearly explained to me and understood by me, I will be given a copy of this consent document.

1. Name and signature / thumb impression of the participant (or legal representative if participant incompetent)

Name _____Signature/Guardian_____ Date _____

2. Name and Signature of impartial witness (required for illiterate patients):

Name _____ Signature _____ Date _____

Address and contact number of the impartial witness:

3. Name and Signature of the investigator or his representative obtaining consent:
Name _____ Signature _____ Date _____

சுய ஒப்புதல் படிவம்

ஆய்வு தலைப்பு :

இரத்த சிவப்பணுவின் அமைப்பில் ஏற்படும் மாற்றத்தின் மூலம் வளர்சிதை மாற்ற நோய்குறியில் ஏற்படக்கூடிய ஆக்ஸிடேட்டிவ் ஸ்டிரஸ்ஸை (ஆக்ஸிஜனேற்ற அழுத்தம்) கண்டறிதல் மற்றும் ஆக்ஸிடேட்டிவ் ஸ்டிரஸ்ஸை குறைப்பதில் வைட்டமின்-E மற்றும் வைட்டமின்-C யின் பங்கு வழக்கமான சிகிச்சை முறையுடன் ஓர் திறந்தநிலை ஒப்பிடுதல் ஆய்வு.

பெயர் : வயது : தேதி : உள்நோயாளி எண் :

..... என்பவராகிய நான் இந்த ஆய்வின் விவரங்களும் அதன் நோக்கங்களும் முழுமையாக அறிந்து கொண்டேன். எனது சந்தேகங்கள் அனைத்திற்கும் தகுந்த விளக்கம் அளிக்கப்பட்டது. இந்த ஆய்வில் முழு சுதந்திரத்துடன் மற்றும் சுயநினைவுடன் பங்கு கொள்ள சம்மதிக்கிறேன்.

எனக்கு விளக்கப்பட்ட விஷயங்களை நான் புரிந்து கொண்டு நான் எனது சம்மதத்தைத் தெரிவிக்கிறேன். இச்சுய ஒப்புதல் படிவத்தை பற்றி எனக்கு விளக்கப்பட்டது.

இந்த ஆய்வினை பற்றிய அனைத்து தகவல்களும் எனக்கு தெரிவிக்கப்பட்டது. இந்த ஆய்வில் எனது உரிமை மற்றும் பங்கினை பற்றி அறிந்து கொண்டேன்.

இந்த ஆய்வில் பிறரின் நிர்பந்தமின்றி என் சொந்த விருப்பத்தின் பேரில்தான் பங்கு பெறுகிறேன் மற்றும் நான் இந்த ஆராய்ச்சியிலிருந்து எந்நேரமும் பின் வாங்கலாம் என்பதையும் அதனால் எந்த பாதிப்பும் ஏற்படாது என்பதையும் நான் புரிந்து கொண்டேன்.

இந்த ஆய்வில் கலந்து கொள்வதன் மூலம் என்னிடம் பெறப்படும் தகவலை ஆய்வாளர் இன்ஸ்டிட்யூசனல் எத்திக்ஸ் கமிட்டியினரிடமோ, அரசு நிறுவனத்திடமோ தேவைப்பட்டால் பகிர்ந்து கொள்ளலாம் என சம்மதிக்கிறேன்.

இந்த ஆய்வின் முடிவுகளை வெளியிடும்போது எனது பெயரோ, அடையாளமோ வெளியிடப்பட்டாது என அறிந்து கொண்டேன். இந்த ஆய்வின் விவரங்களைக் கொண்ட தகவல் தாளைப் பெற்று கொண்டேன். இந்த ஆய்விற்காக இரத்தப் பரிசோதனை செய்துக் கொள்ள சம்மதிக்கிறேன்.

இந்த ஆய்வில் பங்கேற்கும் பொழுது ஏதேனும் சந்தேகம் ஏற்பட்டால், உடனே ஆய்வாளரை தொடர்பு கொள்ள வேண்டும் என அறிந்து கொண்டேன்.

இச்சுய ஒப்புதல் படிவத்தில் கையெழுத்திடுவதன் மூலம் இதிலுள்ள அனைத்து விஷயங்களும் எனக்கு தெளிவாக விளக்கப்பட்டது என்றும் தெரிவிக்கிறேன் என்று புரிந்து கொண்டேன். இச்சுய ஒப்புதல் படிவத்தின் ஒரு நகல் எனக்கு கொடுக்கப்படும் என்றும் தெரிந்து கொண்டேன்.

பங்கேற்பாளர் / பாதுகாவலர் கையொப்பம் தேதி :

ஆய்வாளர் கையொப்பம் தேதி :

APPENDIX IV :INFORMATION TO PARTICIPANTS

Title: **MORPHOLOGICAL CHANGES IN THE RED BLOOD CELLS AS A MARKER OF OXIDATIVE STRESS IN METABOLIC SYNDROME AND ANTIOXIDANTS AS AN ADD ON THERAPY IN THE REVERSAL OF CHANGES - A RANDOMIZED, OPEN LABEL, COMPARATIVE PILOT STUDY**

Principal Investigator:

Name of Participant:

This study is conducted in Rajiv Gandhi Govt. General Hospital, Chennai. You are invited to take part in this study. The information in this document is meant to help you decide whether or not to take part. Please feel free to ask if you have any queries or concerns.

The purpose of this study

The Metabolic syndrome (syndrome X) consists of a constellation of metabolic abnormalities that confer increased risk of cardiovascular disease and diabetes mellitus. The major features of the metabolic syndrome include central obesity, hypertriglyceridemia, low high-density lipoprotein cholesterol, hyperglycemia, and hypertension. Oxidative stress plays an important role in the pathogenesis of metabolic syndrome. Oxidative stress is an imbalance which occurs due to overproduction reactive oxygen or a shortage of antioxidants. In this study we want to test the morphological changes in red blood cells due to oxidative stress in metabolic syndrome and the effect of Antioxidants (Vitamin C & Vitamin E) in reversing these changes.

We have obtained permission from the Institutional Ethics Committee.

The study design

All patients in the study will be divided into 2 groups A & B. You will be assigned to either of the groups. Group A will receive the standard treatment & Group B will receive standard treatment + one tablet of Vitamin C 500 mg and one capsule of Vitamin E 400 mg.

Study Procedures

The study involves evaluation of Blood pressure, Red blood cell morphology, Lipid profile, Blood sugar, Renal function test, Complete blood count, Liver function test and Urine analysis. The planned scheduled visits involve visits at 4^{th}, 8^{th} and 12^{th} week after your initial visit and you will be required to visit the hospital 3 times during the study. At each visit, the study physician will examine you and collect about 7 ml blood. Blood tests will be carried out at screening, 4^{th}, 8^{th} and at the follow-up visit. These tests are essential to monitor your condition, and to assess the safety and efficacy of the treatment given to you. In addition, if you notice any adverse events, you have to report it. You will be required to return unused study medicines when you report for your scheduled visits. This will enable correct assessment of the study results.

Possible benefits to you

Antioxidants with your standard medications will reduce oxidative stress and your future risk of developing complications due to metabolic syndrome.

Possible benefits to other people

The results of the research may provide benefits to the society in terms of advancement of medical knowledge and therapeutic benefit to future patients.

Confidentiality of the information obtained from you

You have the right to confidentiality regarding the privacy of your medical information (personal details, results of physical examinations, investigations, and your medical history). By signing this document, you will be allowing the research team investigators, other study personnel, sponsors, Institutional Ethics Committee and any person or agency required by law like the Drug Controller General of India to view your data, if required.

The information from this study, if published in scientific journals or presented at scientific meetings, will not reveal your identity.

Decision to not participate in the study

Your decision not to participate in this research study will not affect your medical care or your relationship with the investigator or the institution. You will be taken care of and you will not lose any benefits to which you are entitled.

Decision to withdraw from the study once started

The participation in this research is purely voluntary and you have the right to withdraw from this study at any time during the course of the study without giving any reasons. However, it is advisable that you talk to the research team prior to stopping the treatment/discontinuing of procedures etc. The expenditure for the treatment and investigation for this study will not be collected from you. The results of this study will be informed to you at the end of the study.

Signature of Investigator Signature of Participant

Date Date

ஆய்வு தகவல் தாள்

ஆய்வு தலைப்பு :

இரத்த சிவப்பணுவின் அமைப்பில் ஏற்படும் மாற்றத்தின் மூலம் வளர்சிதை மாற்ற நோய்குறியில் ஏற்படக்கூடிய ஆக்ஸிடேட்டிவ் ஸ்டிரஸ்ஸை (ஆக்ஸிஜனேற்ற அழுத்தம்) கண்டறிதல் மற்றும் ஆக்ஸிடேட்டிவ் ஸ்டிரஸ்ஸை குறைப்பதில் வைட்டமின்-E மற்றும் வைட்டமின்-C யின் பங்கு வழக்கமான சிகிச்சை முறையுடன் ஓர் திறந்தநிலை ஒப்பிடுதல் ஆய்வு.

ஆய்வாளர் :

பங்கேற்பாளர் :

இந்த ஆய்வு இராஜீவ் காந்தி அரசு பொது மருத்துவமனையில் நடைபெற உள்ளது. நீங்களும் இந்த ஆய்வில் பங்கேற்க நாங்கள் விரும்புகிறோம். இதிலுள்ள தகவலின் அடிப்படையில் இந்த ஆய்வில் பங்கேற்பதா அல்லது வேண்டாமா என்று நீங்கள் முடிவு செய்து கொள்ளலாம். உங்களது சந்தேகங்களை எங்களிடம் கேட்டு நிவர்த்தி செய்து கொள்ளுங்கள்.

இந்த ஆய்வின் நோக்கம்:

வளர்சிதை மாற்ற நோய்குறி (சிண்ட்ரோம் எக்ஸ்) என்பதின் முக்கிய அம்சங்கள் மைய உடல் பருமன், குறைந்த உயர்அடர்த்தி கொழுப்பு புரதம், சர்க்கரை மிகைப்பு, ஹைபர்டிரை கிளசரிடிமியா மற்றும் உயர் இரத்த அழுத்தம் ஆகியவை. இந்நோய்குறி உள்ளவர்களுக்கு இதய நோய், நீரிழிவு நோய் ஏற்படும் ஆபத்து அதிகரித்துள்ளது.

ஆக்சிஜனேற்ற அழுத்தம் வளர்சிதை மாற்ற நோய்குறி தோன்ற ஒரு முக்கிய பங்கு வகிக்கிறது. ஆக்சிஜனேற்ற அழுத்தம் என்பது எதிர்வினை ஆக்சிஜன் மிகுதி, அல்லது ஆக்சிஜனேற்ற தடுப்பான் பற்றாக்குறை காரணமாக ஏற்படுகிறது. இந்த ஆய்வில் வளர்சிதை மாற்ற நோய்குறி உள்ள நோயாளிகளில் ஆக்சிஜனேற்ற அழுத்தததினால் இரத்த சிவப்பணுக்களில் ஏற்படும் உருமாற்றங்களையும், இந்த மாற்றங்களை குறைக்கும் ஆக்சிஜனேற்ற தடுப்பானின் விளைவு ஆகியவற்றை வழக்கமாக எடுத்துக்கொள்ளும் சிகிச்சை முறையுடன் சேர்த்து கொடுத்து சோதிக்க உள்ளோம்.

இந்த ஆய்விற்கு இன்ஸ்டிட்யூசனல் எத்திக்ஸ் கமிட்டி சம்மதம் பெற்றிருக்கிறோம்.

ஆய்வு வடிவமைப்பு:

இந்த ஆய்வில் கலந்து கொள்பவர்களை A மற்றும் B என இரு குழுக்களாக பிரிக்கப்படுவர். A குழுவில் இருப்பவர்கள் வழக்கமான சிகிச்சையும், B குழுவில் இருப்பவர்கள் வழக்கமான சிகிச்சையுடன் வைட்டமின்-C 500மி.கி. மற்றும் வைட்டமின்-E 400மி.கி. பெறுவர்.

ஆய்வு நடைமுறைகள்

இந்த ஆய்வில் தங்களின் இரத்த அழுத்தம், இரத்த சிவப்பணு உருபனியல், லிப்பிட் சுயவிவரம், இரத்த சர்க்கரை, யூரியா, கிரியேட்டினின், இரத்த செல்களின் முழு எண்ணிக்கை, கல்லீரல் செயல்திறன் ஆகியவை மதிப்பீடு செய்யப்படும். இந்த ஆய்வில் நீங்கள் முதல் வாரம் மற்றும் மூன்று முறை (4,8,12 வாரங்களில்) மருத்துவமனைக்கு வர நேரிடும். அவ்வாறு வரும் ஒவ்வொரு முறையும் தங்களின் உடல்நிலை மருத்துவரால் பரிசோதிக்கப்பட்டு தங்களிடமிருந்து 7மி.லி. இரத்தம் பரிசோதனைக்காக எடுக்கப்படும். இப்பரிசோதனையின் மூலம் நோயின் தன்மையில் ஏற்படும் முன்னேற்றத்தினை அறிந்துக்

கொள்வோம். இந்த ஆய்வினால் ஏதேனும் பக்க விளைவுகள் ஏற்பட்டால் உடனடியாக எங்களிடம் தெரிவிக்க வேண்டும்.

ஆய்வினால் ஏற்படும் நன்மைகள்:

தங்களின் வழக்கமான் மருந்துகளுடன் இணைத்து ஆக்சிஜனேற்ற தடுப்பான்களை கொடுப்பதினால் ஆக்சிஜனேற்ற அழுத்தம் குறைவதுடன் வருங்காலத்தில் வளர்சிதை மாற்ற நோய்குறியால் ஏற்படும் சிக்கல்களும் குறையும். ஆய்வின் முடிவுகள் எதிர்காலத்தில் இந்நோய்குறி உள்ளவர்களுக்கான சிகிச்சை முறையிலும் மற்றும் மருத்துவ அறிவிலும் முன்னேற்றம் ஏற்பட்டு சமூகத்திற்கு நன்மைகளை வழங்கலாம்.

உங்களிடமிருந்து பெறப்பட்ட தகவலின் நம்பகத்தன்மை:

தங்களது மருத்துவ கிசிக்சை குறித்த தகவல்கள் இரகசியமாக பாதுகாக்கப்படும். இந்த தகவல் தாளில் கையெழுத்திடுவதன் மூலம் உங்களை பற்றிய குறிப்புகளோ, எடுத்து கொண்ட சிகிச்சை முறையை பற்றியோ ஆய்வாளரோ, இன்ஸ்டிட்யூசனல் எத்திக்ஸ் கமிட்டியை சார்ந்தவர்களோ தேவை ஏற்பட்டால் அறிந்து கொள்ளலாம் என்று சம்மதிக்கிறீர்கள். ஆய்வின் போதோ, அல்லது முடிவுகளை வெளியிடும்போதோ தங்களது பெயரையோ, அடையாளங்களையோ வெளியிடமாட்டோம் என்பதை தெரிவித்துக் கொள்கிறோம்.

இந்த ஆய்வில் பங்கேற்காவிட்டாலும் நீங்கள் வழக்கமான சிகிச்சையை தொடர்ந்து பெறலாம்.

இந்த ஆய்வில் பங்கேற்பது தங்களுடைய விருப்பத்தின்பேரில் தான் இருக்கிறது. மேலும் நீங்கள் எந்நேரமும் இந்த ஆய்விலிருந்து பின்வாங்கலாம் என்பதையும் தெரிவித்துக் கொள்கிறோம்.

இந்த சிறப்பு சிகிச்சை முடிவுகளை ஆய்வின் போதோ அல்லது ஆய்வின் முடிவின் போதோ தங்களுக்கு அறிவிப்போம் என்பதையும் தெரிவித்துக் கொள்கிறோம்.

ஆய்வாளர் கையொப்பம்

பங்கேற்பாளர் / பாதுகாவலர் கையொப்பம்

தேதி :

INSTITUTIONAL ETHICS COMMITTEE
MADRAS MEDICAL COLLEGE, CHENNAI-3

EC Reg No.ECR/270/Inst./TN/2013
Telephone No. 044 25305301
Fax : 044 25363970

CERTIFICATE OF APPROVAL

To
Dr. R. Gowthami,
Postgraduate in M.D. (Pharmacology)
Madras Medical College
Chennai 600 003

Dear Dr. R. Gowthami,

The Institutional Ethics Committee has considered your request and approved your study titled **"MORPHOLOGICAL CHANGES IN THE RED BLOOD CELL AS A MARKER OF OXIDATIVE STRESS IN METABOLIC SYNDROME AND ANTIOXIDANTS AS AN ADD ON THERAPY IN THE REVERSAL OF CHANGES – A RANDOMIZED, OPEN LABEL, COMPARATIVE PILOT STUDY" No. 06092015.**

The following members of Ethics Committee were present in the meeting held on **08.09.2015** conducted at Madras Medical College, Chennai-3.

1.	Prof.C.Rajendran, M.D.,	: Chairperson
2.	Prof.R.Vimala, M.D., Dean, MMC, Ch-3	:Deputy Chairperson
3.	Prof.Sudha Seshayyan, M.D., Vice-Principal, MMC, Ch-3	: Member Secretary
4.	Prof.B.Vasanthi, M.D., Professor Pharmacology, MMC	: Member
5.	Prof.P.Ragumani, M.S., Professor, Inst.of Surgery, MMC	: Member
6.	Prof. Amudhavalli, Prof. of Biochemistry, MMC	: Member
7.	Prof.Srinivasagalu, Director, Inst.of Inter Med. MMC	: Member
8.	Tmt. J. Rajalakshmi, JAO, MMC	: Lay Person
9.	Thiru S.Govindasamy, B.A., B.L.,	: Lawyer
10.	Tmt.Arnold Saulina, M.A., MSW.,	: Social Scientist

We approve the proposal to be conducted in its presented form.

The Institutional Ethics Committee expects to be informed about the progress of the study and SAE occurring in the course of the study, any changes in the protocol and patients information/informed consent and asks to be provided a copy of the final report.

Member Secretary, Ethics Committee
MEMBER SECRETARY
INSTITUTIONAL ETHICS COMMITTEE
MADRAS MEDICAL COLLEGE