"COMPARATIVE STUDY OF VITAMIN E AND OMEGA 3 FATTY ACIDS IN TYPE 2 DIABETES MELLITUS"

BY

Dr. ASHWITHA SHRUTI DASS, MBBS

Dissertation submitted to the
Sri Devaraj Urs Academy of Higher Education and Research,
Tamaka, Kolar, Karnataka

In partial fulfillment of the requirements for the degree of

DOCTOR OF MEDICINE
IN
PHARMACOLOGY

Under the guidance of

Dr. SARALA. N, MD

DEPARTMENT OF PHARMACOLOGY
SRI DEVARAJ URS MEDICAL COLLEGE, KOLAR

April 2016

DECLARATION BY THE CANDIDATE

I hereby declare that this dissertation entitled **"COMPARATIVE STUDY OF VITAMIN E AND OMEGA 3 FATTY ACIDS IN TYPE 2 DIABETES MELLITUS"** is a bonafide and genuine research work carried out by me under the direct guidance of **Dr. SARALA. N, MD** Professor and HOD, Department of Pharmacology Sri Devaraj Urs Medical College, Tamaka, Kolar.

Date:

Place**:** Kolar

Signature of the candidate

Dr. ASHWITHA SHRUTI DASS

CERTIFICATE BY THE GUIDE

This is to certify that the dissertation entitled **"COMPARATIVE STUDY OF VITAMIN E AND OMEGA 3 FATTY ACIDS IN TYPE 2 DIABETES MELLITUS"** is a bonafide research work done by **Dr. ASHWITHA SHRUTI DASS** in partial fulfillment of the requirement for the degree of **MD** in **PHARMACOLOGY.**

Date:

Place: Kolar

SIGNATURE OF THE GUIDE

Dr. SARALA. N, MD

PROFESSOR AND HEAD

DEPARTMENT OF PHARMACOLOGY

CERTIFICATE BY THE CO-GUIDE

This is to certify that the dissertation entitled **"COMPARATIVE STUDY OF VITAMIN E AND OMEGA 3 FATTY ACIDS IN TYPE 2 DIABETES MELLITUS"** is a bonafide research work done by **Dr. ASHWITHA SHRUTI DASS** in partial fulfillment of the requirement for the degree of **MD** in **PHARMACOLOGY.**

Date:

Place: Kolar

SIGNATURE OF THE CO-GUIDE

Dr. VENKATARATHNAMMA PN, MD

PROFESSOR

DEPARTMENT OF MEDICINE

ENDORSEMENT BY THE HOD, PRINCIPAL/ HEAD OF THE INSTITUTION

This is to certify that the dissertation entitled **"COMPARATIVE STUDY OF VITAMIN E AND OMEGA 3 FATTY ACIDS IN TYPE 2 DIABETES MELLITUS"** is a bonafide research work done by **Dr. ASHWITHA SHRUTI DASS** under the guidance of **Dr. SARALA. N, MD,** Professor and HOD, Department of Pharmacology.

Dr. SARALA. N	**Dr. B.G. RANGANATH**
SEAL & SIGNATURE OF THE HOD	SEAL & SIGNATURE OF THE PRINCIPAL
Date: Place: Kolar	Date: Place: Kolar

COPYRIGHT

DECLARATION BY THE CANDIDATE

I hereby declare that the Sri Devaraj Urs Academy of Higher Education and Research, Tamaka, Kolar, Karnataka shall have the rights to preserve, use and disseminate this dissertation in print or electronic format for academic/research purpose.

Date:

Place: Kolar

SIGNATURE OF THE CANDIDATE

Dr. ASHWITHA SHRUTI DASS

SRI DEVARAJ URS MEDICAL COLLEGE, TAMAKA, KOLAR.

ETHICS COMMITTEE

CERTIFICATE

This is to certify that, the ethics committee of Sri Devaraj Urs Medical College, Tamaka, Kolar has unanimously approved the dissertation work of **Dr. ASHWITHA SHRUTI DASS**, a postgraduate student in the Department of Pharmacology of Sri Devaraj Urs Medical College entitled "**COMPARATIVE STUDY OF VITAMIN E AND OMEGA 3 FATTY ACIDS IN TYPE 2 DIABETES MELLITUS"** to be submitted to the Sri Devaraj Urs Academy of Higher Education and Research, Tamaka, Kolar.

MEMBER SECRETARY

ACKNOWLEDGEMENT

I take this opportunity to express my immense gratitude to everyone who are helped me directly or indirectly for the completion of my dissertation. I would firstly and above all, want to thank ***The Almighty*** *for blessing me with strength and courage to complete this study.*

I am immensely grateful to my guide ***Dr. Sarala. N****, Professor and HOD, Department of Pharmacology, who pushed me beyond horizons to select a topic where my interest lies. She has provided her constant support and rectified my flaws like my guiding light. Her exemplary work ethic, immense experience is impeccable and relentlessly instilled in me the importance of excellence. She has been a vigilant and dynamic mentor throughout my dissertation and post graduate career.*

I am extremely indebted to my co- guide ***Dr. Venkatarathnamma PN****, Professor, Department of Medicine, Sri Devaraj Urs Medical College, Tamaka, Kolar who guided me in completing this dissertation. Her dedication to patients inspired me to be a better, humble doctor and give undivided attention and care to the patients.*

I owe my heartfelt gratitude to ***Dr. Lakshmiah V*** *and* ***Dr. Madhavi Reddy*** *as they helped me in patient recruitment and follow up.*

I owe my deep sense of gratitude to ***Dr. Bhuvana. K,*** *Associate Professor for her positive criticism and friendly advice which encouraged me to carry out my work efficiently. I am thankful to* ***Dr. Asha. B****, Associate Professor and* ***Dr. Meenakshi Lella,*** *former Assistant Professor for their timely help provided in guiding, reviewing my dissertation and sharing their enlightening opinions. I render my hearty thanks to* ***Dr. Mahesh V****, Assistant professor and* ***Mr. Ravishankar****, Statistician Department of Community Medicine who guided me to carry out the statistical analysis with ease.*

I express my sincere thanks to ***Dr. Smitha Rai*** *and* ***Dr. Jayakumar JK*** *for their encouragement and for sharing their informative sights that supported this dissertation.*

I am grateful to ***Dr. Revathi Ramesh*** *for extending her constant support during my dissertation. Her helpful nature and selflessness has inspired me greatly.*

*I would like to thank my batchmate **Dr. Ganashree P.** for her indispensable companionship and high spirited support at every level in completing my dissertation. I also thank my senior post graduates **Dr. Dharmistha Patel, Dr. Tejashree T, Dr.Nandhish C, Dr. Chetan Kumar G**, **Dr. Dheepan Nayagam**, my juniors **Dr. Sahana HV, Dr. Sowmya C and Dr. Mohammed Yaseen** for their valuable feedback and support. I thank them all for the light hearted moments and their ability to diffuse any tension with humour, which has made my time in the department memorable.*

*Above all I owe my wholehearted boundless thanks to my parents **Mrs. Julia Surekha Dass** and **Late Mr. Surendra Kumar Dass**. I am grateful to my loving uncle **Mr. Arun Kumar Guru,** all my uncles and aunties for their constant encouragement, love and endless support which has made me accomplish this work. My family's everlasting belief in my endeavours has encouraged me to come this far.*

*I am highly indebted to my fiancé **Dr. Dominic Augustine** for his relentless support and encouragement. His zeal for learning and dedication to work has been instrumental in completing my dissertation. I am grateful to my in laws **Mr. NA Augustine and Mrs. F Mary Bernadette** for showering me with their blessings and love.*

Last but not the least, I thank all my patients for providing me the opportunity to carry out this study and the non-teaching staff of Department of Pharmacology for their kind co-operation.

Date:
Place: Kolar

Signature of the Candidate
Dr. ASHWITHA SHRUTI DASS

Dedicated with Reverence To My Parents

LIST OF ABBREVIATIONS

DM	Diabetes mellitus
AGE	Advanced glycation end products
PKC	Protein kinase C
SOD	Superoxide dismutase
GSH	Reduced glutathione
NADPH	Nicotinamide adenine dinucleotide phosphate
NAD	Nicotinamide adenine dinucleotide
TNF-α	Tumour necrosis factor-α
TGF-β	Transforming growth factor-β
MCSF	Macrophage colony-stimulating factor
GCSF	Granulocyte colony-stimulating factor
ROS	Reactive oxygen species
NF-κB	Nuclear factor-κB
CTGF	Connective tissue growth factor
VEGF	Vascular endothelium derived growth factor
PECAM	Platelet endothelial cell adhesion molecule
ICAM	Intracellular adhesion molecule
PAI-1	Plasminogen activator inhibitor-1
MAPK	Mitogen activated protein kinase
RAGE	Receptor for advanced glycation end products
GFAT	Glutamine fructose 6 phosphate aminotransferase
PUFAs	Polysaturated fatty acids
TC	Total cholesterol
TG	Triglycerides
LDL	Low density lipoprotein cholesterol
VLDL	Very low density lipoprotein

HDL	High density lipoprotein cholesterol
EPA	Eicosapentaenoic acid
DHA	Docosahexaenoic acid
FDA	Food and drug administration
JNC	Joint national committee
ATP	Adult treatment panel
SBP	Systolic blood pressure
DBP	Diastolic blood pressure
FBS	Fasting blood sugar
PPBS	Post prandial blood pressure
HbA1c	Glycated haemoglobin
BMI	Body mass index
WHR	Waist-hip ratio
MMSE	Mini mental state examination
ANOVA	Analysis of variance

ABSTRACT

INTRODUCTION:

Diabetes mellitus (DM) is a multi-system metabolic disorder where oxidative stress due to hyperglycemia induces overproduction of the oxygen free radicals (superoxide, hydrogen peroxide and hydroxyl radical) which are neutralized by the antioxidants. The free radicals formed targets and damages the vascular endothelial cells resulting in pathogenesis and complications in type 2 DM. Therefore supplementation of antioxidants like either vitamin E or omega 3 fatty acids may help to reduce oxidative stress and thereby improving the glycemic control, insulin sensitivity and lipid profile in type 2 DM.

OBJECTIVE:

To assess the effect of vitamin E and omega 3 fatty acids on fasting blood sugar (FBS), postprandial blood sugar (PPBS), glycated haemoglobin (HbA1c), anthropometric measurements (body mass index [BMI], waist-hip ratio [WHR]), lipid profile (total cholesterol [TC], triglycerides [TG], low density lipoprotein cholesterol [LDL], high density lipoprotein cholesterol [HDL]) and cognitive function using mini mental state examination (MMSE) scale

MATERIALS AND METHODS:

A prospective, randomized, parallel, open label study was conducted on patients attending Medicine and Diabetology outpatient department of R.L. Jalappa Hospital and Research Centre from February 2014 to June 2015. Hundred patients diagnosed with type 2 DM receiving combination of Metformin (500mg) and

glimepiride (1mg) were recruited. They were randomly assigned to receive add on therapy of Vitamin E 400mg (Group1) or Omega 3 fatty acids [eicosapentaenoic acid-180 mg, docosahexaenoic acid-120 mg] (Group 2), once daily for 12 weeks and Group 3 served as control. FBS, PPBS, HbA1c, BMI, WHR, lipid profile, MMSE were done at the baseline and after 12 weeks.

RESULTS:

Eighty seven patients (Group 1=31, Group 2=29 and Group 3=27) completed the study. Significant reduction in FBS, PPBS and HbA1c was observed in all three groups at 12 weeks. There was significant reduction in TC, TG in patients receiving either of the antioxidants and also significant reduction in LDL in patients receiving omega 3 fatty acids at 12 weeks compared to baseline. BMI and WHR were significantly increased in control group. Intergroup analysis showed in patients receiving vitamin E and omega 3 fatty acids the reduction of FBS, PPBS and HbA1c were similar. The patients receiving omega 3 fatty acids had significant reduction in TG compared to control. There was no significant effect on cognitive function.

CONCLUSION:

Vitamin E and Omega 3 fatty acids had beneficial effects on lipid profile, anthropometric measurements however the glycemic control was similar to the patients in control group.

Key words: Diabetes mellitus, glimepiride, metformin, omega 3 fatty acids, vitamin E

CONTENTS

LIST OF TABLES

LIST OF FIGURES

Introduction

INTRODUCTION

Diabetes mellitus (DM) is one of the most prevalent non-communicable diseases worldwide.[1] Currently, oral antidiabetic drugs are the first line treatment in patients suffering from type 2 DM. Even though several antidiabetic drugs are available in the market, the disease still continues to foster with several microvascular and macrovascular complications.

Diabetes mellitus is a multi-system metabolic disorder where one of the main features is hyperglycemia which leads to activation of polyol pathway, increased formation of intracellular advanced glycation end products (AGEs), activation of protein kinase C (PKC) isoforms and overactivity of the hexosamine pathway. These pathways induce excessive production of oxygen free radicals (superoxide, hydrogen peroxide and hydroxyl radical).[2] The free radicals formed leads to increase in oxidative stress which targets and damages the vascular endothelial cells resulting in microvascular (diabetic retinopathy, nephropathy and neuropathy) and macrovascular (coronary artery disease, peripheral arterial disease and stroke) complications in type 2 DM.[3,4]

In healthy individuals, oxidative damage to tissues is prevented by antioxidant enzyme such as superoxide dismutase (SOD), reduced glutathione (GSH), vitamin E and C which have scavenging property. In diabetics, increased oxidative stress results in free radical generation and decreased activity of antioxidants.[4] Therefore supplementation of appropriate adjuvant for enhancing antioxidant activity and thereby improving the glycemic control in type 2 DM. A study has shown that use of antioxidants as an add on therapy to oral antidiabetic drugs was effective in control of hyperglycemia.[5]

Type 2 DM is also characterized by insulin resistance resulting in depletion of the cellular antioxidant defense system secondary to increased oxidative stress.[5] Anthropometric measurements (such as sagittal abdominal diameter, body mass index, waist circumference and waist-hip ratio) are simple, non-invasive and useful tools to assess the insulin sensitivity.[6] There are very few Indian studies which have compared vitamin E and omega 3 fatty acids as an add on therapy for the treatment of type 2 DM.

The aim of this study was to assess and compare the effects of vitamin E and omega 3 fatty acids on glycemic control, insulin sensitivity (using BMI and waist-hip ratio) and lipid profile in type 2 DM patients.

Aims

&

Objectives

AIMS AND OBJECTIVES

1. To assess the effect of vitamin E and omega 3 fatty acids on fasting blood sugar, postprandial blood sugar and glycated haemoglobin

2. To assess the effect of vitamin E and omega 3 fatty acids on anthropometric measurements (body mass index, waist-hip ratio) and lipid profile (total cholesterol, triglycerides, low density lipoprotein cholesterol, high density lipoprotein cholesterol)

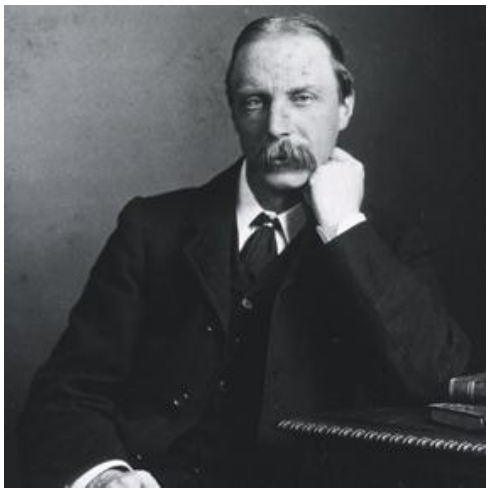
3. To assess the effect of vitamin E and omega 3 fatty acids on cognitive function by mini mental state examination scale

Review Of Literature

REVIEW OF LITERATURE

History of Diabetes Mellitus

Diabetes Mellitus (DM) clinical features were described 3000 years ago by the ancient Egyptians. The word "diabetes" was coined by Araetus of Cappodocia. Later on, the word “mellitus” meaning sweet was coined by Thomas Willis from Britain in 1675 after rediscovering the sweetness of urine and blood of patients which was first noticed by the ancient Indians. Dobson from Britain in 1776 initially confirmed the presence of excess sugar in urine and also blood as a cause of their sweetness.[7,8]



English physiologist Sir Edward Albert Sharpey-Schafer studied the pancreas in detail which lead him to the discovery of a substance i.e. Insulin. The name came from the Latin word ‘insula’ meaning island i.e. islets of Langerhans in the pancreas which produces insulin.[8]

Figure 1. Sir Edward Albert Sharpey

Frederick Banting, and his student assistant Charles Best extracted insulin from dog pancreas. Banting and Best worked in laboratory space at the

Figure 2. Charles Best and Frederick Banting

University of Toronto provided by Professor J.J.R. Macleod. They injected the insulin into dogs whose pancreas had been removed and it was observed that their blood sugar levels was decreased. James Collip purified the extract so that it could be used in humans. In 1923, Banting and Macleod were awarded Nobel Prize in Physiology and Medicine, though the contributions of all four men are important in the discovery of insulin.[7,8]

Table 1. Landmarks in therapy of diabetes[7]

Year	**Landmarks/ Discovery**
1921	Discovery of insulin
1926	Insulin crystallization techniques
1946	Neutral Protamine Hagedorn insulin
1955	First sulfonylurea (carbutamide)
1956	Lente insulin
1957	First biguanide (phenformin)
1963	First premixed insulin
1978	Subcutaneous insulin infusion pump (Pickup, UK)
1982	Recombinant human insulin approved by US FDA
1995	First alpha glucosidase inhibitor approved by US FDA
1996	First rapid-acting insulin analog
1997	First thiazolidinedione (Troglitazone)
2000	Edmonton protocol for islet cell transplant
2003	First long-acting insulin analog approved by US FDA
2005	First glucagon like peptide-1 analog (Exenatide)
2006	First dipeptidyl peptidase-4 inhibitor (Sitagliptin)
2013	First sodium-glucose transport 2 inhibitor (Canagliflozin)

History of Vitamin E

Vitamin E was discovered by Herbert Mclean Evans and Katharine Scott Bishop in 1922 as an unidentified element in vegetable oils required for reproduction in female rats. It was earlier named as 'factor X' and 'antisterility factor' which was later changed to vitamin E.[9]

It was named as alpha-tocopherol from the Greek word 'tocos' (meaning childbirth) and 'ferein' (to bring forth), relating to its essentiality for rats to bear young. The suffix 'ol' was added to the ending to indicate the presence of an OH group in the molecule. Erhard Fernholz explained its structure whereas Paul Karrer and his team first synthesized it in 1938.[9,10]

Widenbauer in 1938 used vitamin E as a therapeutic agent, who used wheat germ oil supplement on 17 premature newborn infants who suffered from growth failure, 11 recovered and were able to resume normal growth rates. [10]

Evan V. Shute and Wilfred E. Shute, siblings from Ontario, Canada, published the first monograph in 1945 which stated that mega doses of vitamin E can slow down and even reverse the development of atherosclerosis. The same research team in 1946, also demonstrated that α-tocopherol improved impaired capillary permeability and low platelet counts in experimental thrombocytopenic purpura. [10,11]

Gyorge and Rose in 1948 observed that rats which received tocopherol supplements suffered less hemolysis than those that did not receive in the course of the experiments conducted on alloxan effects on rats. Vitamin E was given via oral route had positive response which was not observed with intramuscular route. Since

then, supplementation of infant formulas with vitamin E has eradicated its deficiency as a cause for hemolytic anemia.[10,11]

There has been increased manufactured products like pharmaceutical food and its usage in cosmetic industries. The Food and Nutrition Boards of the National Academy of Sciences officially recognized vitamin E as an essential nutrient in 1968.[11]

History of Omega 3 fatty acids

American scientists Burr and Evans in 1923 discovered that rats deprived of polyunsaturated fatty acids developed symptoms of illness. Evans called these polyunsaturated fatty acids as vitamin F as they were substances vital for bodily function which the body could not manufacture by itself. Burr discovered a few years later that a deficiency in linoleic acid could not be corrected by using alpha-linolenic acid and vice versa. Hence there was not a single vitamin F but there are two families of essential fatty acids: omega-3 which was derived from alpha-linolenic acid and omega-6, derived from linoleic acid. The families of fatty acids have the prefix 'lin' as it was extracted from the linseed. Since then researchers have shown keen interest in fatty acids and their discoveries have multiplied.[12]

In 1982, the Swedish workers Bergstrom, Samuelsson and the British researcher Vane received Nobel Prize for their explanation of the association between a deficiency in essential fatty acids and the symptoms caused by it. They showed that the eicosanoids like prostaglandins, prostacyclin, thromboxanes and leukotrienes played a central role in the body as cell mediators such as in immunity, platelet aggregation and inflammation. The common property of all these molecules are that they are all synthesized from two precursors, the omega-3 and omega-6 unsaturated fatty acids.[12,13]

During the nineties, clinical trials confirmed that the omega-3 supplements for patients with risk of heart disease was helpful. 2000 onwards, it was supplemented for improving mental health. Studies showed that increased levels of omega-3 acids in the tissues correlated with a reduction of depression and Alzheimer's disease hence it demonstrated the central role of polyunsaturated fatty acids in human brain function.[13]

TYPE 2 DIABETES MELLITUS

Type 2 DM is one of the leading chronic disease, accounting for 6.8% mortality in the world.[1] It is a multifactorial disease, caused by a combination of genetic factors related to impaired insulin secretion, insulin resistance and factors such as overeating, lack of exercise, obesity, stress and aging.[14,15] These factors are independent risk factors of pathogenesis of type 2 diabetes. Obesity mainly visceral fat with decrease in muscle mass due to a lack of exercise, induces insulin resistance in DM. The changes in dietary energy sources mainly increase in fat intake, decrease in starch intake, increase in the consumption of simple sugars and decrease in dietary fibre intake, contribute to obesity and cause deterioration of glucose tolerance. Hence obesity is one of the main reason that increases the risk of developing diabetes.[7,14,15]

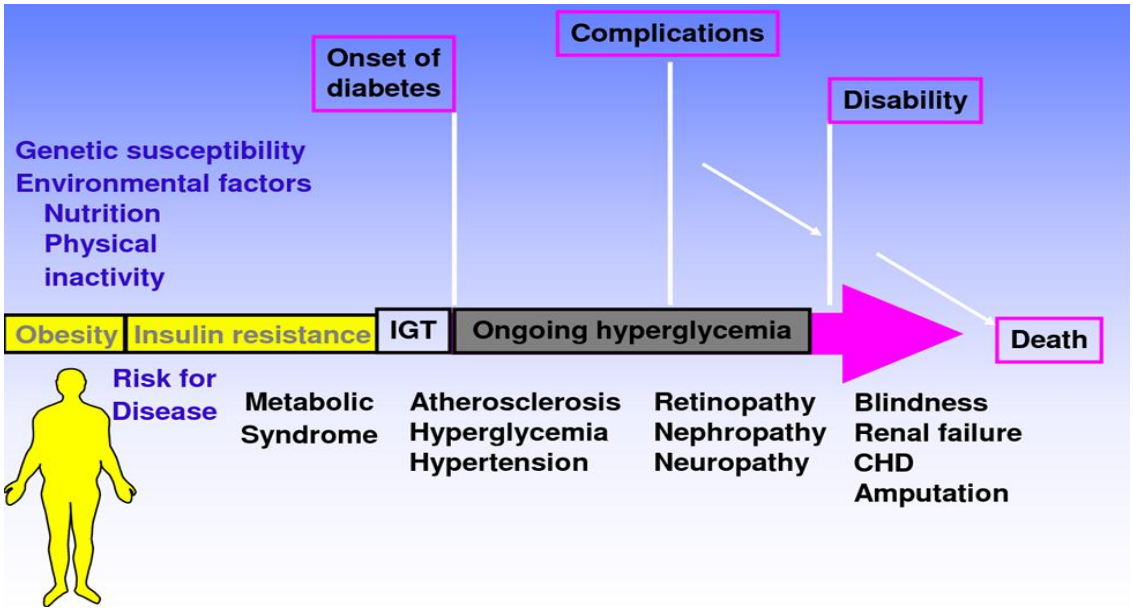


Figure 3. Etiology and pathophysiology of type 2 diabetes

Individuals with type 2 DM have detectable levels of circulating insulin, unlike patients with type 1 DM. On the basis of oral glucose tolerance test the essential components of type 2 DM can be divided into four groups:

1) Normal glucose tolerance.

2) Chemical diabetes also called impaired glucose tolerance.

3) DM with minimal fasting plasma glucose less than 140 mg/dl.

4) DM with overt fasting plasma glucose greater than 140 mg/dl.[14]

The individuals with impaired glucose tolerance have hyperglycemia inspite of high levels of plasma insulin which indicates that the resistance to the action of insulin. In the advancement from impaired glucose tolerance to DM, the level of insulin eventually declines which indicates that the patients with type 2 DM have decreased insulin secretion. Insulin resistance and insulin deficiency are common in type 2 DM patients and the former being the primary cause. [14-22]

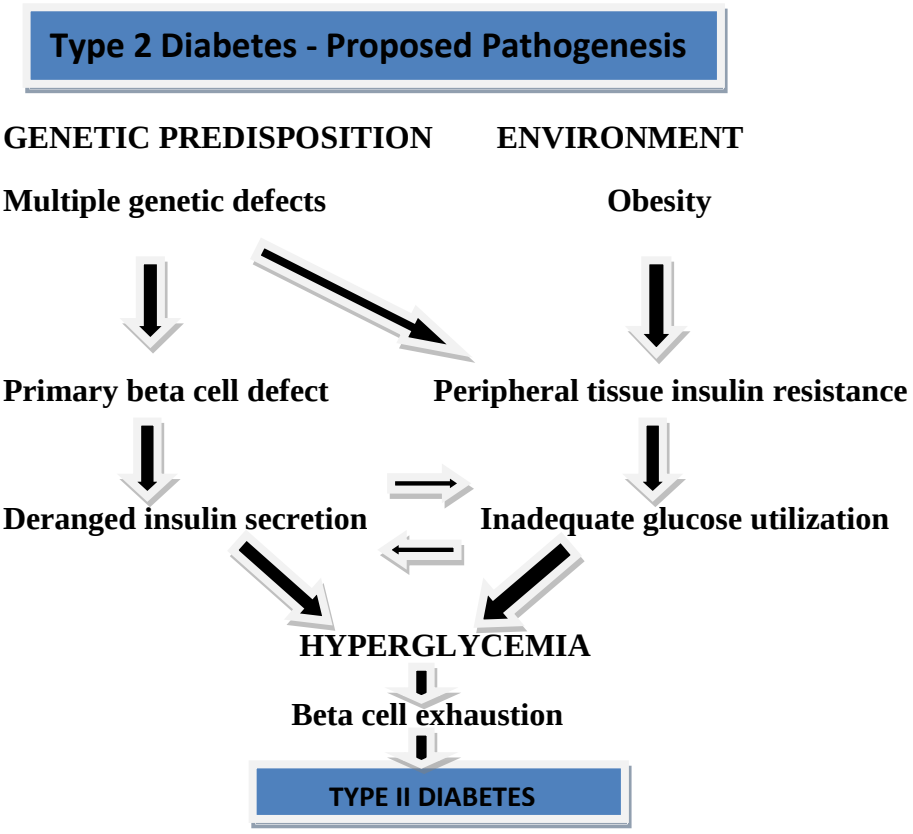


Figure 4. Pathogenesis of type 2 diabetes mellitus

Type 2 DM is characterized by hyperglycemia, hyperinsulinemia and insulin resistance resulting in depletion of the cellular antioxidant defense system secondary to increased oxidative stress. Oxidative stress contributes to the pathogenesis of DM by injury to pancreatic β cells, impairment of insulin action, increased lipid peroxidation and vascular endothelial damage.[1] The complications of DM are the result of an imbalance between free radical generation and their control by natural antioxidants. Antioxidants provide a defense system against free radical induced damage hence playing an important role in the prevention of complications. [19,20]

The major cause of tissue damage in diabetes occurs due to progressive narrowing and occlusion of lumen of the vessels leading to decreased perfusion, ischemia and tissue damage.[2] There is also increased permeability to plasma proteins that may get deposited in the vessel wall. There is expansion of the extracellular matrix around perivascular cells such as the pericytes in the retina and the mesangial cells in the glomeruli leading to thickening of the basement membrane. In large vessels, there is increased deposition of collagen and lipids in atherosclerotic plaques. Endothelial, mesangial and arterial smooth muscle hyperplasia and hypertrophy also results in vascular wall thickening. These processes, together with an increased coagulability in the vessel leads to vascular occlusion and tissue damage. [18-24]

Tissue damage occurs due to increased free radical production leading to enhanced oxidative stress by activating four major molecular mechanisms and these include increased polyol pathway influx, increased formation of advanced glycation end-products, activation of protein kinase C isoforms and increased hexosamine pathway activity. These mechanisms exacerbate insulin resistance and may lead to diabetic complications.[2,23,24]

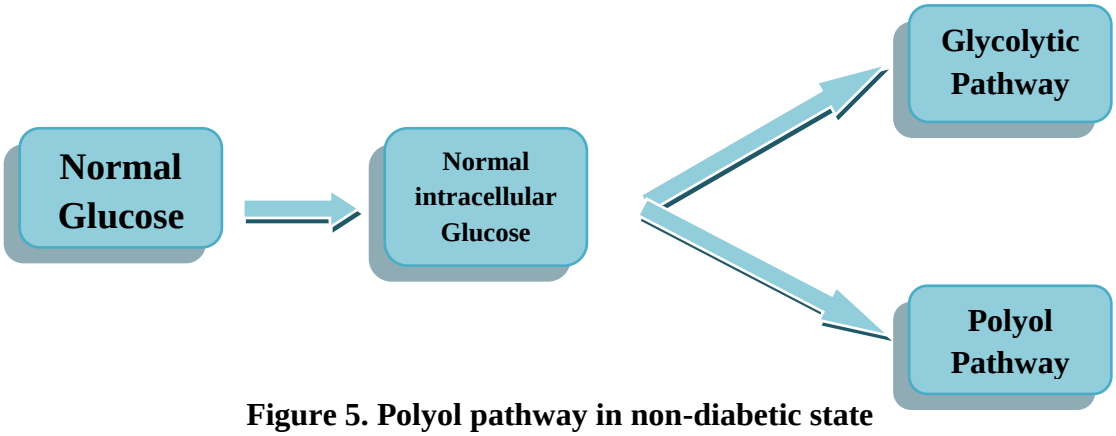
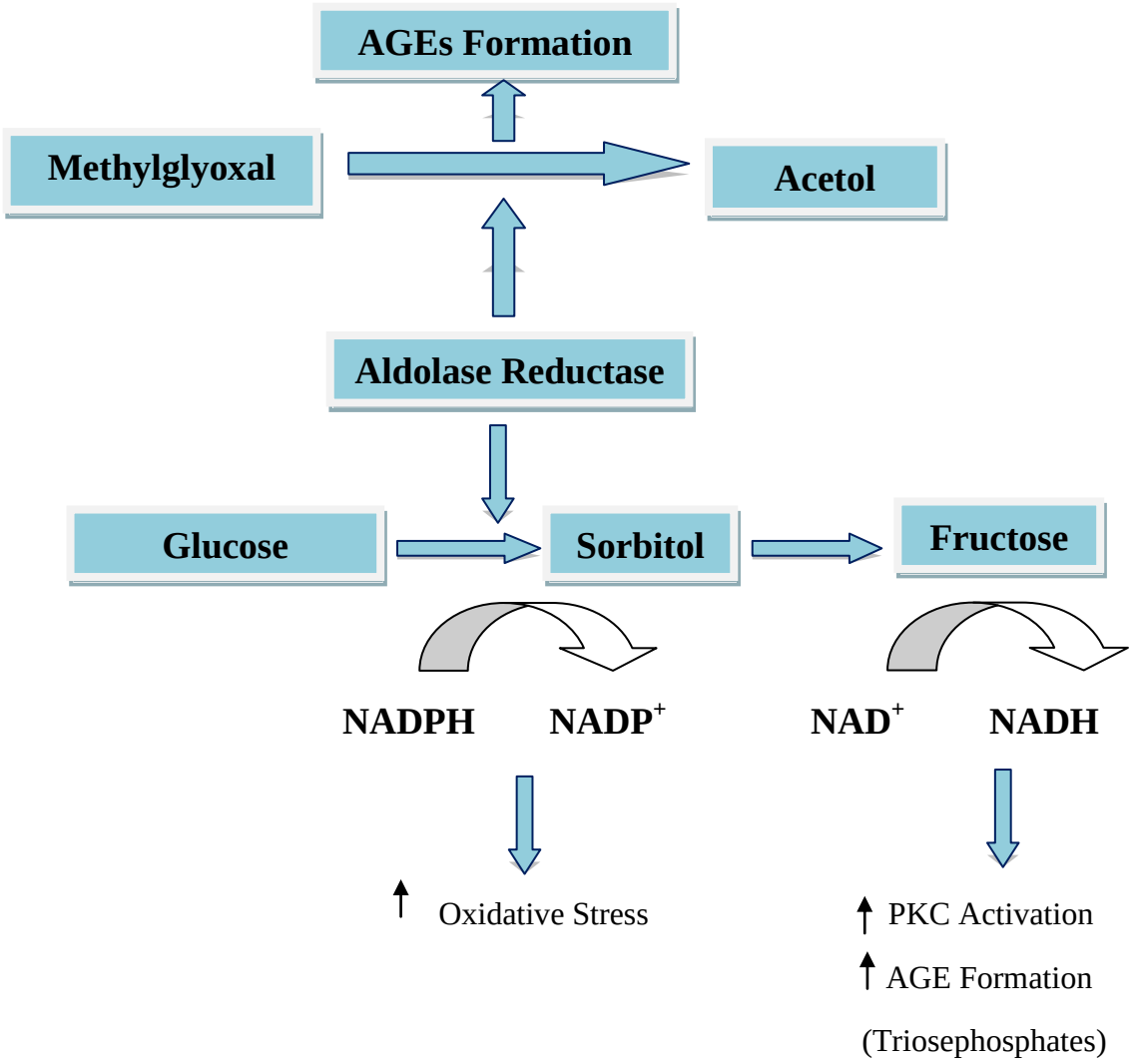


Figure 5. Polyol pathway in non-diabetic state

The polyol pathway is inactive in the non-diabetic state, as most of the glucose is metabolized through the glycolytic pathway. In DM, hyperglycemia and other glucose-derived substrates like methylglyoxal which is acted upon by aldose reductase leads to activation of polyol pathway. Aldose reductase is located in nerves, retina, lens, glomeruli and walls, all these are insulin independent. Therefore these tissues are primary targets of tissue damage resulting in retinopathy, neuropathy, nephropathy, vasculopathy and cataract.[2,24]



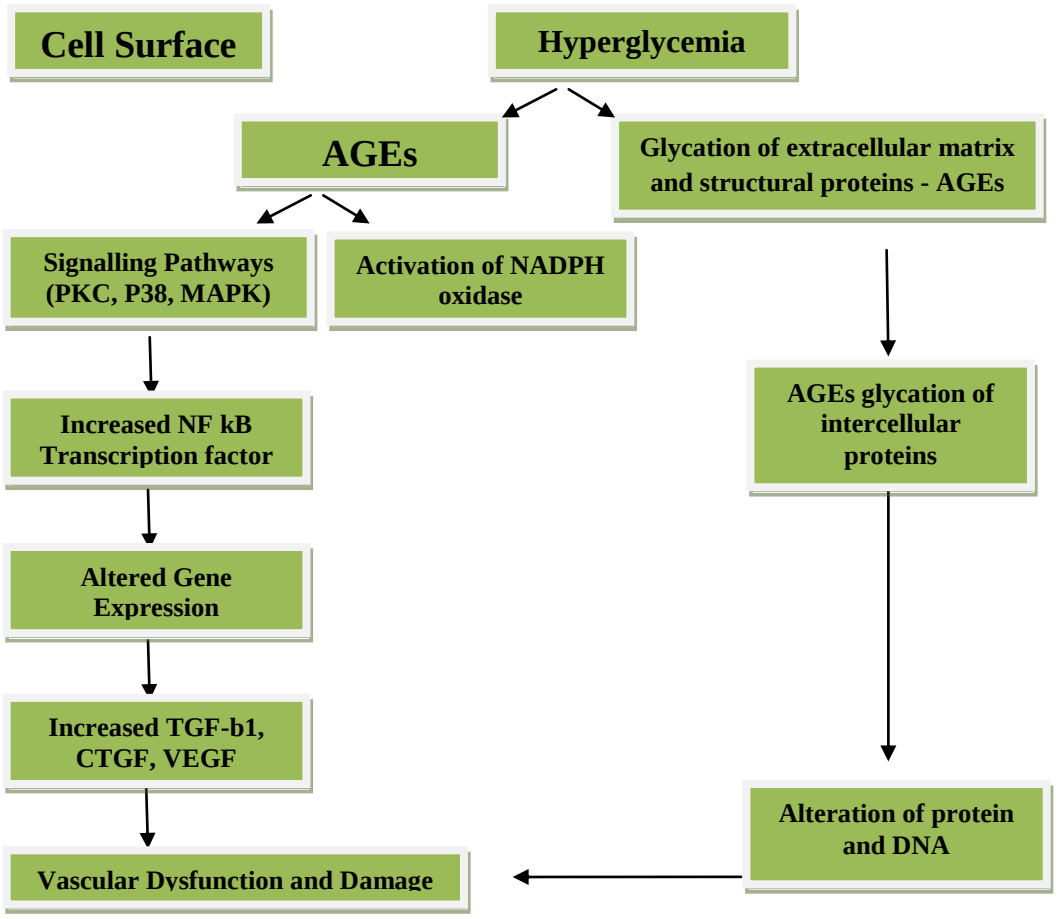
(AGEs: Advanced gylcation end products; PKC: Protein kinase C; NADPH: Nicotinamide adenine dinucleotide phosphate; NAD: Nicotinamide adenine dinucleotide)

Figure 6. The polyol pathway in diabetic mellitus

There is activation of the osmotic damage due to sorbitol accumulation, increased oxidative stress secondary to decreased NADPH levels, PKC activation from increase NADH/NAD+ ratio and increased AGE formation secondary to methylglyoxal, acetol and raised NADH/NAD+ ratio.

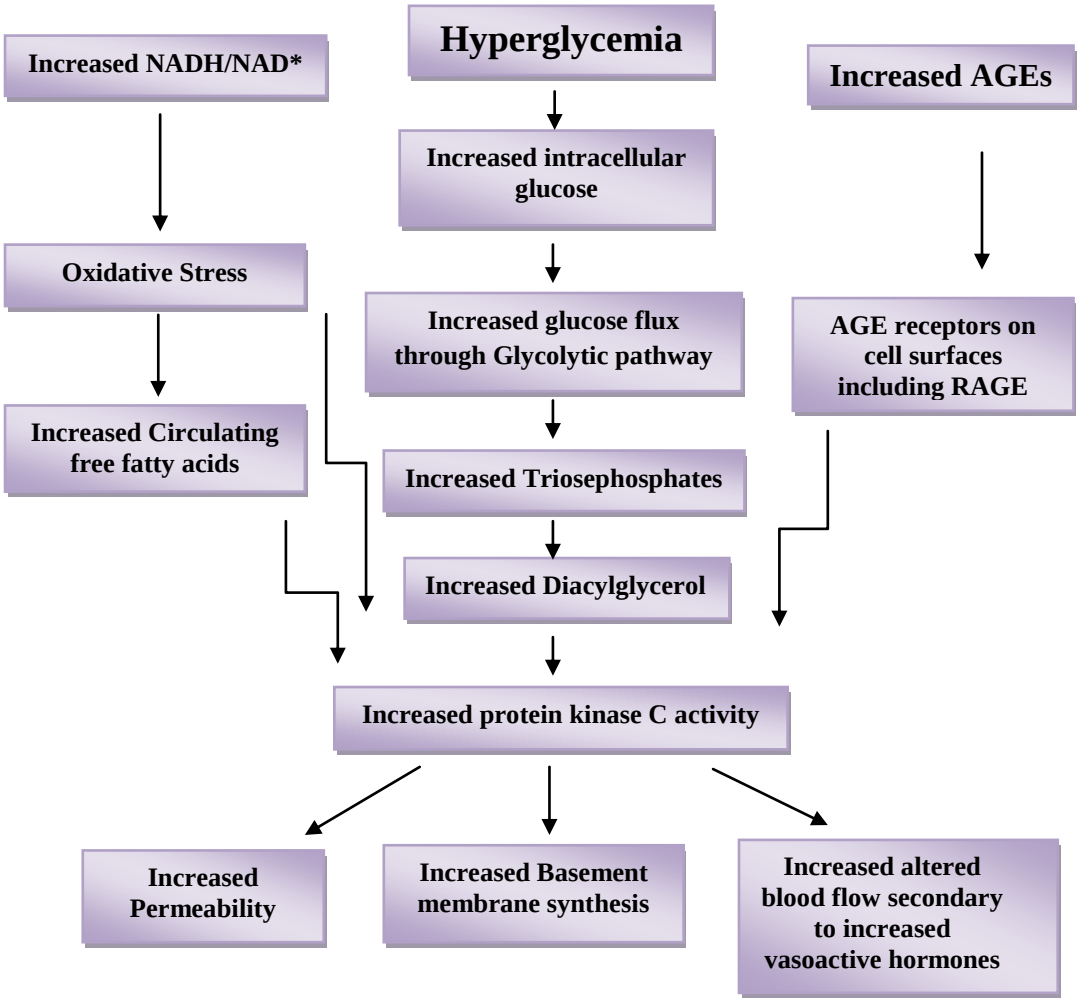
Glucose and the other glycating compounds such as decarbonyl-3 deoxy glucosone, methylglyoxal and glyoxal react with proteins and nucleic acids to form glycation products. Hence hyperglycemia is the primary factor of intacellualr and extracellular AGE formation. AGE can also bind to specific receptors on monocytes and macrophages leading to increased production of cytokines like IL-1, TNF-α, TGF-β, macrophage colony-stimulating factor (MCSF) and granulocyte colony stimulating factor (GCSF) which mediate tissue damage and inflammation. RAGE mediates generation of reactive oxygen species (ROS) and NF-κB activation leading to oxidative stress induced tissue damage.[2,24]

Hyperglycemia mainly activates PKC-β and PKC-δ isoforms present in retina, glomeruli and vascular tissues. It mainly activates β isoform causing increased permeability of vessels, abnormalities in blood flow and pathological angiogiogenesis through VEGF in retina. There is inhibition of nitric oxide production and altered gene expression for vasoactive and growth factors such as endothelin-1, VEGF, TGF-β and connective tissue growth factor (CTGF). Platelet/endothelial cell adhesion molecule (PECAM), intracellular adhesion molecule (ICAM) and plasminogen activator inhibitor-1(PAI-1) promote hypercoagulopathy leading to the vascular complications.[2,24]



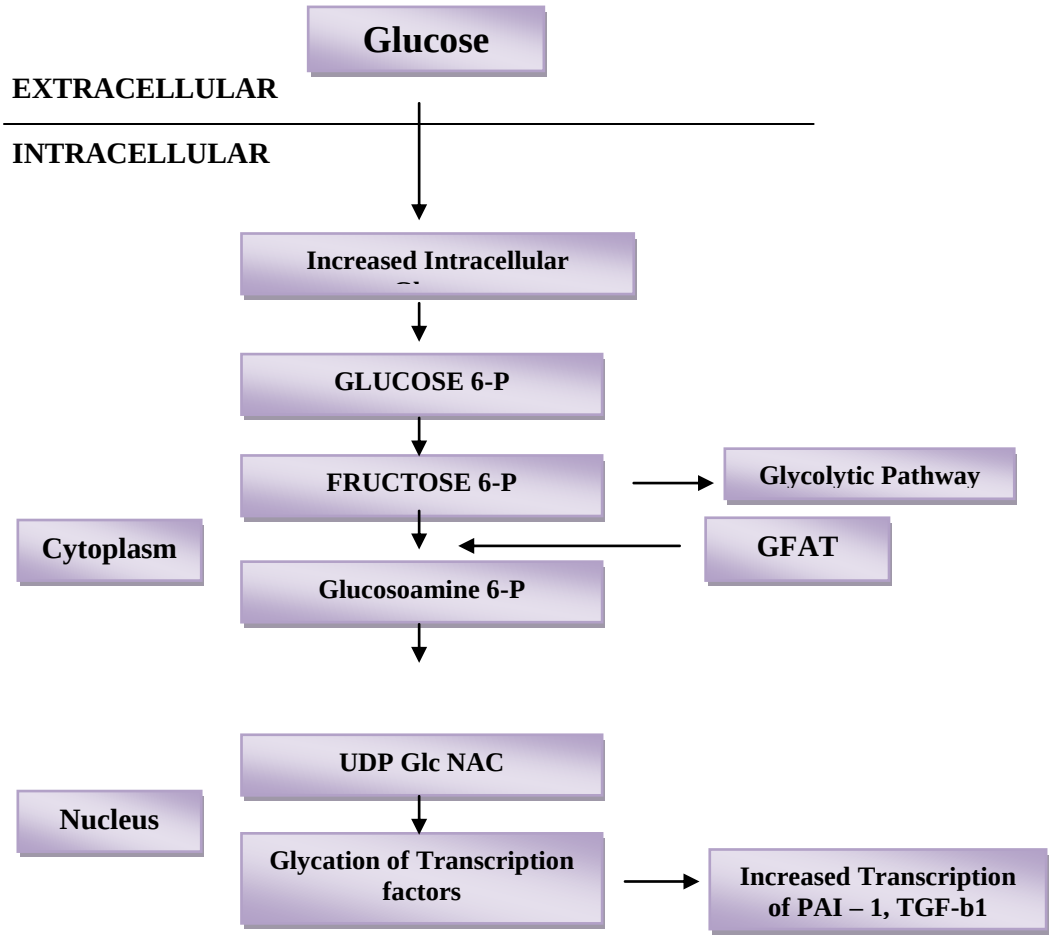
(AGEs: Advanced gylcation end products; PKC: Protein kinase C; TGF-b1: transforming growth factor beta 1, CTGF: Connective tissue growth factor, VEGF: Vascular endothelium derived growth factor, NF kB: Nuclear factor kappa B. p38 MAPK: Mitogen activated protein kinase)

Figure 7. Advanced glycation end products and vascular complications



(AGEs: Advanced gylcation end products; RAGE: Receptor for advanced glycation end products; NADPH: Nicotinamide adenine dinucleotide phosphate; NAD: Nicotinamide adenine dinucleotide)

Figure 8. Activation of protein kinase C in vascular complications



(PAI – 1: Plasminogen activator inhibitor – 1, TGF-b1: transforming growth factor beta 1, GFAT: Glutamine fructose – 6 – phosphate amino-transferase; UDP- Glc NAC: UDP- N-acetyl glucosamine)

Figure 9. Role of hexosamine pathway in diabetic complications

Alterations in the lipid profile were also observed in type 2 DM like reduction in high-density lipoprotein cholesterol (HDL-C), increased levels of plasma triglycerides (TG) and low-density lipoprotein cholesterol (LDL-C). LDL-C is more susceptible to oxidation in patients with type 2 diabetes mellitus. Insulin resistance and impaired insulin secretion in diabetes result in hyperglycemia and dyslipidemia.

Mitochondrial metabolism of the excessive glucose and free fatty acids results in increased superoxide production and oxidative stress. Antioxidants include vitamins like vitamin C, vitamin E (alpha- tocopherol), omega 3 fatty acids and beta-carotene, enzymes like catalase, superoxide dismutase and glutathione peroxidase and transition metal binding proteins like ceruloplasmin have been reported to be major contributors to serum total antioxidant activity. Low levels of antioxidants may be due to their increased consumption during the process of combating excessive free radicals generated in diabetes. As a result there is depletion of antioxidant reserves which include vitamins C and E. Other factors that have been associated with low plasma antioxidant levels include low intake of antioxidant- rich foods (particularly fruits and vegetables), poor health status, cigarette smoking and low physical activity.[25-39]

Brain is one of the organ system that is more susceptible to damage by free radicals because of its high oxygen consumption rate. Hyperglycemia causes cognitive decline due to oxidative stress and it alters the regional blood flow causing osmotic changes in cerebral neurons. MMSE is used to assess the cognitive function.[33]

Therefore the dietary supplementation with antioxidants was found to be beneficial in reducing insulin resistance and risk of complications of type 2 diabetes mellitus by protecting the vascular endothelium. Primary among these are alpha lipoic acid, vitamin C, vitamin E and omega-3 fatty acids.[36-39] Hence in this study we intended to compare the effect of vitamin E and Omega 3 fatty acids in T2DM based on their antioxidant property. In this study, patients on oral anti-diabetic drugs metformin and glimepiride were chosen to assess the additional beneficial effect of the antioxidants.

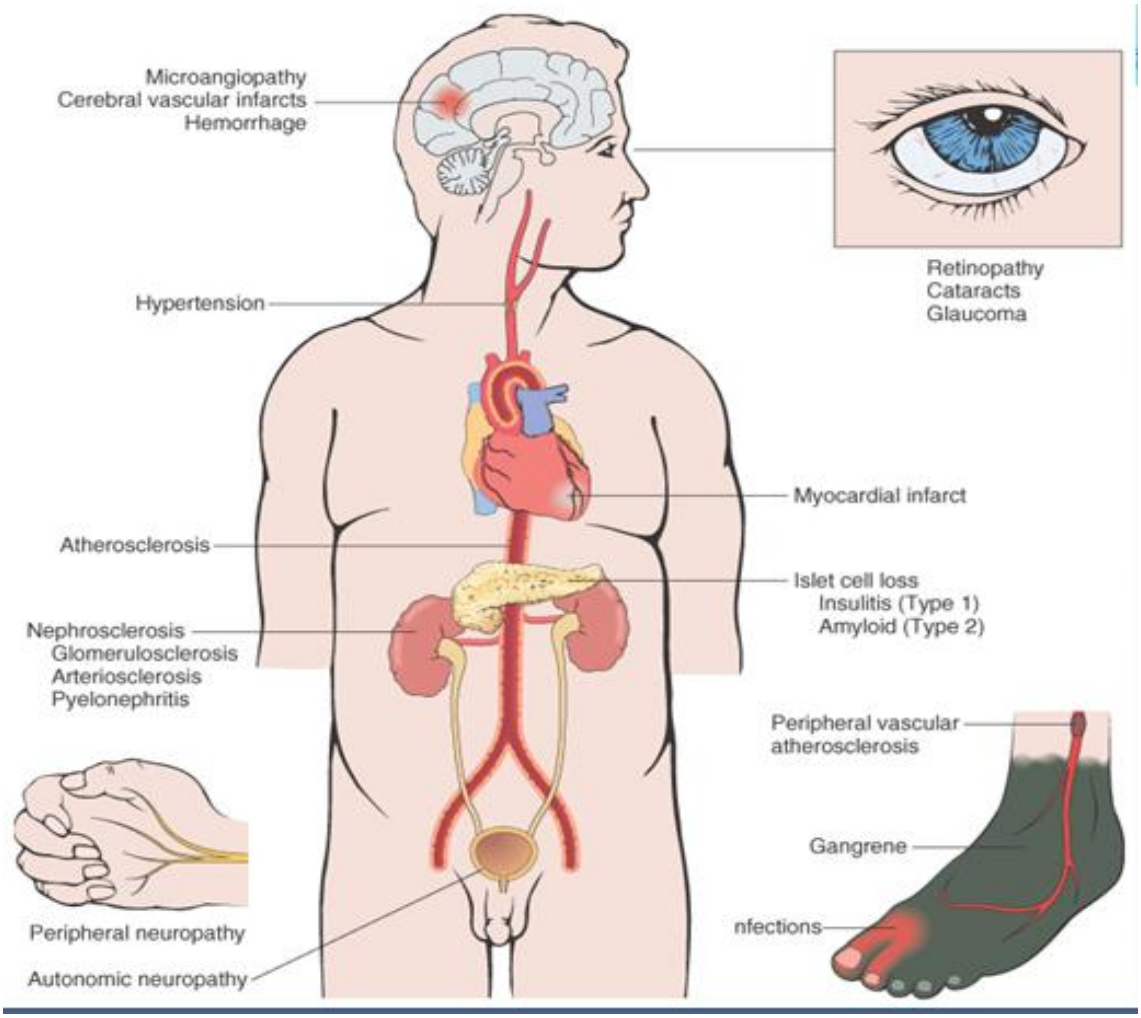


Figure 10. Complications of diabetes mellitus

VITAMIN E:

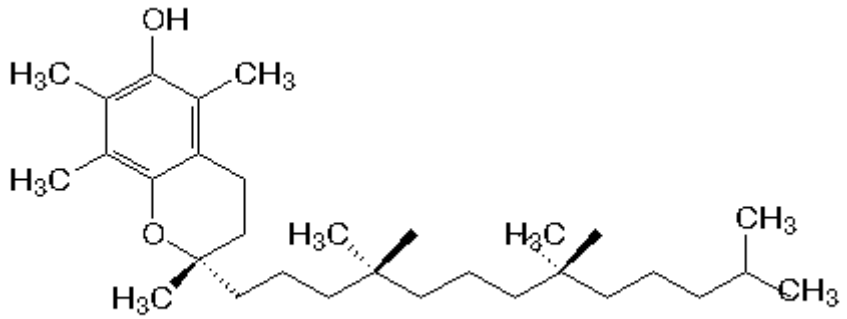


Figure 11. Chemical structure of Vitamin E[9]

Vitamin E is an antioxidant that occurs naturally in foods such as nuts, seeds and leafy green vegetables. It is a fat-soluble vitamin important for many processes in the body. It belongs to FDA pregnancy category C.[9]

Actions:

It prevents propagation of free-radical reaction like lipid peroxidation by scavenging peroxyl radicals and protects polyunsaturated fatty acids (PUFAs) and other oxygen-sensitive substances such as vitamin A and ascorbic acid from oxidation.[26,27,30,33]

It enhances immune response in healthy geriatric individuals. It is important in maintaining the stability of cell membrane. In addition to its antioxidant property, it improves the endothelial function by reducing effect of oxidized lipoproteins, proliferation of smooth muscle cells, platelet adhesion and aggregation.[36-42]

In a study which used the obese zucker rat, an animal that exhibits many of the features of type 2 diabetic mellitus showed improvements in glucose metabolism and insulin action by addition of vitamin E that was mediated by a reduction in oxidative

stress. They found that glucose-stimulated hyperinsulinemia and lipid peroxidation in the obese zucker rats could be significantly reduced with the dietary vitamin E.[27]

The antioxidant property of vitamin E may have beneficial effect in delaying the onset or slowing the progression of Alzheimer's disease. Vitamin E has a potential role in improving the cognitive impairment in patients with type 2 diabetes mellitus by reversing the damage caused by oxidative stress.[33,36,40]

Pharmacokinetics:

Vitamin E absorption from the GI tract depends on biliary and pancreatic secretions, micelle formation, uptake into erythrocytes and chylomicron secretion. It is not well absorbed, only about 20–60% absorbed from dietary sources. Fraction absorbed decreases as dosage increases. It is distributed into all tissues and stored in adipose tissue. It crosses the placenta and also is distributed into human milk. Only the R-stereoisomer of α-tocopherol is secreted by the liver. It is extensively metabolized, principally in the liver, to glucuronides of tocopheronic acid and its γ-lactone. It is excreted principally in the feces via biliary excretion, also in urine.[56]

Indications:

Vitamin E deficiency, malabsorption in cystic fibrosis, cholestasis and severe liver disease, age-related macular degeneration, chemotherapy-induced toxicity, dementia, ischaemic heart disease, prophylaxis of malignant neoplasms, motor neurone disease, muscle spasm, muscular dystrophies, pre-eclampsia, respiratory tract infections, retinitis pigmentosa, retinopathy of prematurity and tardive dyskinesia. [56]

Drug Interactions:

Oral anticoagulant- warfarin has a risk of hemorrhage with large doses of vitamin E due to effect on warfarin metabolism CYP2C9, CYP3A4 and CYP1A2. Vitamin E dosages ≥10 units/kg daily may delay response to iron therapy in children. Orlistat and mineral oil impairs the absorption of vitamin E. Vitamin A increases absorption, utilization and storage of vitamin E. Colestyramine and Colestipol interfere with the absorption of vitamin E.[56,58]

Adverse effects:

Oral doses >300 units daily have rarely caused nausea, diarrhea, intestinal cramps, fatigue, emotional disturbances, weakness, headache, blurred vision and rash. Topical application of vitamin E have caused growth of white hair at site of alopecia and contact dermatitis. It has also caused gonadal dysfunction, breast soreness, decrease in serum thyroxine and triiodothyronine.[56,58]

OMEGA 3 FATTY ACIDS:

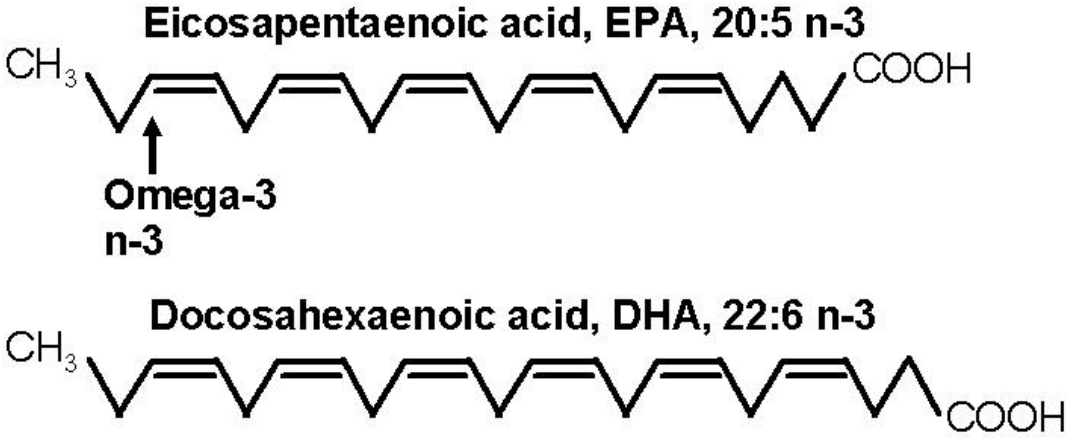


Figure 12. Chemical structure of Omega 3 fatty acids[55]

The omega-3 polyunsaturated fatty acids EPA (Eicosapentaenoic Acid) and DHA (Docosahexaenoic) are found in oils from fish and vegetables. It belongs to FDA category C.[43-45]

Actions:

The mechanism of action of Omega-3-acids is not completely understood. Potential mechanisms of action include inhibition of acyl-CoA: 1,2-diacylglycerol acyltransferase, increased mitochondrial peroxisomal β-oxidation in the liver, decreased lipogenesis in the liver and increased plasma lipoprotein lipase activity.[44,45] Omega-3 fatty acids may reduce the synthesis of triglycerides in the liver because EPA, DHA are poor substrates for the enzymes responsible for TG synthesis and EPA, DHA inhibit esterification of other fatty acids.[46-48]

Ingestion of poly unsaturated fatty acids (PUFA) rich diets which were enriched in omega-3 fatty acids has shown to have anti-obesity effect by reducing the

hepatic output of triglycerides, induction of fatty acid oxidation in liver, skeletal muscle and suppressing hepatic lipogenesis. It facilitates the insulin action by enhancing the membrane fluidity. It also suppresses cyclic endoperoxides, increases improvement of vascular smooth muscle cell sensitivity to nitric oxide and decreased formation of reactive oxygen species.[49-56] Omega 3 fatty acids (docosahexaenoic acid) is a major constituent of neuronal membrane, therefore its deficiency leads to degenerative changes.[45,47,54]

Pharmacokinetics:

It is well absorbed orally, studies show insignificant change in absorption due to food. It is metabolized into eicosanoids including leukotrienes and prostaglandins, then esterified and hydrolyzed from tissue and transformed into polyunsaturated fatty acids. It undergoes oxidative catabolism to carbon dioxide and water.[56,58]

Indications:

Atherosclerosis, hyperlipidemia, rheumatoid arthritis, psoriasis, inflammatory bowel disease, cystic fibrosis, prophylaxis of malignant neoplasms, huntingtons disease and bipolar disorder[56]

Drug interactions:

Oral anticoagulants and omega-3-acids caused prolongation of bleeding time in some clinical trials, hence bleeding time should be monitored periodically. Asthmatic patients sensitive to aspirin need to be monitored as omega 3 fatty acids may affect prostaglandin synthesis.[56,58]

Adverse effects:

It rarely causes eructation, dyspepsia, vomiting, anorexia, constipation, dry mouth, dysphagia, colitis, fecal incontinence, gastritis, gastroenteritis, increased appetite, intestinal obstruction, melena, pancreatitis and tenesmus. Backache, chest pain, chills, fever, altered taste, lymphadenopathy, hemorrhagic diathesis, fungal and viral infection have also been reported.[57]

Rashes, alopecia, eczema, pruritus, sweating, tachycardia, arthralgia, myalgia, depression, dizziness, insomnia, bronchitis, increased cough, dyspnea, epistaxis, laryngitis, pharyngitis, rhinitis and sinusitis have been reported.[56,57]

Materials & Methods

MATERIALS AND METHODS

Location of the study:

A prospective, randomized, parallel and open label study was conducted on patients attending the outpatient department of Medicine and Diabetology, R.L. Jalappa Hospital & Research Centre attached to Sri Devaraj Urs Medical College, Tamaka, Kolar, from February 2014 to June 2015.

Data collection:

A proforma containing detailed information of each patient, was designed according to the study protocol and ethical clearance was obtained from Institutional Ethics Committee. Patients who were willing to give the written informed consent were included in the study.

Inclusion Criteria:

1. Patients of either gender aged between 35-60 years
2. Type 2 diabetes mellitus patients (fasting blood sugar -125 to 250 mg/dl) receiving combination of metformin and glimepiride
3. Diabetic patients with any one or both parameters mentioned below

   Central obesity: waist-hip ratio >1.0 (males) ≥ 0.9 (females)

   Hypertension: (JNC 7) systolic blood pressure (SBP):120- 159 mmHg and diastolic blood pressure (DBP): 80-99 mmHg on treatment
4. Diabetic patients with (ATP III) total cholesterol 200-239 mg/dl and triglycerides: 150-499 mg/dl

Exclusion Criteria:

1. Patients with type 2 diabetes mellitus on insulin therapy
2. Type 1 diabetes mellitus patients
3. Patients with renal or hepatic dysfunction
4. Patients with history of myocardial infarction or cardiac intervention
5. History of hypersensitivity to the test drugs
6. Pregnant and lactating women

Method of collection of data:

Patients clinically diagnosed with type 2 diabetes mellitus (fasting blood sugar 125-250gm/dl) receiving combination of metformin with glimepiride were included in the study. They were randomly divided into three groups, by simple randomization (lottery method).

Group 1 received metformin (500mg) + glimepiride (1mg) + vitamin E (400mg) for 12 weeks.

Group 2 received metformin (500mg) + glimepiride (1mg) + omega 3 fatty acids (eicosapentaenoic acid-180 mg, docosahexaenoic acid-120 mg) for 12 weeks.

Group 3 received metformin (500mg) + glimepiride (1mg) for 12 weeks.

Anthropometric measurements, fasting blood sugar, postprandial blood sugar, glycated hemoglobin, lipid profile, mini mental examination scale consisting of 11 questions with maximum score of 30 were assessed at the baseline and at the end of 12 weeks. Patients were instructed to take the study drugs once daily orally, 30

minutes before food and to follow the diabetic diet. Patient's compliance was assessed by advising them to return the used tablet strips during follow-up.

All the adverse events were assessed in accordance with the WHO causality assessment scale, as follows:

Certain: if adverse effect has a pausible time relationship to drug intake, subsides on stopping the drug and manifests on readministration.

Probable: if it has a reasonable time relationship to drug intake and if the adverse effect subsides on withdrawing the drug.

Possible: if it has a reasonable time relationship to drug intake, if the adverse effect can be explained by disease or other drugs.

Unlikely: if it has an improbable time relationship to drug intake, if the adverse effect can be explained by disease or other drugs.

Sample size calculation: To detect the mean difference of 1.24 in the HbA1c at the end of 12 weeks with an effect size of 0.765, alpha error of 5%, power of 80% with a dropout rate of 10%, the required sample size was 31 in each group.

Statistical methods:

The demographic data was analyzed using descriptive statistics. The fasting blood sugar, postprandial sugar, glycated hemoglobin, lipid profile, anthropometric measurements within the group were analyzed using paired t test and ANOVA for between the groups. Adverse effects was analyzed using the Chi Square test. p value of <0.05 was considered statistically significant.

Results

RESULTS

A total of 100 patients were recruited in the study. Among them, 87 completed the study, 10 patients were lost to follow up and three patients withdrew from the study.

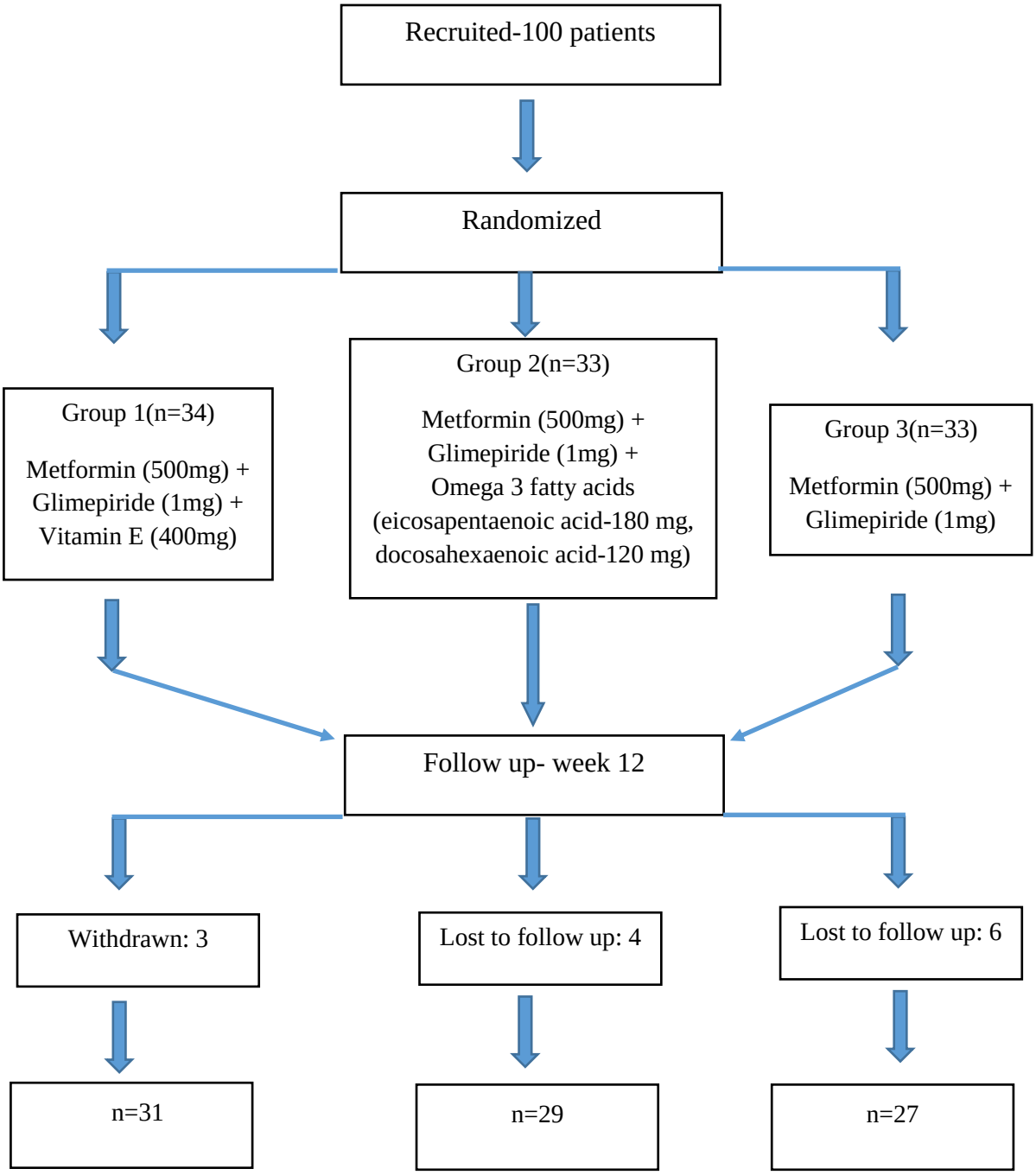


Figure 13. Patient recruitment, randomization and follow up

Table 2. Demographic data at baseline

Baseline characteristics	Group 1(n=31)	Group 2(n=29)	Group 3(n=27)	p value
Number of patients	31	29	27	-
Age (years)	51.38 ± 9.79	51.76 ± 7.09	52.73 ± 8.19	0.79
Gender (M/F)	17/14	11/18	16/11	0.33
Duration of DM (months)	51.85 ± 53.69	46.24 ± 33.94	66.21 ± 71.49	0.32
Weight (kg)	64.19 ± 12.43	66.61 ± 13.41	65.03 ± 11.25	0.53
Height (m)	1.59 ± 0.07	1.57 ± 0.06	1.59 ± 0.07	0.35
Pulse rate (beats/min)	82.62 ± 5.42	79.45 ± 6.52	82.42 ± 5.38	0.05
SBP (mm Hg)	136.18 ± 14.14	133.82 ± 17.46	134.85 ± 14.38	0.82
DBP (mm Hg)	85.59 ± 9.27	83.94 ± 9.66	84.73 ± 8.65	0.76

Values: Mean ± SD

All the demographic characteristics were comparable between the groups at baseline

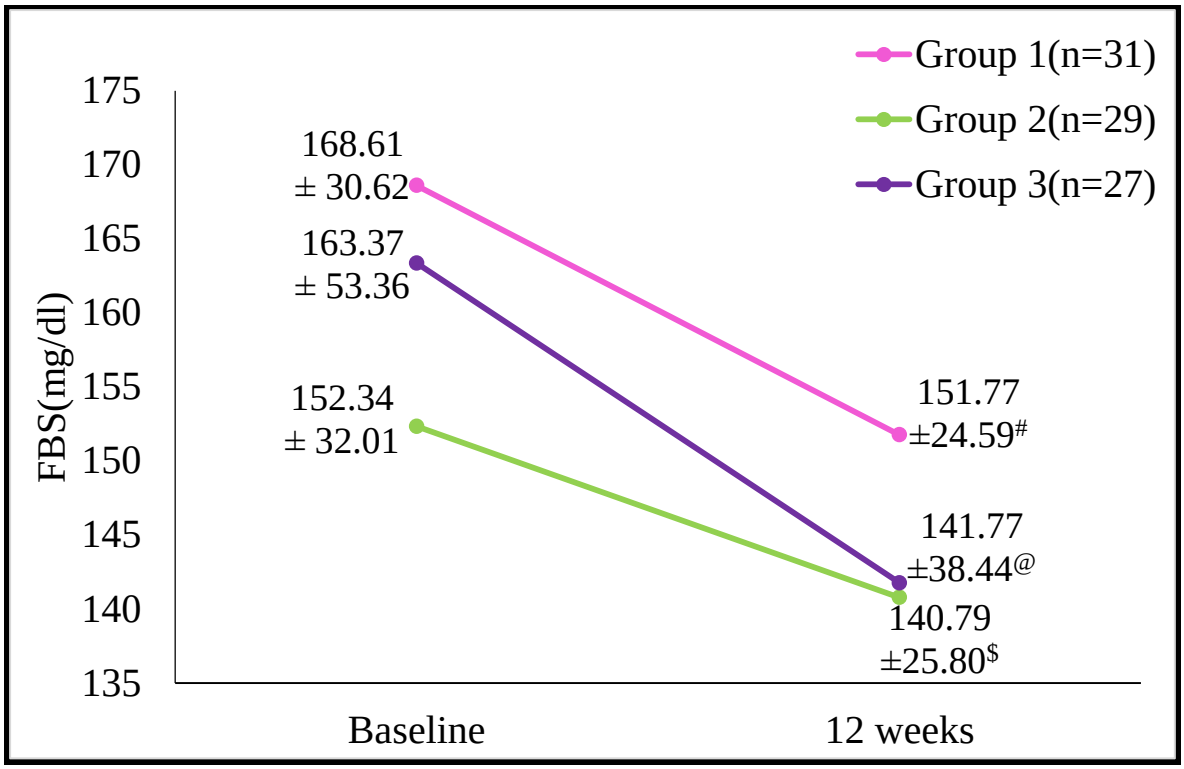


Figure 14. Comparison of fasting blood sugar (FBS) within the groups

Values: Mean ±SD, paired't' test

#Group 1 (p=0.0001)

$Group 2 (p=0.003)

@Group 3(p=0.0001)

Fasting blood sugar was significantly reduced in patients in all 3 groups at 12 weeks compared to baseline

Table 3. Comparison of fasting blood glucose between the groups

FBS(mg/dl)	Group 1(n=31)	Group 2(n=29)	Group 3(n=27)	p-value
Baseline	168.61±30.62	152.34±32.01	163.37±53.36	0.27
12 weeks	151.77±24.59	140.79±25.80	141.77±38.44	0.29

The reduction of fasting blood glucose at 12 weeks from baseline between the groups was not statistically significant.

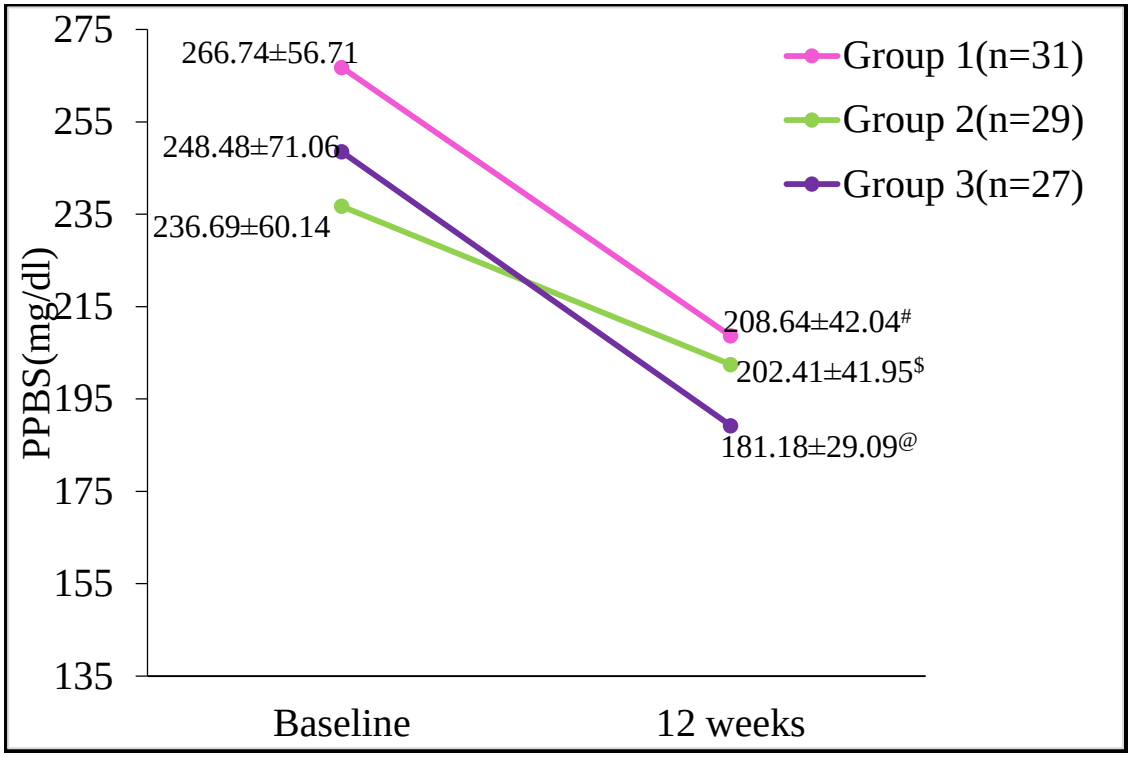


Figure 15. Comparison of post prandial blood sugar (PPBS) within the groups

Values: Mean ±SD, paired't' test

#Group 1 (p=0.0001)

$Group 2 (p=0.0001)

@Group 3(p=0.0001)

Post prandial blood glucose was significantly reduced in patients in all 3 groups after 12 weeks from baseline.

Table 4. Comparison of post prandial blood sugar between the groups

PPBS(mg/dl)	Group 1(n=31)	Group 2(n=29)	Group 3(n=27)	p-value
Baseline	266.74±56.71	236.69±60.14	248.48±71.06	0.17
12 weeks	208.32±42.04	202.41±41.95	189.18 ± 29.09	0.16

The reduction of post prandial blood glucose at 12 weeks from baseline between the groups was not statistically significant.

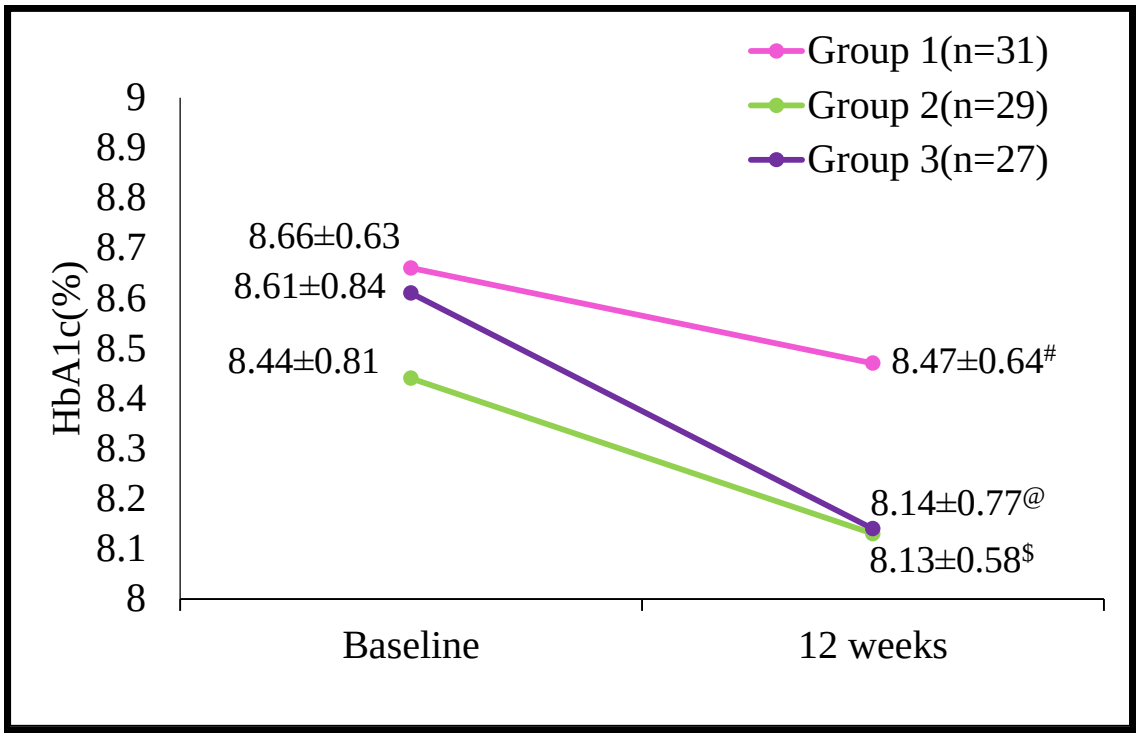


Figure 16. Comparison of glycated hemoglobin (HbA1c) within the groups

Values: Mean ±SD, paired't' test

#Group 1 (p=0.0001)

$Group 2 (p=0.002)

@Group 3(p=0.001)

HbA1c was significantly reduced of patients of all 3 groups at 12 weeks compared to baseline.

Table 5. Comparison of glycated hemoglobin between the groups

HbA1c (%)	Group 1(n=31)	Group 2(n=29)	Group 3(n=27)	p-value
Baseline	8.66± 0.63	8.44±0.81	8.61±0.84	0.51
12 weeks	8.47±0.64	8.13±0.58	8.14±0.77	0.09

The reduction of glycated hemoglobin at 12 weeks from baseline between the groups was not statistically significant.

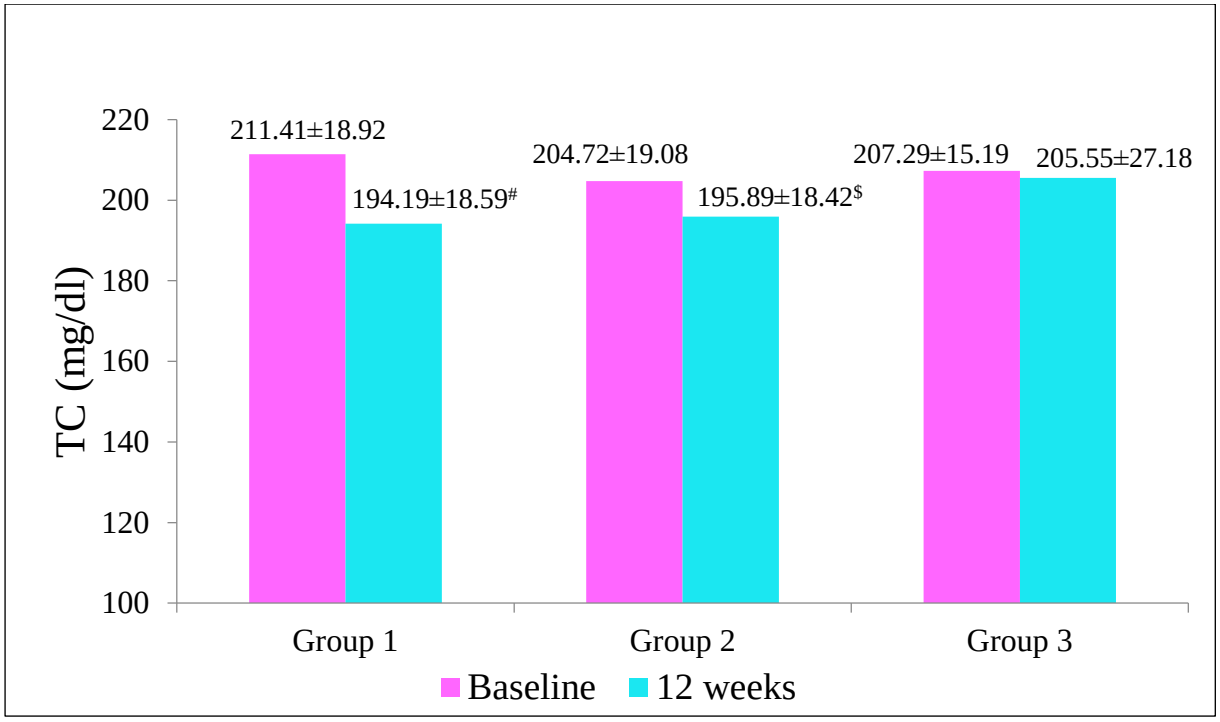


Figure 17. Comparison of total cholesterol (TC) within the groups

Values: Mean ±SD, paired‘t’ test

#Group 1(p=0.0001)

$Group 2(p=0.01)

Total cholesterol was significantly reduced in patients of group 1 and group 2 and there was no significant reduction in patients of group 3 (p=0.71) at 12 weeks compared to baseline.

Table 6. Comparison of total cholesterol between the groups

TC(mg/dl)	Group 1(n=31)	Group 2(n=29)	Group 3(n=27)	p-value
Baseline	211.41±18.92	204.72±19.08	207.29±15.19	0.34
12 weeks	194.19±18.59	195.89±18.42	205.55±27.18	0.11

The reduction of total cholesterol at 12 weeks from baseline between the groups was not statistically significant.

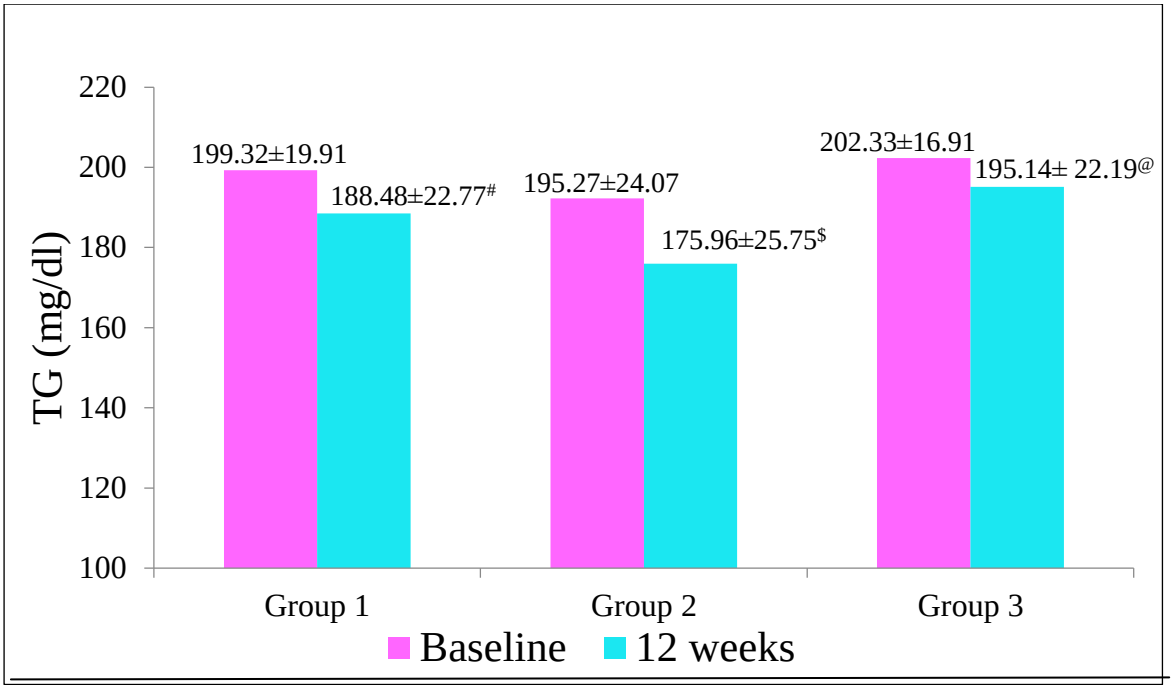


Figure 18. Comparison of triglycerides (TG) within the groups

Values: Mean ±SD, paired't' test

#Group 1 (p=0.02)

$Group 2 (p=0.001)

@Group 3(p=0.04)

Triglycerides was significantly reduced in patients of all 3 groups at 12 weeks compared to baseline.

Table 7. Comparison of triglycerides between the groups

TG(mg/dl)	Group 1(n=31)	Group 2(n=29)	Group 3(n=27)	p-value
Baseline	199.32±19.91	192.27±24.09	202.333±16.91	0.17
12 weeks	188.48±22.77	175.96±25.75	195.14± 22.19	0.01*

Post Hoc Bonferroni test was carried out and it was observed that triglycerides was significantly reduced in patients of group 2 compared to group 3 (p=0.01) at 12 weeks.

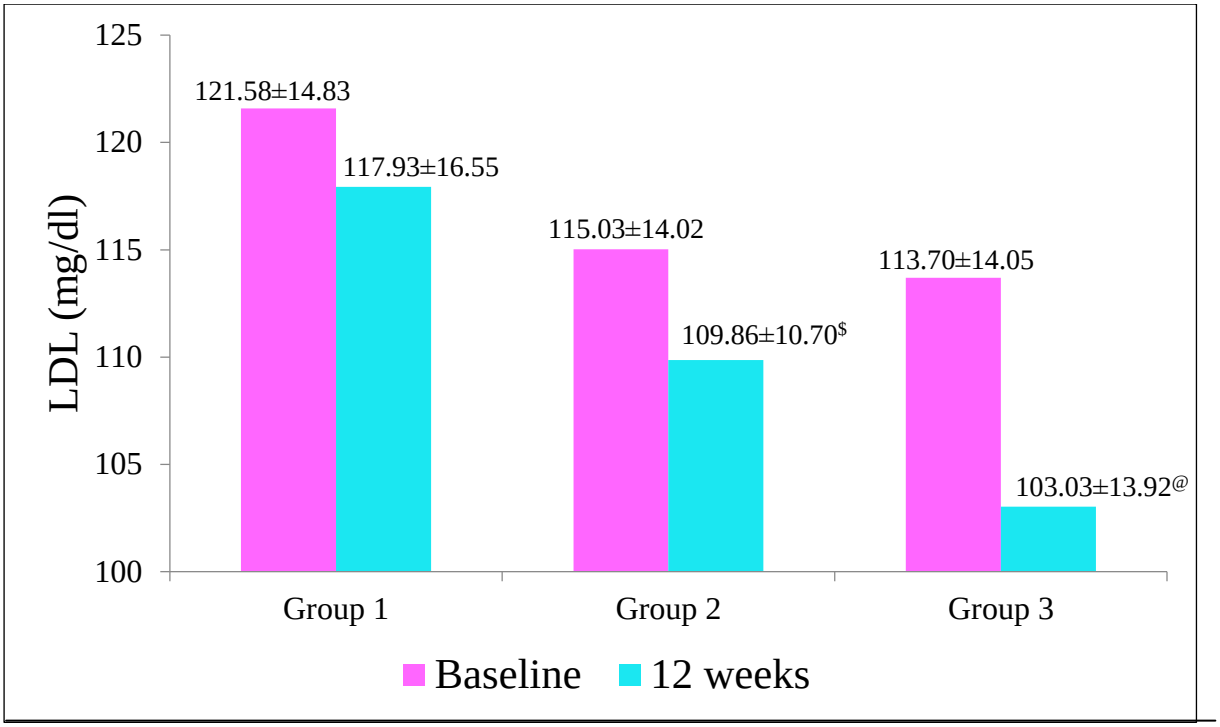


Figure 19. Comparison of low density lipoprotein (LDL) within the groups

Values: Mean ±SD, paired't' test

$Group 2 (p=0.04)

@Group 3(p=0.002)

LDL was significantly reduced in patients of group 2 and 3 but it was insignificant in group 1 (p=0.12) after 12 weeks.

Table 8. Comparison of low density lipoprotein between the groups

LDL(mg/dl)	Group 1(n=31)	Group 2(n=29)	Group 3(n=27)	p-value
Baseline	121.58±14.83	115.03±14.02	113.70±14.05	0.08
12 weeks	117.93±16.55	109.86±10.70	103.03±13.92	0.001*

Post Hoc Bonferroni test was carried out and it was observed that LDL was significantly reduced in patients of group 3 compared to group 1 (p=0.0001) at 12 weeks.

There was no statistical significant increase in HDL in patients of all three groups at 12 weeks [group 1 (p=0.56), group 2 (p=0.90) and 3 (p=0.27)]

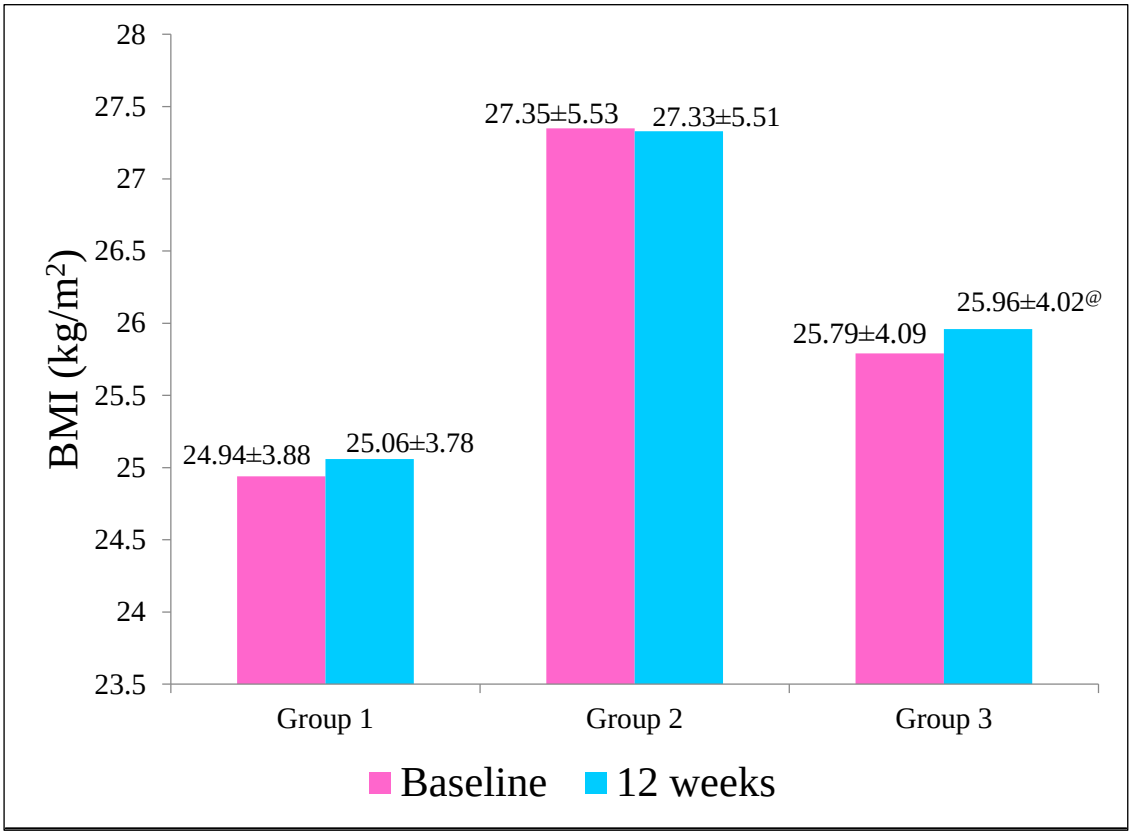


Figure 20. Comparison of body mass index (BMI) within the groups

Values: Mean ±SD

@Group 3(p=0.008)

BMI was significantly increased in group 3 but there was no significant increase in groups 1 (p=0.10) and 2 (p=0.82) after 12 weeks.

The mean reduction in BMI between between groups at 12 weeks was statistically insignificant.

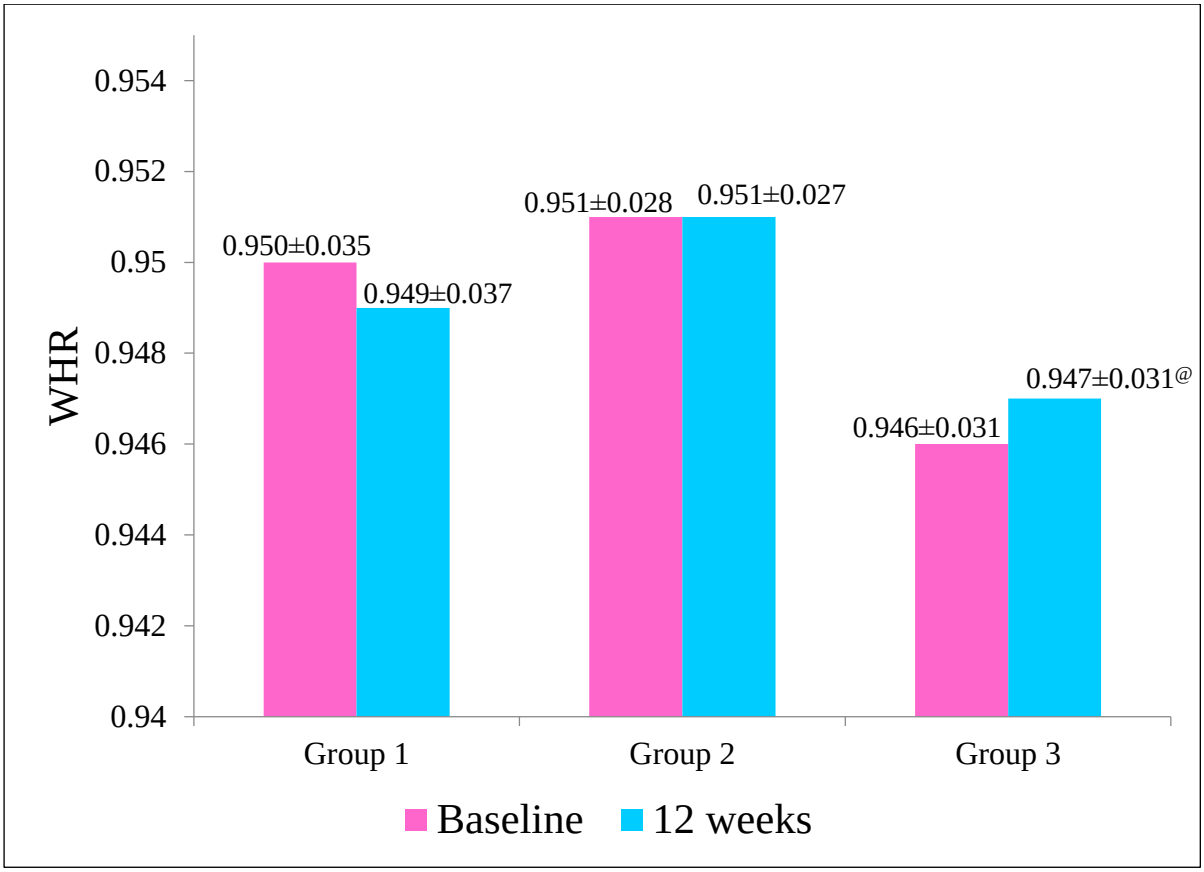


Figure 21. Comparison of waist-hip ratio (WHR) within the groups

Values: Mean ±SD

[@]Group 3(p=0.04)

WHR was significantly increased in group 3 and no significant change was observed in group 1(p=0.42) and 2 (p=1.00) after 12 weeks.

There was no statistical significant difference in WHR at 12 weeks between the groups.

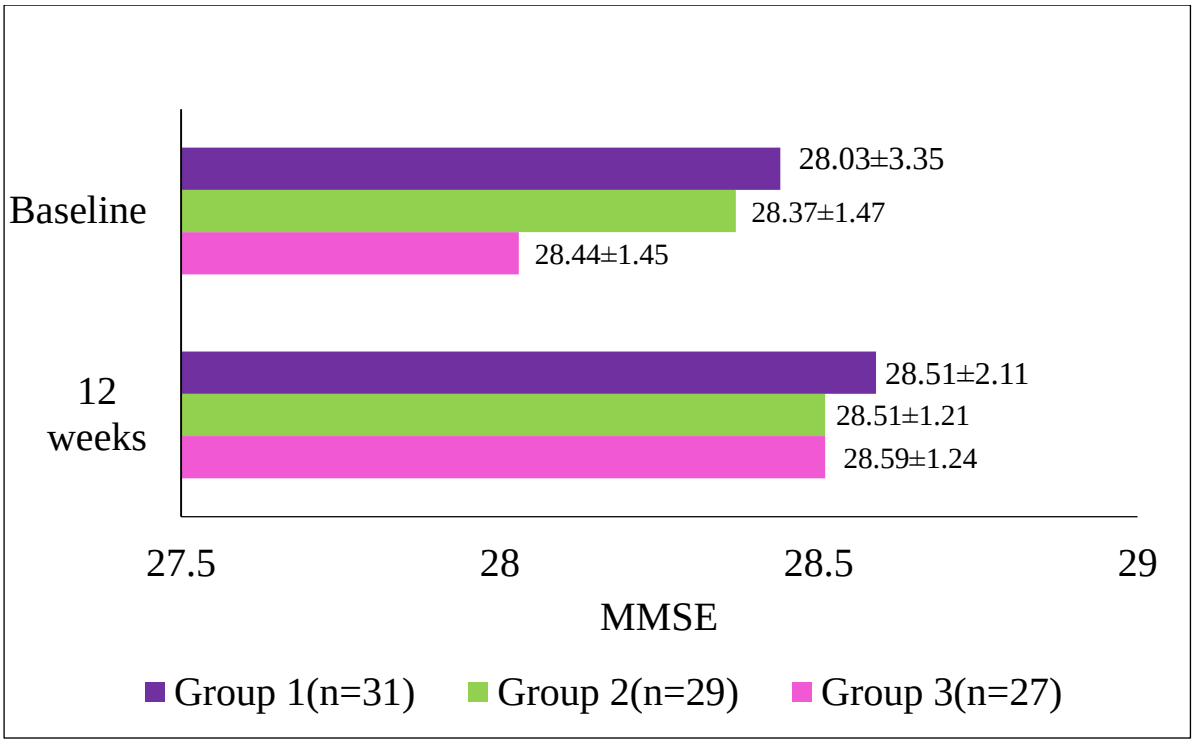


Figure 22. Comparison of mini mental state examination (MMSE) scores within the groups

Values: Mean ±SD, paired‘t’ test

MMSE scores were not significantly increased from baseline to 12 weeks in any of the groups [Group 1(p=0.08), Group 2(p=0.16), Group 3(p=0.10)].

Discussion

DISCUSSION

Diabetes mellitus (DM) is one of the most prevalent non-communicable disease.[4] The risk of developing DM is increased due to oxidative stress which in turn plays an important role in the pathogenesis of DM and its complications. Oxidative stress due to hyperglycemia induces overproduction of the oxygen free radicals which are neutralized by the antioxidants. In addition, chronic hyperglycemia decreases the antioxidant defense mechanism system due to the activation of the polyol and hexosamine pathway, increased formation of advanced glycation end products (AGEs), activation of the protein kinase C isoforms.[23] Supplementation of antioxidants can reduce oxidative stress in the type 2 DM patients which may result in improvement in the glycemic control, insulin sensitivity and lipid profile in type 2 DM patients.

The staple food consumed by this locality people is rice, ragi and wheat, therefore supplementation of antioxidants through diet like vegetables, fish and fruits may be inadequate. In this study, we assessed the effect of antioxidants [vitamin E and omega 3 fatty acids (OFA)] on fasting blood sugar (FBS), postprandial blood sugar (PPBS), glycated hemoglobin (HbA1c), anthropometric measurements [body mass index (BMI), waist hip ratio (WHR)], lipid profile- [total cholesterol (TC), triglycerides (TG), low density lipoprotein cholesterol (LDL), high density lipoprotein cholesterol (HDL)] and cognitive function using mini mental state examination (MMSE) scale.

Hundred patients diagnosed with type 2 DM receiving combination of metformin (500mg) and glimepiride (1mg) were recruited, among them 87 completed the study. Patients were randomly assigned to receive add on therapy of Vitamin E

400mg (Group 1) or OFA [eicosapentaenoic acid-180 mg, docosahexaenoic acid-120 mg] (Group 2), once daily for 12 weeks and Group 3 served as control. FBS, PPBS, HbA1c, BMI, WHR, lipid profile, MMSE were assessed at baseline and after 12 weeks. Majority of the patients were in the 5th decade (Table 2) and their common complaint was generalized fatigue and body pain at the start of study. The demographic characteristics were comparable (Table 2).

There was significant reduction in FBS, PPBS, HbA1c of the patients at 12 weeks compared to baseline (Figure 14, 15 and 16) but the glycemic control between the groups was similar (Table 3, 4 and 5). This implies that add on therapy in our study did not significantly contribute to reduction of blood sugar levels. This result was similar to Xu et al study, a meta-analysis of the randomized controlled trials, which described twelve studies and their conclusion was that vitamin E supplementation did not significantly reduce FBS and HbA1c.[37] Systematic reviews conducted by Hartweg et al and Hendrich also concluded that there were no significant reduction in FBS and HbA1c with OFA supplementation.[44,45] But the study conducted by Udupa et al showed significant reduction of HbA1c in patients receiving add-on therapy (alpha lipoic acid, omega 3 fatty acid and vitamin E) after 12 weeks and maximum reduction was attributed to omega 3 fatty acid.[27] A study conducted by Haliga et al observed that the addition of flaxseed (rich in OFA) and/or vitamin E to the high-fat diet had produced significant reduction of serum glucose levels in diabetic hamsters.[42]

Significant reduction in TC, TG was observed in patients receiving either of the antioxidants and also significant reduction in LDL in patients receiving OFA at 12 weeks compared to baseline (Figure 17, 18 and 19). Intergroup analysis showed that patients receiving OFA had significant reduction in TG compared to control (Table

7). BMI and WHR were significantly increased in the patients who received only anti diabetic drugs which signifies that the omega 3 fatty acids and vitamin E prevented weight gain (Figure 20 and 21). There was no significant effect on cognitive function in patients of any of the groups (Figure 22).

Hartweg et al and Hendrich studies reported that OFA supplementation significantly reduced TG compared to control whereas Hendrich study also observed reduction in LDL.[44,45] In a study conducted by Haliga et al, the addition of vitamin E alone or in combination with flaxseed to the high-fat diet showed the reduction in serum concentrations of TC, TG and LDL but failed to increase the HDL level in diabetic hamsters.[42] In Udupa et al study, at the end of 12 weeks there was significant reduction in the TC, BMI and waist circumference in patients who received add-on therapy (alpha lipoic acid, omega 3 fatty acid and vitamin E).[27] Three patients in vitamin E group developed abdominal pain which was probable according to causality assessment hence they were withdrawn from the study (Figure 13). All other drugs were well tolerated.

In our study, the glycemic control achieved with supplementation of vitamin E or OFA was similar to the control group. Both vitamin E and OFA had beneficial effect on lipid profile and anthropometric measurements. The cognitive functions were similar at baseline and end of the study. Probably longer duration of supplementation with these antioxidants may improve the glycemic control but our study was of 12 weeks duration.

Conclusion

CONCLUSION

- In this study, 100 patients with type 2 DM and receiving metformin (500mg) and glimepiride (1mg) were recruited.
- They received either Vitamin E 400mg (Group1) or Omega 3 fatty acids [eicosapentaenoic acid-180 mg, docosahexaenoic acid-120 mg] (Group 2), once daily for 12 weeks and Group 3 served as control.
- The demographic characteristics were comparable between the groups.
- FBS, PPBS and HbA1c were significantly reduced in all three groups at 12 weeks.
- The antioxidants reduced TC, TG but OFA also reduced LDL.
- Anthropometric measurements (BMI and WHR) were increased only in control group.
- The glycemic control between the groups was similar but TG were significantly reduced by OFA.
- In patients receiving vitamin E, three of them withdrew from the study because of abdominal pain.
- Vitamin E and OFA had beneficial effects on lipid profile and anthropometric measurements. The glycemic control was similar to the patients in control group, probably longer duration of add-on therapy may be required to produce better effect.

Summary

SUMMARY

Diabetes mellitus (DM) and its complications have been implicated to hyperglycemia induced oxidative stress. Thus, antioxidants may be used to reduce the blood sugar.In our study, 100 patients diagnosed with type 2 DM receiving combination of metformin (500mg) and glimepiride (1mg) were recruited. They were randomly assigned to receive add on therapy of Vitamin E 400mg (Group1) or Omega 3 fatty acids [eicosapentaenoic acid-180 mg, docosahexaenoic acid-120 mg] (Group 2), once daily for 12 weeks and Group 3 served as control.

Fasting blood sugar (FBS), postprandial blood sugar (PPBS), glycated haemoglobin (HbA1c), anthropometric measurements (BMI, waist-hip ratio[WHR]), lipid profile [Total cholesterol (TC), triglycerides (TG), low density lipoprotein cholesterol (LDL), high density lipoprotein cholesterol (HDL)] and mini mental state examination (MMSE) scale were done at the baseline and 12 weeks. Adverse effects were also noted.

Eighty seven patients (Group 1 =31, Group 2 =29 and Group 3 =27) completed the study, 44 were males and 43 were females. Most of the patients were in the 5^{th} decade. All the demographic characteristics were comparable between the groups at baseline.

After 12 weeks, there was significant reduction in FBS, PPBS and HbA1c in all three groups. There was significant reduction in TC, TG in patients receiving either of the antioxidants and also significant reduction in LDL in patients receiving OFA at 12 weeks compared to baseline. BMI and WHR were significantly increased in control group.

Intergroup analysis showed that in patients receiving vitamin E and omega 3 fatty acids the reduction of FBS, PPBS and HbA1c were similar to control group. TG was significantly reduced by OFA compared to control. Three patients in vitamin E group had abdominal pain which was probable after causality assessment hence they were withdrawn from the study. The other study drugs were well tolerated.

The glycemic control achieved with supplementation of vitamin E or OFA was similar to the control group, probably longer duration of treatment may be required to observe beneficial effect. Both vitamin E and OFA had beneficial effect on lipid profile and anthropometric measurements.

Bibliography

BIBLIOGRAPHY

1. Powers AC. Diabetes mellitus. In:Longo D, Fauci A, Kasper D, Hauser S, Jameson J, Loscalzo J,editors. Harrison's principles of internal medicine.18th ed. New York: Mc Graw Hill; 2012. p. 2968-3003.
2. Madhu SV. Diabetes complications: Overview. In: Chandalia HB, Sridar GR, Das AK, Madhu SV, Mohan V, Rao PV. RSSDI textbook of diabetes mellitus. 3rd ed. New Delhi: Jaypee The Health Sciences Publishers Ltd; 2014.p.773-86.
3. Maitra A. The Endocrine system In: Kumar V, Abbas KA, Fausta N, Aster CJ. Robbins and Cotran pathologic basis of disease. 8th ed. Philadelphia: Elsevier Inc; 2010. p. 1130–48.
4. Sarita N, Shinde, Vithal N, Dhadke, Adinath N, Suryakar. Evaluation of oxidative stress in type 2 diabetes mellitus and follow up along with vitamin E supplementation. Indian J Clin Biochem 2011; 26: 74-7.
5. Riserus U, Arnlov J, Brismar K, Zethelius B, Berglund L, Vassley B. Sagittal abdominal diameter is a strong anthropometric marker of insulin resistance and hyperproinsulinemia in obese men. Diabetes care 2004; 27: 2041-46.
6. Singh Y, Garg MK, Tandon N, Marwaha RK. A study of insulin resistance by HOMA-IR and its cut-off value to identify metabolic syndrome in urban Indian adolescents. J Clin Res Pediatr Endocrinol 2013; 5: 245-51.
7. Unnikrishnan R. Landmarks in the history of diabetes. In: Chandalia HB, Sridar GR, Das AK, Madhu SV, Mohan V, Rao PV. RSSDI textbook of diabetes mellitus. 3rd ed. New Delhi: Jaypee The Health Sciences Publishers Ltd; 2014.p.3-19.

8. American Diabetes Association (Internet) 2015 [cited on 2015 june 02]. Available from http://www.diabetes.org/research-and-practice/student-resources/history-of-diabetes.html
9. Wolf G. The discovery of the antioxidant function of vitamin E: the contribution of Henry A Mattill. Am Soc J Nutr Sci 2005;135:363-6.
10. Niki E, Traber M. A history of vitamin E. Ann Nutr Metab 2012;61:207-12.
11. American Nutrition Association (Internet) 2015 [cited on june 11]. Available from http://www.americannutritionassociation.org/vitamin-e-factor-summary-review/history.html
12. Isodisnatura.ca (Internet) 2015 [cited on june 11]. Available from http://www.isodisnatura.ca/history_of_omega-3s.html
13. University of Maryland Medical Center(Internet) 2015[cited on june 16] available from http://umm.edu/health/medical/altmed/supplement/omega3-fatty-acids/history.html
14. Das AK, Tripathy BB. Etiology of diabetes: an overview. In: Chandalia HB, Sridar GR, Das AK, Madhu SV, Mohan V, Rao PV. RSSDI textbook of diabetes mellitus. 3rd ed. New Delhi: Jaypee The Health Sciences Publishers Ltd; 2014.p.193-8.
15. Saxena TK, Maheshwari S, Saxena M. Aetiopathogenesis of type 2 Diabetes Mellitus:could chronic stress play an important role? JAPI 2014;62:484-9.
16. Ozougwu JC, Obimba KC, Belonwu CD, Unakalamba CB. The pathogenesis and pathophysiology of type 1 and type 2 diabetes mellitus. J Physiol Pat 2013;4:46-57.
17. Kaku K. Pathophysiology of type 2 diabetes and its treatment policy.JMAJ 2010;53:41-6.

18. Tripathy D, Tripathy BB, Chandalia HB. Pathogenesis of type 2 diabetes. In: Chandalia HB, Sridar GR, Das AK, Madhu SV, Mohan V, Rao PV. RSSDI textbook of diabetes mellitus. 3rd ed. New Delhi: Jaypee The Health Sciences Publishers Ltd; 2014.p.215-44.
19. Tushuizen ME, Diamant M, Heine RJ. Postprandial dysmetabolism and cardiovascular disease in type 2 diabetes. Postgrad Med J 2005;81:1-6.
20. Kuroki T, Isshiki K, King GL. Oxidative stress: The lead or supporting actor in the pathogenesis of diabetic complications. J Am Soc Nephrol 2003;14:S216-20.
21. Singh Y, Garg MK, Tandon N, Marwaha RK. A study of insulin resistance by HOMA-IR and its cut-off value to identify metabolic syndrome in urban Indian adolescents. J Clin Res Pediatr Endocrinol 2013;5:245-51.
22. Tiwari BK, Pandey KB, Abidi AB, Rizvi SI. Markers of oxidative stress during Diabetes Mellitus. J Biomarkers 2013:1-8.
23. Folli F, Corradi D, Fanti P, Davalli A, Paez A, Giaccari A et al. The role of oxidative stress in the pathogenesis of type 2 diabetes mellitus- micro and macrovasular complications: Avenues for a mechanistic-based therapeutic approach. Current Diabetes Reviews 2011;7:313-24.
24. Rask-Madsen C, He Z, King GL. Mechanisms of diabetic microvascular complications. In: Kahn CR, Weir GC, King GL, Jacobson AM, Moses AC, Smith RJ. Joslin's Diabetes mellitus. 14th ed. Philadelphia:Lippincott Williams & Wilkins 2006.p.823-39.
25. Montonen J, Knekt P, Jarvinen R, Reunanen A. Dietary antioxidant intake and risk of type 2 diabetes. Diabetes Care 2004;27:362-6.

26. Desai NK, Bhabher PH, Damadia D, Bhatt JD. Role of antioxidant therapy in management of type 2 diabetes mellitus. Nat J Int Res Med 2013;4:128-33.
27. Udupa AS, Nahar PS, Shah SH, Kshirsagar MJ, Ghongane BB. Study of comparative effects of antioxidants on insulin sensitivity in type 2 diabetes mellitus. J Clin Diagnos Res 2012; 6: 1469-73.
28. Robertson RP, Harmon J, Tran PO, Poitout V. β cell glucose toxicity, lipotoxicity and chronic oxidative stress in type 2 diabetes. Diabetes 2004;53:S119-24.
29. Oliveira AM, Rondo PHC, Luzia LA, Abronzo FH, Illison VK. The effect of lipoic acid and alpha tocopherol supplementation on the lipid profile and insulin sensitivity of patients with type 2 diabetes mellitus:A randomized double blind placebo control trial. Diabetes Res Clin Pr 2011; 92: 253-60.
30. Ramos RV, Lauro GA, Elina MB, Donaji BA. Vitamins and type 2 diabetes mellitus. End Met Imm Disorders- Drug Targets 2015;15:54-63.
31. Wright E, Scism-Bacon JL, Glass LC. Oxidative stress in type 2 diabetes: the role of fasting and postprandial glycaemia. Int J Clin Pract 2006;60:308-14.
32. Whillier S, Kuchel PW, Raftos JE. Oxidative Stress in Type 2 Diabetes Mellitus and the Role of the Endogenous Antioxidant Glutathione, Role of the Adipocyte in Development of Type 2 Diabetes, Dr. Colleen Croniger (Ed.), ISBN: 978-953-307-598-3, InTech Publisher, http://www.intechopen.com/books/role-of-the-adipocyte-in-development-of-type-2-diabetes/oxidative-stress-intype-ii-diabetes-mellitus-and-the-role-of-the-endogenous-antioxidant-glutathione(accessed 22 June 2015)

33. Vijayakumar APR, Kumar JB, Jenny VM, Sushanta KD. Supplementation of vitamin E improves cognitive status and oxidative stress in type 2 Diabetes Mellitus. IRJP 2011; 2: 169-72.
34. Anthonia OO, Emmanuel E, Chioma U, Olajumoke O. Treatment of diabetes mellitus‐associated neuropathy with vitamin E and Eve primrose. Indian J Endocrin Met 2014;18: 846-9
35. Mahmoodi MR, Kimiagar M, Mehrabi Y. The effects of omega-3 plus vitamin E and zinc plus vitamin C supplementation on cardiovascualar risk markers in postmenopausal women with type 2 diabetes.Ther adv endocrinol metab 2014;5:67-76.
36. Sharma A, Kharb S, Chugh SN, Kakkar R, Singh GP. Evaluation of oxidative stress before and after control of glycemia and after vitamin E supplementation in diabetic patients. Metabolism 2000;49:160-2.
37. Xu R, Zhang S, Tao A, Chen G, Zhang M. Influence of vitamin E supplementation on glycemic control: A meta-analysis of randomized controlled trials. PLOS ONE 2014;9:1-9.
38. Pandey A, Tripathi P, Pandey R, Srivatava R, Goswami S. Alternative therapies useful in the management of diabetes : A systematic review. J Pharm Bioall Sci 2011;3:504-12.
39. Odum EP, Ejilemele AA, Wakwe VC. Antioxidant status of type 2 diabetic patients in Port Harcourt,Nigeria. Niger J Clin Pract 2012;15:55-8.
40. Lonn E, Yusuf S, Hoogwerf B, Pogue J, Yi Q, Zinman B et al. Effects of vitamin E on cardiovascular and microvascular outcomes in high-risk patients with diabetes. Diabetes Care 2002;25:1119-27.

41. Tabei SM, Fakhen S, Djalali M, Javanbakht MH, Zarei M, Derakhshanian H et al. Effect of vitamins A, E, C and omega 3 fatty acids supplementation on the level of catalase and superoxide dismutase activities in streptozotocin-induced diabetic rats. Bratisl Lek Listy 2015;116:115-8.
42. Haliga RE, Mocanu V, Badescu M. Antioxidative and antiatherogenic effects of flaxseed, α-tocopherol and their combination in diabetic hamsters fed with a high fat diet. Exp Therap Med 2015;9:533-8.
43. Flachs P, Rossmeisl M, Kopecky J. The effect of n-3 fatty acids on glucose homeostasis and insulin sensitivity. Physiol. Res. 2014; 63: 93-118
44. Hartweg J, Perera R, Montori VM, Dinneen SF, Neil AH, Farmer AJ. Omega 3 polyunsaturated fatty acids for type 2 diabetes mellitus. Cochrane Database of Systematic Reviews 2008; Issue 1:Art. No.:CD 003205. DOI:10.1002/14651858.CD003205.pub2
45. Hendrich S. Omega-3 fatty acids: Clinical trials in people with type 2 diabetes. Am J Clin Nutr Adv Nutr 2010;1:3-7.
46. Marai P, Massalha S. Effect of omega 3 polyunsaturated fatty acids and vitamin D on cardiovascular diseases. IMAJ 2014; 16: 117-21.
47. Kumar PR, Essa MM, Al-Adawi S, Dradekh G, Memon M, Akbar M et al. Omega 3 fatty acids could alleviate the risks of traumatic brain injury-a mini review. J Tradit Complement Med Jun 2014;4:89-92.
48. Yessoufou A, Nekova MP, Gbankot A, Mashalla Y, Mortairou K. Beneficial effects of omega 3 polyunsaturated fatty acids in gestational diabetes: consequnences in macrosomia and adulthood obesity. J Diabetes Res 2015:1-11.

49. Wu JH, Micha R, Imamura F, Pan A, Biggs ML, Ajaz O et al. Omega 3 fatty acids and incident type 2 diabetes : A systematic review and meta-analysis. Br J Nutr 2012;107:S214-27.
50. Brostow DP, Odegaard AO, Koh WP, Duval S, Gross MD, Yuan J et al. omega 3 fatty acids and incident type 2 diabetes: The Singapore Chinese health study. Am J Clin Nutr 2011:1-7.
51. Karlstrom BE, Jarvi AE, Byberg L, Berglund L, Vessby BD. Fatty fish in the diet of patients with type 2 diabetes:comparison of the metabolic effects of foods rich in n-3 and n-6 fatty acids. Am J Clin Nutr 2011;94:26-33.
52. Caterina RF, Madonna R, Bertolotto A, Schmidt EB. n-3 fatty acids in the treatment of diabetic patients. Diabetes Care 2007;30:1012-26.
53. Yamamoto T, Kajikawa Y, Otani S, Yamada Y, Takemoto S, Hirota M. Protective effect of eicosapentaenoic acid on insulin resistance in hyperlipidemic patients and on the postoperative course of cardiac surgery patients: The positive possible involvement of adiponectin. Acta Med Okayama 2014;68:349-61.
54. Stirban A, Nandrean S, Gotting C, Tamler R, Pop A, Negrean M et al. Effect of omega-3 fatty acids on macro and microvascular function in subjects with type 2 diabetes mellitus. Am J Clin Nutr 2010; 91: 808-13.
55. Ogawa S, Abe T, Nako K, Okamura M, Senda M, Sakamoto T et al. Eicosapentaenoic acid improves glycemic control in elderly bedridden patients with type 2 diabetes. Tohoku J Exp Med 2013;231:63-74.
56. Sweetman SC. Martindale The complete drug reference. 38th ed. London: Pharmaceutical Press; 2013 .p. 1460-2122.

57. Rx list:Internet drug index (Internet) 2013[cited 2013 october 18]. Available from http://www.rxlist.com/lovaza-drug.html

58. Nutescu A, Haines ST, Wittkowsky AK. Venous thromboembolism. In Chrisholm-Burns MA, Wells B, Schwinghammer T, Malone PM, Kolesar J, Dipiro JT. Pharmacotherapy principles and practice 3rd ed. New York: Mc Graw Hill; 2013.p. 197-228.

Annexures

PROFORMA

OP No.: Date:

Serial No.:

- Name:
- Age:
- Gender:
- Occupation:
- Socioeconomic status:
- Address with Phone no.:
- Chief complaints
- Past history of same complaints and treatment if taken:
- Family history:
- Personal History:
- General Physical Examination:
  - PR: BP: RR:
  - Height:
  - Weight

- Pallor: Icterus: Cyanosis:

  Clubbing: Lymphadenopathy: Oedema:

- Systemic examination:
  - CVS:
  - RS:
  - CNS:
  - PA:

- Diagnosis:

INVESTIGATIONS

Anthropometric measurements:

	BASELINE	12 WEEKS
Weight (kg)		
Height (m)		
BMI(kg/m^2)		
Waist circumference(cm)		
Hip circumference(cm)		
Hip - waist ratio		

Blood sugar:

	BASELINE	12 WEEKS
Fasting blood sugar (mg/dl)		
Post prandial blood sugar (mg/dl)		
HbA1c(%)		

Lipid profile:

	BASELINE	12 WEEKS
Total cholesterol(mg/dl)		
Triglycerides(mg/dl)		
LDL(mg/dl)		
VLDL(mg/dl)		
HDL(mg/dl)		

Renal function tests: Serum creatinine-

Blood urea:

Liver function tests: Total bilirubin-

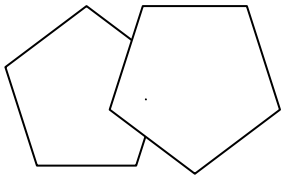
Alkaline phosphatase-

SGOT-

SGPT-

THE MINI –MENTAL STATE EXAMINATION

	Score	Maximum
Orientation • What is the year, season, date, day and month? • Where are we- State, country, town, hospital and floor?		5 5
Registration • Name three objects: one second to say each, then ask the patient to repeat after the examiner has listed out the 3 objects. Give 1 point for each correct answer. Then repeat them until he/she learns all three. Count trials and record. Trials _____		3
Attention and Calculation • Serial 7's . 1 point for each correct answer. Stop after 5 answers. Alternatively spell " world" backward		5
Recall • Ask for the three objects repeated above. Give 1 point for each correct answer		3
Language • Name a pencil and watch • Repeat the following "No ifs, ands or buts" • Follow a 3 stage-command: "Take a paper in your hand, fold it in half and put it on the floor"		2 1 3

• Read and obey the following: CLOSE YOUR EYES • Write a sentence • Copy the design shown. 		1 1 1
Total score		30

Score: 24-30 indicates no cognitive impairment

18-23 indicates mild cognitive impairment

0-17 indicates severe cognitive impairment

Master chart

Master Chart: Metformin + Glimepiride + Vitamin E

Sl No	OP No	Age(years)	Gender	Duration of DM (months)	Pulse Rate(bpm)	SBP (mm/Hg)	DBP (mm/Hg)	Weight(kg)		Height (m)	BMI(kg/m^2)		WC (cm)		HC (cm)		WHR		FBS (mg/dl)		PPBS (mg/dl)		HbA1c (%)		TC (mg/dl)		TG (mg/dl)		LDL (mg/dl)		HDL (mg/dl)		S. Creatinine (mg/dl)	B.Urea (mg/dl)	Total bilirubin (mg/dl)	Alkaline phosphatase (IU/L)	SGOT(IU/L)	SGPT(IU/L)	MMSE	
								Baseline	12 weeks		Baseline	12 weeks	Baseline	12 weeks	Baseline	12 weeks	Baseline	12 weeks	Baseline	12 weeks	Baseline	12 weeks	Baseline	12 weeks	Baseline	12 weeks	Baseline	12 weeks	Baseline	12 weeks	Baseline	12 weeks							Baseline	12weeks
1	876899	60	0	9	96	140	70	82	81	1.6	32.03	31.64	106	105	118	118	0.89	0.88	136	128	220	191	9.1	9	230	205	206	198	139	132	44	49	0.83	37	0.23	96	28	36	30	30
2	1015518	53	1	60	82	130	80	52	53	1.57	21.09	21.54	82	82	89	89	0.92	0.92	142	156	292	312	9.4	9.2	202	189	186	178	128	148	48	43	0.74	29	0.28	98	27	34	30	30
3	763229	60	1	24	82	120	70	44	46	1.56	18.08	18.93	79	79	81	81	0.97	0.97	129	116	231	198	7.2	7.2	226	221	176	167	112	128	49	47	0.8	31	0.6	94	28	26	29	29
4	13166	46	1	72	86	130	90	55	53	1.64	20.44	19.77	83	83	89	88	0.93	0.94	202	192	343	293	10.4	10.2	184	198	189	209	121	131	30	45	0.83	31	0.4	96	24	32	30	30
5	15404	60	1	60	84	130	80	69	68	1.57	27.99	27.64	101	101	107	107	0.94	0.94	172	167	352	278	9	8.8	208	195	184	181	104	127	48	46	0.7	32	0.4	98	32	33	30	30
6	98788	38	1	36	86	150	90	71	71	1.63	26.72	26.72	96	96	104	104	0.92	0.92	201	179	311	276	8	8.4	202	223	211	193	140	137	36	31	0.8	30	0.6	118	34	28	28	27
7	1012341	33	0	6	83	140	100	52	53	1.5	23.11	23.55	86	85	95	96	0.9	0.88	172	156	298	204	8.7	8.5	199	187	189	207	126	131	42	38	0.81	36	0.54	99	37	31	30	30
8	356678	40	0	1	84	130	80	62	62	1.54	28.14	28.14	95	95	108	108	0.87	0.87	135	130	319	168	8.7	8.3	209	185	212	206	137	149	46	44	0.75	38	0.39	104	31	33	28	28
9	665123	40	0	2	88	140	80	55	55	1.63	20.7	20.7	90	90	103	103	0.87	0.87	199	167	386	249	9.3	9.1	230	178	198	195	113	107	36	38	0.81	29	0.31	107	29	37	14	20
10	74445	36	1	2	80	160	110	66	67	1.72	22.37	22.64	94	94	92	92	1.02	1.02	223	189	372	261	8.6	8.4	233	167	222	214	136	125	38	41	0.76	33	0.38	97	33	29	26	26
11	960865	45	0	36	80	120	90	60	60	1.49	27.03	27.03	100	100	102	102	0.98	0.98	167	154	239	203	8.1	7.9	198	223	162	156	108	103	45	48	0.9	39	0.37	101	39	30	26	26
12	4166	60	1	96	86	110	70	77	76	1.65	27.61	27.91	110	110	116	116	0.94	0.94	128	149	255	184	8.6	8.5	192	163	189	157	125	113	33	38	0.84	26	0.41	98	34	35	27	28
13	82070	59	0	60	88	160	90	73	73	1.67	26.17	26.17	108	108	113	113	0.95	0.95	147	178	199	232	9.1	9.6	209	191	166	173	113	99	38	42	0.78	38	0.49	95	27	37	30	30
14	29095	60	0	108	80	130	90	58	57	1.56	23.83	23.42	93	93	96	96	0.96	0.96	140	121	236	198	8.5	8.5	213	209	197	218	136	123	48	49	0.83	36	0.39	108	28	33	20	26
15	29538	34	1	84	74	120	90	65	66	1.74	21.47	21.79	98	98	99	99	0.98	0.98	132	112	288	151	7.9	7.8	231	193	216	182	124	140	39	37	0.74	28	0.24	97	33	42	29	29
16	22276	35	0	4	84	130	80	44	45	1.58	17.62	18.02	81	81	89	89	0.91	0.91	148	156	226	215	7.8	7.5	194	173	223	210	103	127	41	53	0.94	37	0.55	99	39	30	30	30
17	93433	45	1	3	74	120	80	85	85	1.68	30.12	30.12	106	106	107	107	0.99	0.99	164	125	241	159	7.8	7.6	235	199	236	183	101	93	37	32	1	31	0.32	103	40	31	30	30
18	35322	52	0	12	70	120	80	51	52	1.51	22.36	22.8	95	95	97	97	0.97	0.97	177	136	224	219	8.4	8.2	194	213	186	246	129	110	37	51	0.93	27	0.51	117	33	44	30	30
19	27970	60	1	60	78	140	90	58	57	1.6	22.65	22.26	91	91	97	97	0.93	0.93	138	123	194	165	7.9	7.7	182	166	207	173	142	127	49	44	1	27	0.43	109	28	36	30	30
20	65109	44	1	48	84	140	100	78	79	1.64	29.1	29.37	104	104	106	106	0.98	0.98	209	173	367	208	9.3	8.9	185	177	198	151	147	134	48	50	0.85	39	0.65	91	36	42	30	30
21	78721	50	1	12	78	130	80	75	75	1.61	28.95	28.95	94	94	98	98	0.95	0.95	127	149	225	177	8.9	8.2	189	181	212	184	132	116	41	46	0.71	37	0.42	109	37	31	29	29
22	23439	55	0	8	80	140	80	61	63	1.47	28.23	29.15	95	96	98	99	0.96	0.96	204	176	235	209	8.6	8.1	229	193	161	181	115	109	36	47	0.88	41	0.37	98	25	35	30	30
23	112186	44	1	24	80	120	80	66	66	1.6	25.78	25.78	101	101	103	103	0.98	0.98	164	111	229	141	8.4	7.9	231	211	173	163	125	118	49	41	0.82	28	0.41	96	25	38	30	30
24	110518	58	1	120	78	140	90	78	78	1.71	26.67	26.67	108	108	110	110	0.97	0.97	197	153	250	186	9.3	9	234	189	217	195	106	93	46	41	0.73	27	0.45	95	39	25	27	28
25	117987	60	1	12	78	140	80	63	62	1.53	26.91	26.48	96	96	97	97	0.98	0.98	218	189	312	236	8.8	8.1	199	171	193	219	138	115	46	38	0.76	35	0.47	108	35	37	27	28
26	112224	60	0	60	80	130	80	51	51	1.57	20.69	20.69	83	83	85	85	0.97	0.97	183	162	284	189	8.5	8.3	235	192	215	182	117	102	42	39	0.97	38	0.55	105	37	44	27	27
27	73352	60	1	180	90	150	90	75	75	1.62	28.57	28.57	108	108	111	111	0.97	0.97	128	124	178	173	8.9	8.7	180	176	189	166	102	99	44	41	0.91	32	0.39	94	26	42	30	30
28	3605	60	0	36	88	150	80	60	61	1.6	23.43	23.82	88	88	90	90	0.97	0.97	173	129	194	167	8.8	8.7	233	229	231	198	129	113	31	32	0.89	29	0.34	103	27	33	27	28
29	158945	60	0	60	92	150	100	49	50	1.56	20.13	20.54	83	83	85	85	0.97	0.97	170	156	289	204	9	8.9	208	191	213	193	114	109	45	48	0.76	33	0.44	107	34	46	27	27
30	159204	60	0	240	88	140	90	60	62	1.58	24.03	24.83	94	94	99	99	0.94	0.94	204	176	235	209	8.3	8.2	226	221	224	214	123	118	48	43	0.71	36	0.36	99	38	31	28	28
31	159195	40	1	12	82	120	80	95	95	1.74	31.37	31.37	107	107	110	110	0.97	0.97	198	173	245	203	9.4	9.3	234	211	198	151	84	80	39	37	0.83	31	0.52	119	41	28	30	30

Master Chart: Metformin + Glimepiride + Omega 3 Fatty acids

Sl No	OP No	Age(years)	Gender	Duration of DM (months)	Pulse Rate(bpm)	SBP(mm/Hg)	DBP(mm/Hg)	Weight(kg)		Height (m)	BMI(kg/m^2)		Waist Circumference (cm)		Hip Circumference (cm)		WHR		FBS (mg/dl)		PPBS (mg/dl)		HbA1c (%)		TC (mg/dl)		TG (mg/dl)		LDL (mg/dl)		HDL (mg/dl)		S. Creatinine (mg/dl)	B.Urea (mg/dl)	Total bilirubin (mg/dl)	Alkaline phosphatase (IU/L)	SGOT(IU/L)	SGPT(IU/L)	MMSE	
								Baseline	12 weeks		Baseline	12 weeks	Baseline	12 weeks	Baseline	12 weeks	Baseline	12 weeks	Baseline	12 weeks	Baseline	12 weeks	Baseline	12 weeks	Baseline	12 weeks	Baseline	12 weeks	Baseline	12 weeks	Baseline	12 weeks							Baseline	12weeks
1	981585	53	0	36	80	160	90	97	98	1.67	34.78	35.13	123	124	128	129	0.96	0.96	205	192	340	327	9.6	9.5	175	196	173	189	136	128	44	48	0.72	33	0.6	106	34	31	30	30
2	13896	60	0	36	82	130	80	50	51	1.59	19.77	20.17	89	89	93	93	0.95	0.95	117	112	280	264	8.6	8.3	219	188	182	167	140	137	43	49	0.9	24	0.45	112	38	31	30	30
3	2095	54	1	6	84	130	80	80	78	1.68	28.34	27.63	110	110	117	117	0.94	0.94	140	134	196	163	7	7.1	208	215	188	191	104	108	48	57	0.8	32	0.41	128	24	29	30	30
4	5511	55	0	36	80	100	60	48	49	1.55	19.97	20.39	75	76	81	81	0.92	0.93	132	147	232	178	9.9	8.3	209	218	213	219	108	89	49	46	0.9	29	0.36	146	30	32	28	28
5	78890	55	1	48	84	120	80	69	68	1.6	26.9	26.56	93	93	97	97	0.95	0.95	131	124	194	163	7.8	7.2	226	196	207	184	99	101	39	41	0.67	33	0.29	98	28	41	30	30
6	87636	45	0	12	86	120	80	79	78	1.65	29.01	28.65	93	93	110	110	0.84	0.84	126	118	224	182	7.5	8.3	186	174	178	145	109	103	46	38	0.9	27	0.4	123	31	28	30	30
7	566289	40	1	1	86	186	100	72	71	1.59	28.47	28.08	94	94	101	101	0.93	0.93	135	121	224	164	7.9	7.9	193	188	189	154	105	114	41	37	0.8	36	0.63	98	39	33	25	27
8	45436	50	0	12	72	120	80	50	50	1.44	24.11	24.11	91	91	94	94	0.96	0.96	172	137	225	186	8.2	7.8	218	225	194	181	117	109	39	43	0.8	24	0.43	105	33	36	27	27
9	71459	56	0	8	72	130	90	85	84	1.54	35.84	35.41	110	110	114	114	0.96	0.96	133	119	171	162	7.3	7.6	227	214	176	145	112	101	45	45	1	25	0.67	111	37	42	28	28
10	17734	50	0	72	80	140	90	76	76	1.52	32.89	32.89	106	106	108	108	0.98	0.98	129	138	201	218	8.5	8.1	191	203	193	172	106	96	35	39	0.9	28	0.42	101	35	38	28	28
11	82358	40	1	12	84	130	90	70	70	1.65	25.71	25.71	99	99	102	102	0.97	0.97	162	124	216	179	9.2	8.5	179	153	169	152	101	108	38	42	0.8	41	0.38	83	42	37	27	27
12	84890	47	0	96	84	140	90	53	54	1.6	20.7	21.09	87	88	95	96	0.91	0.91	228	196	378	241	9.7	9.1	197	175	195	163	116	93	47	41	0.9	28	0.45	97	33	40	27	27
13	42551	60	1	60	72	130	70	65	64	1.53	27.76	27.33	98	98	101	101	0.97	0.97	143	127	229	193	8.6	8.2	188	197	177	169	127	118	45	40	0.7	34	0.67	101	27	30	28	28
14	17777	36	0	96	80	130	90	49	48	1.57	19.87	19.47	87	87	94	94	0.92	0.92	118	109	292	174	8.4	7.8	225	216	204	173	113	106	49	44	0.7	31	0.8	89	36	39	30	30
15	95904	60	0	6	78	110	80	53	54	1.54	22.34	22.76	93	93	97	97	0.95	0.95	187	125	200	183	9.2	8	194	184	109	124	102	117	46	42	0.8	25	0.42	96	41	44	30	30
16	100067	50	0	60	78	130	80	85	86	1.6	33.2	33.59	103	104	107	108	0.96	0.96	206	188	319	263	9.6	9.1	239	235	218	206	143	123	37	35	0.91	31	0.6	101	26	37	29	29
17	98872	45	1	2	78	120	90	65	65	1.55	27.05	27.05	102	102	105	105	0.97	0.97	189	168	286	237	8.9	8.2	183	167	178	125	125	119	36	38	0.77	22	0.56	109	44	28	28	28
18	814450	36	0	48	80	140	80	58	58	1.53	24.77	24.77	90	90	94	94	0.95	0.95	135	118	196	170	8.1	7.9	201	184	197	187	106	109	38	32	0.54	26	0.36	108	28	34	28	28
19	75973	60	0	60	78	140	80	46	46	1.6	17.96	17.96	88	88	92	92	0.95	0.95	147	175	185	236	8.4	8.8	196	183	195	173	94	116	42	39	0.82	36	0.58	97	35	36	25	27
20	30971	46	1	84	74	130	90	57	57	1.58	22.83	22.83	90	90	93	93	0.96	0.96	201	186	356	264	9.5	9.1	219	193	235	216	122	105	36	39	0.78	28	0.51	98	42	38	30	30
21	112093	60	1	120	78	130	80	80	79	1.7	27.68	27.33	105	105	107	107	0.98	0.98	184	156	293	257	9	8.6	228	206	170	223	104	108	45	41	0.85	37	0.44	103	32	30	29	29
22	87827	46	1	96	78	140	90	64	63	1.67	22.94	22.58	92	91	94	94	0.97	0.96	102	121	161	182	7.1	7.4	210	189	209	217	127	93	33	35	0.8	33	0.63	99	41	43	30	30
23	22867	55	1	36	80	150	80	87	87	1.52	37.65	37.65	114	114	117	117	0.97	0.97	167	145	242	193	8.9	8.3	181	196	214	173	116	109	49	46	0.71	26	0.37	98	26	28	28	28
24	28637	60	0	24	80	140	80	64	64	1.55	26.63	26.63	98	98	101	101	0.97	0.97	128	116	159	153	7.7	7.6	194	209	173	178	129	103	47	52	0.7	35	0.61	106	32	35	27	27
25	120703	50	0	3	78	130	90	77	78	1.44	37.13	37.61	110	110	115	115	0.95	0.95	138	154	289	205	8.5	8.1	238	212	234	195	136	118	36	38	0.91	29	0.65	109	33	36	28	28
26	83835	56	0	36	80	130	80	67	67	1.53	28.62	28.62	102	102	106	106	0.96	0.96	122	137	173	195	7.8	8	236	176	213	181	99	106	36	41	0.7	28	0.38	90	39	24	28	28
27	148575	60	0	60	78	140	80	52	53	1.46	24.39	24.86	97	97	102	102	0.95	0.95	145	127	201	183	8.1	7.9	193	183	208	184	101	117	35	39	0.73	35	0.54	95	40	42	30	30
28	164918	55	1	84	80	130	90	78	79	1.54	32.88	33.31	106	106	108	108	0.98	0.98	131	124	179	162	7.6	7.5	189	197	196	163	105	114	46	42	0.85	24	0.41	102	27	36	28	28
29	165144	55	0	96	102	140	80	80	79	1.56	32.87	32.46	108	108	111	111	0.97	0.97	165	145	223	193	8.3	7.8	195	209	189	154	134	118	37	35	0.98	31	0.39	112	35	29	27	27

Master Chart: Metformin + Glimepiride

Sl No	OP No	Age(years)	Gender	Duration of DM (months)	Pulse Rate(bpm)	SBP(mm/Hg)	DBP(mm/Hg)	Weight(kg)		Height (m)	BMI(kg/m^2)		WC(cm)		HC(cm)		WHR		FBS (mg/dl)		PPBS (mg/dl)		HbA1c (%)		TC (mg/dl)		TG (mg/dl)		LDL (mg/dl)		HDL (mg/dl)		S. Creatinine (mg/dl)	B.Urea (mg/dl)	Total bilirubin (mg/dl)	Alkaline phosphatase (IU/L)	SGOT(IU/L)	SGPT(IU/L)	MMSE	
								Baseline	12 weeks		Baseline	12 weeks	Baseline	12 weeks	Baseline	12 weeks	Baseline	12 weeks	Baseline	12 weeks	Baseline	12 weeks	Baseline	12 weeks	Baseline	12 weeks	Baseline	12 weeks	Baseline	12 weeks	Baseline	12 weeks							Baseline	12weeks
1	78939	60	0	120	80	130	80	62	62	1.53	26.48	26.48	98	98	111	111	0.88	0.88	132	144	210	243	9.9	10.3	226	208	219	196	140	132	42	45	0.9	28	0.3	95	23	33	30	30
2	1008016	60	1	120	82	130	70	60	60	1.67	21.51	21.51	94	94	96	96	0.97	0.97	154	146	236	256	8.1	9	203	196	187	178	101	109	45	44	1	29	0.6	112	36	31	30	30
3	16450	47	1	36	84	120	80	70	71	1.61	27	27.39	97	98	101	101	0.96	0.97	129	117	226	193	7.1	8.3	215	189	233	224	132	76	37	39	0.82	31	0.6	132	38	29	30	30
4	768	60	0	12	80	140	90	62	63	1.42	30.74	31.24	98	98	113	113	0.86	0.86	129	137	236	194	8.2	7.8	227	211	189	178	137	106	45	41	1	32	0.4	124	29	24	28	28
5	20577	48	0	6	82	120	80	72	73	1.63	27.09	27.47	96	96	103	103	0.93	0.93	145	132	234	189	8.4	7.8	231	221	213	209	114	106	44	41	0.9	28	0.5	104	30	36	30	30
6	1000983	60	0	180	80	130	90	74	74	1.62	28.19	28.19	101	101	109	109	0.92	0.92	197	171	288	194	10.5	9.1	194	209	189	206	116	135	37	35	0.7	28	0.32	106	31	43	25	27
7	78482	40	0	60	86	120	80	55	55	1.58	22.03	22.03	95	95	107	107	0.88	0.88	142	114	289	167	9.3	8.2	206	186	178	167	102	95	36	31	0.9	33	0.6	96	33	27	28	28
8	63377	55	1	6	90	160	90	61	62	1.57	24.74	25.15	91	91	95	96	0.95	0.95	167	136	245	183	9.4	7.9	234	252	189	192	98	88	36	39	1	29	0.5	121	28	53	27	27
9	73319	37	0	2	80	130	90	65	66	1.48	29.67	30.13	103	103	111	111	0.92	0.93	286	188	377	231	9.7	8.8	195	209	216	194	99	87	41	44	1	36	0.53	105	32	39	28	28
10	74003	52	1	96	100	180	100	78	78	1.56	32.05	32.05	110	110	113	113	0.97	0.97	171	153	248	189	8.9	8.3	204	221	186	164	116	108	40	47	0.72	32	0.34	113	36	34	27	27
11	82153	60	1	300	84	130	90	62	62	1.67	22.23	22.23	93	93	97	97	0.95	0.95	167	128	308	177	9.1	8.9	192	178	196	192	113	97	31	36	0.8	22	0.35	95	38	47	28	28
12	3788	60	1	60	74	130	70	64	64	1.52	27.7	27.7	91	91	93	93	0.97	0.97	198	171	256	216	9	8.1	207	267	188	236	128	106	39	42	0.98	29	0.31	103	41	42	27	28
13	29745	44	1	120	74	130	90	72	72	1.68	25.51	25.51	102	102	104	104	0.98	0.98	127	109	156	164	8.4	7.8	213	238	213	202	98	93	38	85	0.7	41	0.4	101	28	35	27	28
14	24754	60	0	240	80	120	80	47	47	1.58	18.82	18.82	96	96	100	100	0.96	0.96	252	217	396	215	8.3	7.6	188	209	198	186	109	98	41	37	0.86	35	0.4	95	20	41	27	27
15	56398	46	0	48	86	120	80	53	53	1.58	21.23	21.23	97	97	103	103	0.94	0.94	187	161	253	198	8	7.5	210	202	194	187	102	110	36	39	0.99	24	0.6	129	41	38	30	30
16	75573	50	1	60	78	150	80	82	82	1.56	33.69	33.69	102	102	105	105	0.97	0.97	112	135	131	176	8.1	7.8	189	182	212	198	111	86	44	43	0.87	26	0.3	116	41	37	30	30
17	91487	60	1	60	78	130	80	68	68	1.62	25.91	25.91	96	96	101	101	0.95	0.95	133	102	199	153	8.3	7.9	176	165	176	148	98	99	41	38	0.83	28	0.4	97	36	46	30	30
18	87279	40	1	48	80	140	110	68	67	1.72	22.9	22.64	96	96	101	100	0.95	0.96	117	106	189	146	8.2	6.9	232	221	231	215	103	95	38	40	0.96	25	0.6	95	26	31	30	30
19	60692	65	0	6	78	130	90	55	56	1.48	25.1	25.56	97	97	103	103	0.94	0.94	146	121	241	165	7.6	7.1	216	227	189	178	99	127	39	37	0.66	29	0.1	118	34	33	28	28
20	112262	40	0	1	84	140	80	63	64	1.56	25.88	26.29	101	101	106	106	0.95	0.95	113	97	152	149	7.8	7.2	201	194	218	225	136	108	43	38	0.95	31	0.45	99	28	33	30	30
21	96887	59	1	24	90	130	80	74	74	1.51	32.45	32.45	99	99	104	104	0.95	0.95	112	128	219	178	8.4	8.1	188	181	193	208	107	86	39	41	0.69	29	0.43	95	39	36	28	28
22	129427	60	1	8	84	130	90	60	60	1.56	24.65	24.65	93	93	95	95	0.97	0.97	276	235	336	180	10.3	9.4	197	159	194	167	103	96	37	34	0.89	31	0.56	116	38	30	30	30
23	136463	60	1	4	80	130	80	45	48	1.6	17.57	18.75	88	89	91	92	0.96	0.96	281	230	426	242	8.3	8.5	222	201	226	196	129	108	40	37	0.66	20	0.47	88	35	41	27	27
24	137472	48	0	3	78	130	80	52	54	1.56	21.36	22.18	97	98	100	100	0.97	0.98	132	118	219	196	8.2	7.9	199	263	215	241	133	117	33	43	0.7	30	0.42	117	37	43	28	28
25	88783	52	1	120	80	120	70	75	75	1.73	25.05	25.05	101	104	104	104	0.97	0.97	109	117	190	153	7.3	7.1	209	186	196	182	101	98	36	39	0.9	31	0.54	121	24	37	27	27
26	148273	60	1	60	84	130	80	67	67	1.6	26.17	26.17	99	103	103	103	0.96	0.96	165	113	240	183	8.6	7.8	205	186	194	187	126	108	38	40	1	38	0.43	95	43	28	30	30
27	39391	50	1	48	80	140	90	96	96	1.77	30.64	30.64	108	108	111	111	0.97	0.97	132	102	209	178	9.1	8.9	218	189	231	213	119	108	41	38	0.8	36	0.37	98	46	45	28	28

Key words: Sl. No- serial number, OP No- Outpatient number, Gender-0=female, 1=male, DM- Diabetes mellitus, bpm-beats per minute, SBP- systolic blood pressure, DBP- diastolic blood pressure, BMI- body mass index, WC- waist circumference, HC- hip circumference, WHR- waist-hip ratio, FBS- fasting blood sugar, PPBS- post prandial blood pressure, HbA1c- glycated hemoglobin, TC- total cholesterol, TG- triglycerides, LDL- low density lipoprotein cholesterol, HDL- high density lipoprotein cholesterol, SGOT- serum glutamic oxaloacetic transaminase, SGPT- serum glutamic-pyruvate transaminase MMSE- mini mental state examination