TO COMPARE THE ANTI-INFLAMMATORY EFFECT OF ORAL HYPOGLYCEMIC DRUGS IN TYPE 2 DIABETES MELLITUS

DISSERTATION

SUBMITTED FOR

M.D. IN PHARMACOLOGY

THE TAMILNADU Dr. M.G.R MEDICAL UNIVERSITY

DEPARTMENT OF PHARMACOLOGY

PSG INSTITUTE OF MEDICAL SCIENCES & RESEARCH

PEELAMEDU, COIMBATORE- 641 004

TAMILNADU, INDIA

APRIL 2015

PSG INSTITUTE OF MEDICAL SCIENCES & RESEARCH

COIMBATORE

CERTIFICATE

This is to certify that this dissertation entitled **"TO COMPARE THE ANTI-INFLAMMATORY EFFECT OF ORAL HYPOGLYCEMIC DRUGS IN TYPE 2 DIABETES MELLITUS"**, is a work done by **Dr.A.UMAMAHESWARI,** Postgraduate under the guidance of **Dr.K.BHUVANESWARI, M.D.,** Professor and Head, Department of Pharmacology, PSG IMSR.

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March 18, 2013

To
Dr A Umamaheswari
Postgraduate
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Coimbatore

Ref.: Proposal titled: *'To compare the anti-inflammatory effect of oral hypoglycemic drugs in type 2 diabetes mellitus'*

Sub.: Ethics Committee Approval for the study

The Institutional Human Ethics Committee, PSG IMS & R, Coimbatore -4, has reviewed your proposal on 29th January, 2013 in its full board review meeting held at College Council Room, PSG IMS&R, between 2.00 pm and 5.00 pm, and discussed your application to conduct the study entitled:

"To compare the anti-inflammatory effect of oral hypoglycemic drugs in type 2 diabetes mellitus"

The following documents were received for review:

1. Duly filled application form
2. Proposal
3. Informed Consent forms in English and Tamil
4. Case report form
5. CV
6. Budget

The members who attended the meeting at which your study proposal was discussed are as follows:

Sl. No.	Name of the Member of IHEC	Qualification	Area of Expertise	Gender	Affiliation to the Institution Yes/No	Present at the meeting Yes/No
1	Dr. S. Bhuvaneshwari (Member-Secretary, IHEC)	MD	Clinical Pharmacology	Female	Yes	Yes
2	Mrs R. Geetha	+ 2	Lay person	Female	No	Yes
3	Mr Gowpathy Velappan	BA., BL	Legal Advisor	Male	No	Yes
4	Mrs G Malarvizhi	M Sc	Nursing	Female	Yes	No
5	Mr. R. Nandakumar (Vice-Chairperson, IHEC)	BA., BL	Legal Expert	Male	No	Yes
6	Dr. G. Rajendiran	DM	Clinician (Cardiology)	Male	Yes	No
7	Dr. V. Ramamurthy	Ph D	Biotechnology	Male	Yes	Yes

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8	Dr. M. Ramanathan	M Pharm, Ph D	Non-Medical (Pharmacy)	Male	Yes	No
9	Dr. P. Sathyan (Chairperson, IHEC)	DO, DNB	Clinician (Opthalmology)	Male	No	Yes
10	Dr. Seetha Panicker	MD	Clinician (Obstetrics & Gyanaecology)	Female	Yes	Yes
11	Dr. S. Shanthakumari	MD	Pathology, Ethicist	Female	Yes	Yes
12	Dr. Y.S. Sivan	Ph D	Social Scientist (Sociology)	Male	Yes	Yes
13	Dr. Sudha Ramalingam (Alternate Member-Secretary, IHEC)	MD	Public Health, Epidemiology, Genetics, Ethicist	Female	Yes	Yes
14	Mrs. K. Uma Maheswari	M Sc, M Phil. B Ed	Botany	Female	No	Yes
15	Dr. D. Vijaya	M Sc, Ph D	Basic Medical Sciences (Biochemistry)	Female	Yes	Yes

After due consideration, the committee has decided to approve the above proposal.

The approval is valid for one year.

We request you to intimate the date of initiation of the study to IHEC, PSG IMS&R and also, after completion of the project, please submit completion report to IHEC. Please submit the CTRI number to the IHEC immediately on receipt of the same.

We hereby confirm that neither you nor any of your study team members have participated in the voting/ decision making procedure of the committee. The members of the committee who have participated in the voting/ decision making procedure of the committee do not have any conflict of interest in the referenced study.

This Ethics Committee is organized and operates according to Good Clinical Practice and Schedule Y requirements.

Non-adherence to the Standard Operating Procedures (SOP) of the Institutional Human Ethics Committee (IHEC) and national and international ethical guidelines shall result in withdrawal of approval (suspension or termination of the study). SOP will be revised from time to time and revisions are applicable prospectively to ongoing studies approved prior to such revisions.

PIs are required to send progress reports (in the form of an extended abstract with publications if any) to the IHEC every six months (and a month before expiry of approval date, if renewal of approval is being sought).

Request for renewal must be made at least a month ahead of the expiry of validity along with a copy of the progress report.

18.3.13

Dr S Bhuvaneshwari
Member - Secretary
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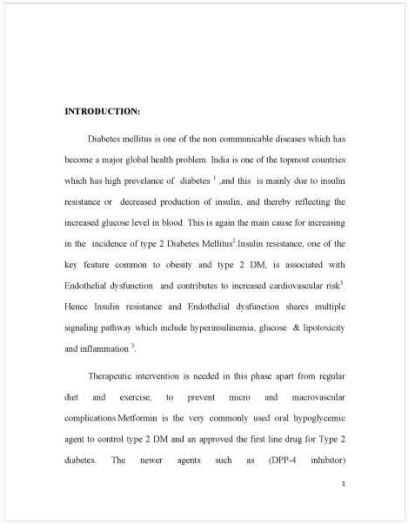

INTRODUCTION:

Diabetes mellitus is one of the non communicable diseases which has become a major global health problem. India is one of the topmost countries which has high prevelance of diabetes [1] ,and this is mainly due to insulin resistance or decreased production of insulin, and thereby reflecting the increased glucose level in blood. This is again the main cause for increasing in the incidence of type 2 Diabetes Mellitus[2].Insulin resistance, one of the key feature common to obesity and type 2 DM, is associated with Endothelial dysfunction and contributes to increased cardiovascular risk[3]. Hence Insulin resistance and Endothelial dysfunction shares multiple signaling pathway which include hyperinsulinemia, glucose & lipotoxicity and inflammation [3].

Therapeutic intervention is needed in this phase apart from regular diet and exercise, to prevent micro and macrovascular complications.Metformin is the very commonly used oral hypoglycemic agent to control type 2 DM and an approved the first line drug for Type 2 diabetes. The newer agents such as (DPP-4 inhibitor)

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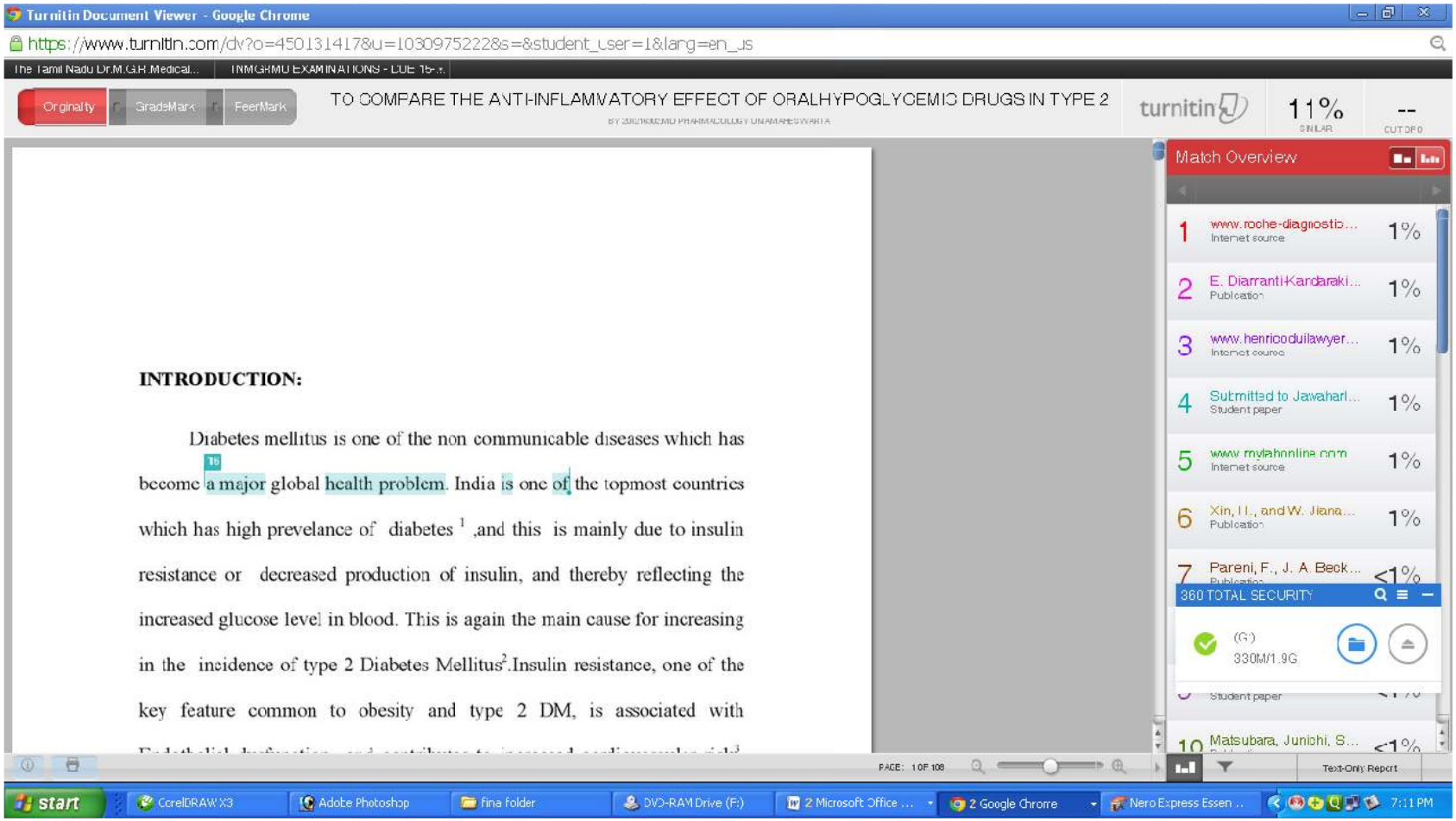
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I also thank my family members and special mention to my husband and father for the moral support they have rendered me throughout the study.

CONTENTS

Title: To Compare The Anti-Inflammatory Effect Of Oral hypoglycemic Drugs In Type 2 Diabetes Mellitus

Aim: To compare the anti-inflammatory effects of Metformin sulfonylurea and Sitagliptin combination with Metformin Sulfonylurea and Acarbose combination in Type2DM patients by using Anti-inflammatory markers (IL6, hs CRP) and also To compare the clinical outcome between these two groups by using the following parameters FBS, PPBs, HbA1C, Plasma Insulin.

Materials and Method : In this study 30 type 2 diabetes patients on Metformin and Sulfonylurea combination ,HbA1c value >7.5 were recruited and randomized into two groups in which one group was added on Acarbose and the other group was added with Sitagliptin along with Metformin, Sulfonylurea combinations and followed for 3 months.

Result :

In this study Sitagliptin combination reduced the mean value of FBS, PPBS, HbA1c, Plasma Insulin , Insulin Resistance ,hsCRP and IL-6 , which were similar to the results of previous studies. In Acarbose combination, there was a reduction in the mean values of FBS,PPBS, HbA1c, Plasma Insulin, Insulin Resistance, hsCRP but not IL-6. Hence long term follow up and large sample size may be required to see the progression in the reduction of inflammatory markers and the role of these drugs in the insulin resistant states.

Conclusion : This study explained the synergism when Sitagliptin given along with Metformin Sulfonylurea combination and has proved a definite role in reducing chronic inflammation.

INTRODUCTION:

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Therapeutic intervention is needed in this phase apart from regular diet and exercise, to prevent micro and macrovascular complications. Metformin is the very commonly used oral hypoglycemic agent to control type 2 DM and approved as the first line drug for Type 2 diabetes. The newer agents such as (DPP-4 inhibitor) Sitagliptin, Vildagliptin, Saxagliptin, Linagliptin have been recently recommended by ADA in the therapeutic intervention of Diabetes Mellitus.

Gliptins are very effective in raising the insulin level by increasing the availability of Incretin and decrease the level of glucagon and thereby

reduce the plasma glucose concentration[2].Hence used as an additive therapy in type 2 DM. Recently studies show that Gliptins have been proved to exert an anti-inflammatory action[4]. Acarbose, α-glucosidase inhibitor helps in reducing the postprandial hyperglycemia and has its effects towards the reduction of inflammatory markers. Metformin also has anti inflammatory action apart from its action in reducing the blood glucose level. Hence, the study objective is to compare the anti-inflammatory effects of these oral hypoglycemic agents in Diabetes Mellitus.

OBJECTIVES OF THE STUDY

PRIMARY OBJECTIVE:

To compare the anti-inflammatory effects of Metformin sulfonylurea and Sitagliptin combination with Metformin Sulfonylurea and Acarbose combination in Type2DM patients by using Anti-inflammatory markers (IL6, hs CRP)

SECONDARY OBJECTIVE :

To compare the clinical outcome between these two groups by using the following parameters

- FBS, PPBS
- HbA1C
- Plasma Insulin

REVIEW OF LITERATURE

Prevalence of type II DM is increasing worldwide. By 2010, it was found that 285 million people around world have diabetes, of which 80% are from developed countries areas. Among those aged 20-79 years 6.6% have diabetes globally. The region with highest prevalence rate, at 11.7%, is North America and the Caribbean. The largest increase will be in countries like India and China where there is practice of increase consumption of high energy food, increasing adoption of sedentary lifestyle and urbanization. The prevalence of diabetes is expected to reach about 438 million by 2030, an increase of 54% compared to predicted figures of 2010[5].

Currently India has more number of people with diabetes mellitus. With a prevalence of 40.9 million in 2007, which may rise to 69.9 million by 2025[6].

A Survey was carried out in six cities in 2001on age –standardized prevalence rate, of which there was incidence of 12% for diabetes and 14% for IGT. Patients under 40 years had prevalence of 5% and 13%of diabetes and IGT respectively[7].

A recent study conducted by Chennai Urban Rural Epidemiology Study (CURES-17) reported that prevalence of diabetes was found to be

14.3%[8]. Another secular trend is the shift towards younger onset of diabetes, especially in urban areas, where up to 36% of those with diabetes are aged 44 years or less [8,9].

The Chennai Population Study (CUPS) has revealed the incidence of diabetes to be 20.2 cases per 1000 person-year and that of pre-diabetes was found to be 13.1 per 1000 person –year [10].

Identification of high risk and awareness should be increased among the public, because lifestyle modification has been shown to be effective in reducing progression from IGT to diabetes in Indian population [11].

Type 1 Diabetes (β-cell destruction)

- Most commonly Autoimmune
- Rarely Idiopathic

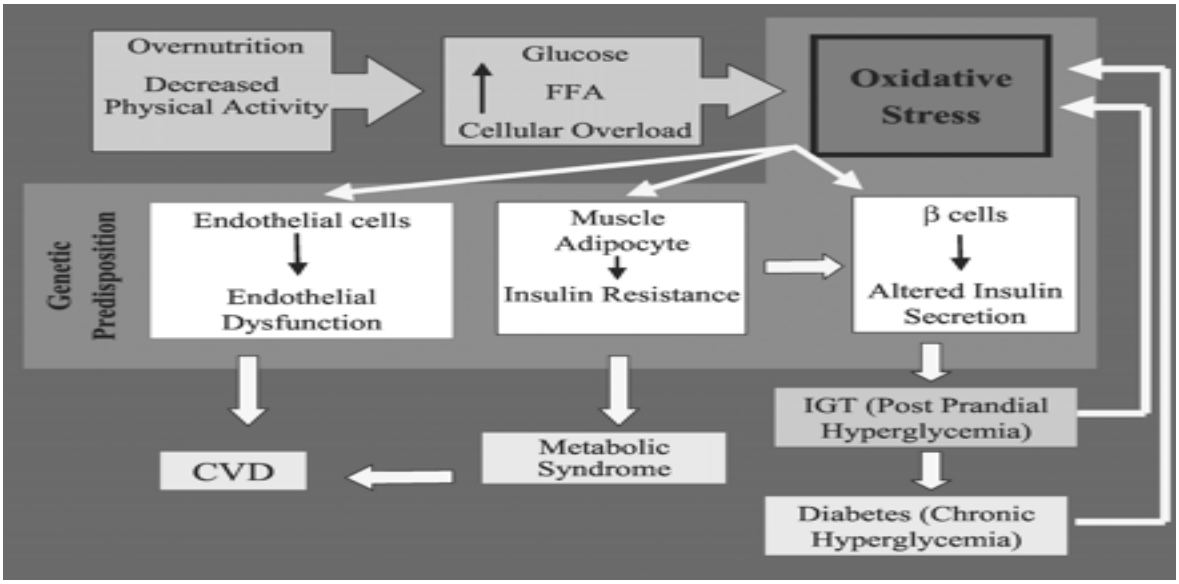
Type 2 Diabetes (insulin hypo secretion with insulin resistance)

Other types

- Various endocrinopathies
- Drug induced
- Chemical induced
- Infections

There are many risk factors associated with increased incidence of T2DM. These include age, dietary excess, sedentary lifestyle dietary factors such as increase intake of animal fats, carbonated drinks, history of gestational diabetes, PCOS, several mental illness, hyperlipidemia, Obesity Positive family history, presence of Hypertension. The cluster of some of these factors like high blood pressure, increased blood glucose, high triglyceride, decreased HDL and obesity is termed as Metabolic Syndrome. These risk factors are also due to westernization and urbanization [12].

Figure.1:Influence of risk factors on Diabetes



Obesity has 80-85% risk of developing T2DM [13]. Central adiposity is linked with insulin resistance and improper function of β cell, through high level of free fatty acids also linked with dyslipidemia and high BP.

Metabolic syndrome has risk as high as 2-5 folds for developing Type 2 DM [14].

Recent studies have shown that sleep duration of about 5hrs or less leads to 47% increase in the incidence of diabetes over a period of 10 years [15].Underlying mechanism between decreased sleep and onset of diabetes is not clear, but still it may be related to activation of the sympathetic nervous system, decrease cerebral glucose utilization, changes in hypothalamic –pituitary-adrenal axis as well as other neuroendocrine deregulation [15].

Some commonly used medications may cause various metabolic changes and high risk of diabetes [16]. Usage of high dose Thiazide diuretics are known to aggravate insulin resistance and β blockers can affect the insulin secretion. Atypical antipsychotics, have found to cause hyperglycemia and diabetes[17]. Increasing use of highly effective antiretroviral therapy (HAART)has reduced the mortality of HIV patients however it also causes impaired glucose levels, resistance to insulin and lipid metabolism with more risk for Type 2 DM [18].

Environmental toxins like persistent chlorinated compounds and brominated flame retardants have association with diabetes. These substances get accumulated in adipose tissue and functions like endocrine disruptors, which causes improper regulation of lipid and glucose

metabolism [19, 20]. Maternal undernutrition, low infant birth weight, along with rapid postnatal growth, is found to be linked with high risk of diabetes in the child. This mismatch is found to be programmed during intra-uterine development and the nutritionally rich postnatal environment. Offspring of women who are obese or with diabetes have an increased risk of diabetes [21, 22]. Hence earlier life events like low birth weight and fetal malnutrition may also have its effects in causing diabetes and cardiovascular disease in later life [23].

INSULIN SECRETION

Pancreatic islet is a vascularized, highly innervated organ that contain four endocrine cell types secreting different hormone

- Alpha(α) cells – glucagon
- Beta(β) cells – glucose
- Delta(δ) cell - Somatostatin, and
- Eeta(ε) cells that secrete ghrelin.

Insulin and glucagon are important pharmacological agents in the treatment of diabetes.

Insulin normally formed as single polypeptide known as preproinsulin,then processed to proinsulin and then to insulin and C-peptide. These processes take place in golgi complex, endoplasmic

reticulum and importantly the distinctive secretory granules of the β cell. Insulin has a t1/2 of 5-6 minutes due to extensive hepatic clearance [24].

C-peptide in contrast with no known physiological function or receptor has a $t_{1/2}$ of ~30 minutes. Because almost all of the C-peptide released into the portal vein reaches the peripheral circulation where it can be measured, this peptide is useful in assessment of β-cell secretion, and to differentiate endogenous and exogenous hyperinsulinemia.

REGULATION OF INSULIN SECRETION

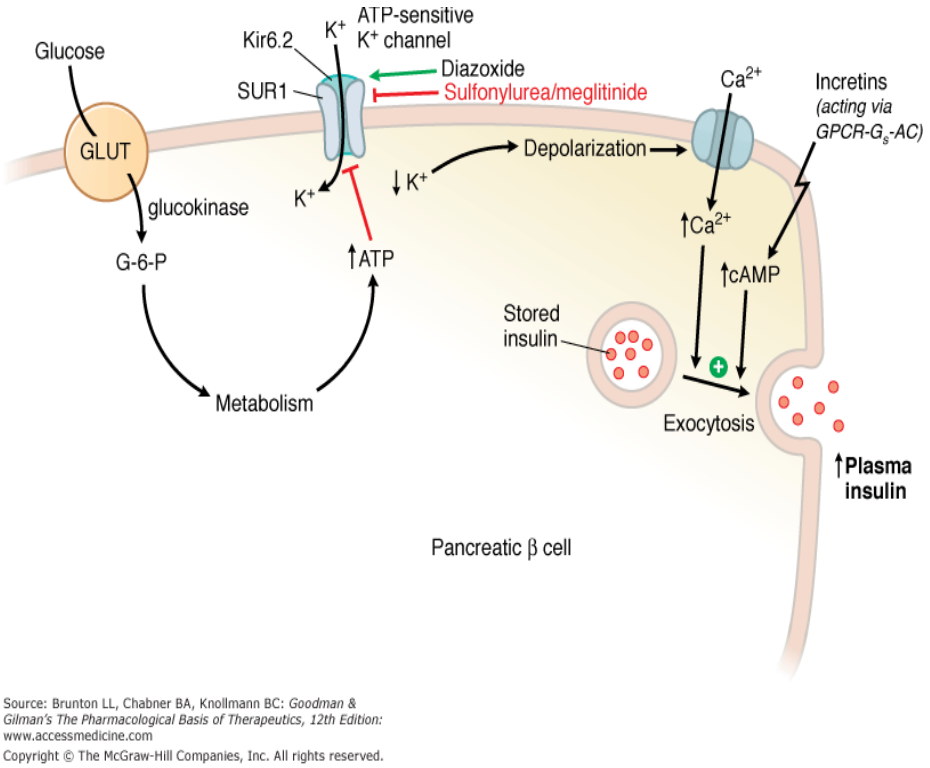
The main secretagogue of insulin is glucose, and secretion of insulin is tightly associated to the extracellular glucose concentration. Insulin secretion is much greater when the similar amount of glucose is delivered orally compared to intravenously (incretin effect). Islets are mainly supplied by adrenergic, cholinergic nervous system.

- α_2 adrenergic stimulation - inhibits secretion of insulin
- β_2 adrenergic receptor agonists,vagal nerve stimulation - enhance release.
- Glucagon and somatostatin - inhibit insulin secretion.

Conditions such as hypoglycemia, hypoxia, exercise, surgery, hypothermia, and severe burns decreases the insulin secretion by stimulation of α_2 receptors. Hence, α_2 adrenergic antagonists increase

basal insulin concentration, and β_2 antagonists decrease plasma insulin concentration [24].

Figure .2: Insulin secretion



During resting state of pancreatic cell it is hyperpolarized. Glucose, entering via GLUT transporters (primarily GLUT1 in humans, GLUT2 in rodents), is metabolized(phosphorylated by glucokinase) increases glucose stimulated insulin secretion.Glucose-6-phosphate enters the glycoltic pathway produces NADP and elevates cellular ATP, this further inhibits K^+ entry by K_{ATP} channel, reduced K^+ conductance ends in depolarization, which leads to Ca^{2+}-dependent exocytosis of insulin from secretory granules. The K_{ATP} channel, composed of SUR1 and Kir 6.2

subunits, is the point of action of several group of drugs: ATP binds and inhibits Kir 6.2 whereas meglitinides, sulfonylureas binds as well as inhibits SUR1; all these three agents thereby promote insulin secretion.

These intracellular events are regulated by a number of processes, such as alteration in cAMP production, metabolism of amino acid and transcription factor levels. GPCRs for Gastro Intestinal Peptide, Glucagon, and Glucagon like Peptide-1 attaches to G_s stimulating adenylylcyclase and insulin secretion; receptors for somatostatin and α_2 adrenergic agonists couple to G_i to reduce cellular cAMP production and secretion. Incretins enhance insulin secretion. Diazoxide and ADP-Mg^{2+} (low ATP) binds as well as activates SUR1, thereby inhibiting secretion of insulin [24].

INSULIN ACTION AND ITS SIGNALING PATHWAY

Insulin binds to the receptor that activates downstream signaling. Insulin binding stimulates tyrosine kinase activity of dimer, results in tyrosine phosphorylation and specific substrates like the Insulin Receptor Substrate (IRS) proteins, Gab-1 and SHC; within the membrane, a caveolar pool of insulin receptor phosphorylates caveolin (Cav), Adaptor protein having PH and SH2 domains(APS), and Cbl -protein.

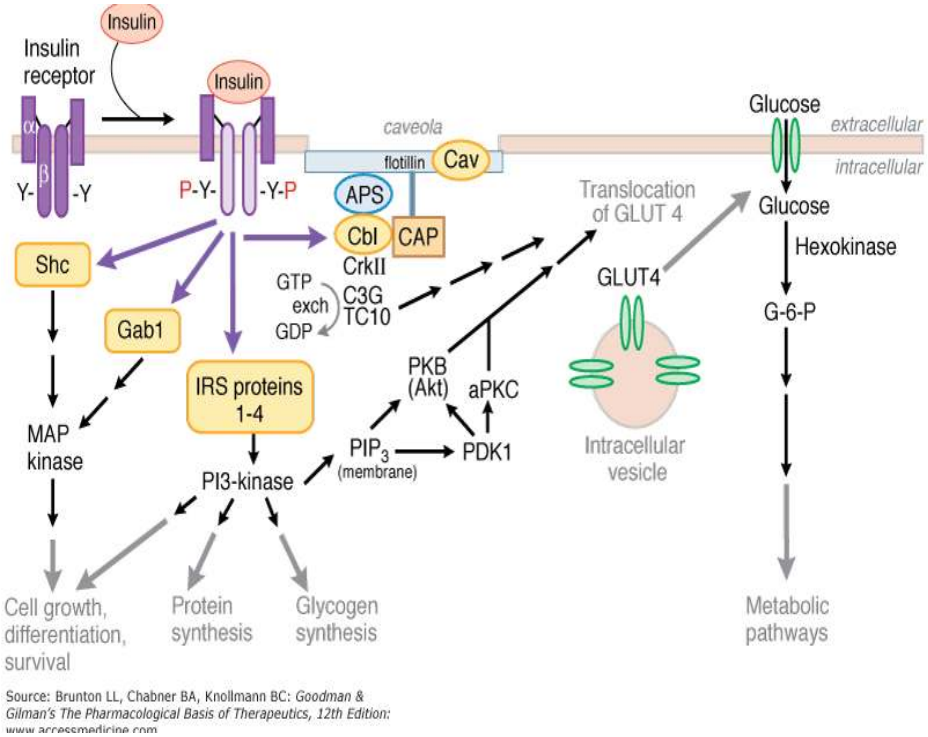


Figure .3: Insulin signaling pathway and its action.

These tyrosine-P proteins react with signaling cascade through SH2 and SH3 and mediate effects of insulin.Grb-2 associated binder (Gab-1) and SHC, within the membrane activates the following signals and activates mitogen-activated protein kinase (MAP kinase) pathway there by it produces cell growth,differentiation and survival.

In target tissues such as skeletal muscle and adipocytes, a key event is the translocation of the GLUT4 from vesicles to the plasma membrane. This translocation is stimulated by both the caveolar and non-caveolar pathways. In the non-caveolar pathway, the activation of PI3K is crucial, and PKB/Akt (anchored at the membrane by PIP3) and/or an atypical

form of PKC is involved. IRS proteins are also involved in protein synthesis and glycogen synthesis[24].

In the caveolar pathway, caveolar protein flotillin localizes the signaling complex to the caveola; the signaling pathway involves series of SH2 domain interactions that add the adaptor protein chicken tumor virus regulator of kinase II (CrkII,) C3G, and GTP-binding protein, TC10.

GLUT4 is present in insulin-responsive tissues like skeletal muscle, adipose tissue that forms main sites of glucose disposal during meal ingestion. GLUT4 is one of a family of 13 glucose transporters in humans that shares 12 membrane-spanning domains. GLUT4 is noteworthy among these transporters as the most dependent on discrete stimuli by insulin or other effectors; normally GLUT4 resides in the intracellular space. Once the insulin receptors are stimulated, GLUT4 is shifted quickly to plasma membrane to facilitate inward transport of glucose. Insulin signaling also reduces GLUT4 endocytosis, increasing the residence time of the protein in the plasma membrane.

Following the facilitated diffusion into cells along a concentration gradient, glucose is phosphorylated to glucose-6-phosphate (G-6-P) by a family of hexokinases. Hexokinase II is found in relation with in adipose

tissue. Like GLUT4, hexokinase II is regulated transcriptionally by insulin. G-6-P is a branch-point substrate that can enter several pathways. G-6-P can be isomerized to G-1-P by phosphoglucomutase, and then the G-1-P can be stored as glycogen (insulin enhances the activity of glycogen synthase); G-6-P can enter the glycolytic pathway (leading to ATP production); G-6-P can also enter the pentose phosphate pathway [24].

INSULIN ACTION

The insulin receptor is seen on all mammalian cells, explaining the wide range of responses to insulin. The tissues involved in regulation of blood glucose are liver, skeletal muscle, and fat .However, recent evidence suggests that specific regions of the brain and the pancreatic islet are also important targets for insulin.

Systemically, the insulin action is anabolic, and insulin signaling is important for uptake, use, and storage of glucose, amino acids and lipids. Importantly, it not only activates lipogenesis, glycogenesis and protein synthesis, but also inhibits catabolism. On a cellular level, insulin activates the transport of ions and substrates, promotes translocation, regulates the action of specific enzymes, and controls mRNA translation, gene transcription.

Other action of insulin such as stimulating glucose and ion transport and phosphorylation or dephosphorylation of specific enzymes occurs within seconds or minutes. It takes over minutes to hours for actions, like promoting synthesis of protein and regulating transcription of gene. The actions of insulin on cell proliferation and differentiation occurs over days. Metabolic effects such as inhibition of lipolysis or hepatic glucose production occur rapidly, within minutes of increasing concentrations of plasma insulin; detectable increases in glucose clearance from the blood may take nearly an hour.

The variability in the kinetics of insulin action is probably due to variable access to insulin receptors in different tissues, distinct intracellular signaling pathways, and the inherent kinetics of the various processes controlled by insulin

INSULIN RESISTANCE AND TYPE2DM:

Type 2 DM is characterized by improper secretion of insulin, resistance to insulin, increased glucose production in liver, and abnormal lipid metabolism. Obesity is very common in type 2 DM.

Abnormal insulin secretion, insulin resistances are important factors involved in development of type 2 DM. Most studies say that

insulin resistance occurs before the insulin secretory defect but type 2 DM develops only when insulin secretion becomes less.

Earlier stages of diabetes, glucose tolerance found to be normal, because pancreatic beta cell produces a compensatory increase in insulin output which leads to insulin resistance after a certain period of time. Certain individuals can't sustain hyperinsulinemic state produced by progressive increase in insulin resistance and compensatory hyperinsulinemia in the pancreatic islets cell . Later this leads to a state called Impaired Glucose Tolerance(IGT), where there will be high postprandial glucose.

A reduction in secretion of Insulin and high hepatic glucose formation leading to overt diabetes with increasing blood glucose values, this indicates failure of beta cells.

IMPAIRED INSULIN SECRETION

The β cell sensitivity to glucose is impaired in type2 DM; also there is reduced response to other stimuli such as insulinotropic GI hormones and neural signaling which ends in delayed production of insulin, which cause blood sugar to rise after meals, and failure to reduce the liver glucose release during fasting. Apart from the defect in functional properties of the cell, the absolute mass of cells is also reduced

in type 2 diabetes patients. The deficit is proceeded by a gradual loss of βcell mass over a period of time. This is futher related to toxic effects of hyperglycemia. Hence due to progressive reduction of βcell mass and function, patients with type2DM require steady increase in therapy to maintain glucose level.

Higher fasting glucose levels and insulin resistance shows a elevated levels of fasting Insulin. Increased amount of proinsulin is the other factor contributing to apparently high insulin levels early in the course of the disease. Proinsulin, the precursor to insulin, is inefficiently processed in the diabetic islet. Whereas healthy subjects have only 2-4% of total circulating insulin as proinsulin, type 2 diabetic patients can have 10-20% of the measurable plasma insulin in this form. Proinsulin has a considerably attenuated effect for lowering blood glucose compared to insulin[24].

Secretion of insulin and sensitivity are related to one another. In type 2 Diabetes mellitus, insulin secretion first increases in relation to insulin resistance to keep up normal glucose levels. In early stages, the insulin secretion deficit is mild.

Abnormalities in proinsulin processing are reflected by increased secretion of proinsulin in type 2 diabetes. Finally, the insulin secretion defect leads to less insulin secretion.

The reasons for reduction in insulin secretion in type 2 DM are unclear.

- A secondary genetic defect with insulin resistance-causes beta cell dysfunction.
- Amylin is found in the islets of individuals with long-standing type II Diabetes mellitus.
- Glucose toxicity - the metabolic environment of diabetes like, long standing increased glucose levels impairs islet function leads to an increase in glucose levels.
- Lipotoxicity- the increased levels of FFA and dietary lipid may also reduce islet function[24].

Insulin resistance causes the impaired usage of glucose by sensitive tissues; it also elevates hepatic glucose output. Hence these are responsible to the increase blood sugar level. Elevated hepatic glucose output is the cause for increased Fasting Plasma Glucose levels; also reduced peripheral utilization of glucose ends PP hyperglycemia.

Combined effect of genetic susceptibility and obesity causes the insulin resistance which leads to the reduced capacity of insulin to act prominently on target tissues a main feature of diabetes .Insulin resistance is low, because high levels of plasma insulin will alter the blood glucose level to normal. Greater impairment in glycogen formation is seen in

skeletal muscle (non oxidative glucose utilization) whereas no alteration of glucose metabolism in insulin-independent tissues.

The main molecular mechanism underlying insulin resistance in diabetes does not have a clear explanation. Tyrosine kinase activity and insulin receptor levels in skeletal muscle are decreased, but still changes are mostly secondary to increase insulin secretion and not primary defect. Hence, defects in insulin-mediated phosphorylation/dephosphorylation appear to have a main role in resistance (PI-3-kinase signaling defect might reduce the membrane translocation of GLUT4). Other impairments like the accumulation of lipid in skeletal muscle cells might impair phosphorylation of mitochondrial oxidation and decrease insulin-generated production of ATP in mitochondria. Additionally, these impairments may produce reactive oxygen species such as lipid peroxides.

Importantly, all insulin signaling pathways are not resistant, like those involving, cell growth control and differentiation through the MAP kinase (mitogenic-activated protein kinase) pathway. Hence as a result, hyperinsulinemia may enhance the insulin action through these pathways, which led to aggravation of diabetes-related conditions such as atherosclerosis.

In diabetes hepatic insulin resistance suppresses gluconeogenesis which shows the failure of hyperinsulinemia. This results in elevation of fasting blood glucose and reduced hepatic glycogen storage during the postprandial state [2].

In the earlier period of diabetes, after the raise of impairment in insulin secretation and resistance of insulin in the skeletal muscle, increased hepatic glucose production is being established.In adipose tissue as a result of resistance, increased free fatty acid and lipolysis occurs from adipocytes, which in turn leads to increased triglycerides,VLDL levels and reduced HDL (high density lipoprotein) synthesis in hepatocytes leading to dyslipidemia which occurs in type 2 DM patients after a certain period of time. This lipid storage in the hepatic cells may cause nonalcoholic fatty liver disease [2].

The metabolic syndrome a insulin resistance state or syndrome X used to describe metabolic derangements that includes, hypertension, resistance of insulin, decreased HDL and increased triglycerides, type 2 diabetes or IGT /IFG, central or visceral obesity, and increased cardiovascular risk with two types:

(1) Type A, affects women of young age and is described by severe obesity, features of hyperandrogenism, and hyperinsulinemia.

(2) Type B, affects middle-aged women, has severe features of hyperandrogenism, hyperinsulinemia, and autoimmune diseases.

Persons with type A insulin resistance syndrome have an undefined defect in the pathway of insulin-signaling; type B persons with resistance of Insulin syndrome show auto antibodies towards the receptor of insulin. The actions of these auto antibodies may prevent insulin binding or activate insulin receptor, leading to intermittent low blood glucose level. Polycystic ovary syndrome (PCOS) is a common disorder, defined by hyperandrogenism and chronic anovulation. Resistance of insulin is seen in PCOS women, and thus increases the risk for type 2 DM, irrespective of the actions of obesity [2].

INFLAMMATION AND INSULIN RESISTANCE

Obesity has low grade inflammation of white adipose tissue (WAT) which results due to chronic activation of innate immune system finally leading to resistance of insulin, impaired glucose tolerance and diabetes.

The obesity in diabetes, with central or visceral fat plays a major role in the pathogenesis of DM. This leads to increased levels of fat cell products like, nonesterified free fatty acids, retinol-binding protein 4, leptin, TNF-α, resistin, adiponectin and free fatty acids in circulation.

Adipokines not only regulates, appetite, body weight and energy expenditure, it additionally modulate the sensitivity of insulin. Adipokines and increase in free fatty acids production cause resistance of insulin in hepatic and skeletal muscle. For example, FFA affects usage of glucose in skeletal muscle increase synthesis of glucose in liver, and causes dysfunction of the beta cell. In contrast adiponectin,an insulin sensitizing peptide produced in the adipocytes is decreased in obesity and this may lead to resistance of Insulin activity in the liver. Products of adipocyte causes, a state of inflammation and explains why inflammatory markers like C-reactive protein and IL-6 are often increased in type 2 DM . In addition, inflammatory cells have been found infiltrating adipose tissue [2].

TNF-α

It is a proinflammtory cytokine produced from macrophages and lymphocytes. It is also synthesized weakly by adipose tissue in humans.TNF-α may have important part in pathophysiology of insulin resistance mediated by the serine phosphorylation of the insulin receptor substrate (IRS-1) instead of phosphorylation of tyrosine and thereby stopping the pathway of insulin signaling. Moreover adipose tissue is not related in increasing levels of TNF-α in circulation, it can be hypothesized that other mechanism like leptin derived from adipose

tissue may produce TNF-α from macrophages. Hence further investigations are required in searching the of TNF-α activities in obese people [25].

Interleukin-6

Interleukin -6 is synthesized from cells like fibroblast, monocytes, endothelial cells and including adipose tissue. Adipose tissue which synthesis IL-6 is increased in obese people. Secretion of IL-6 is high in visceral adipose tissue. It is a multiactional cytokine which is delt in the production of hepatic C-reactive protein, one of the independent factor for cardiovascular risk and its complications. Hence there is association between protein of IL-6 in adipose tissue and levels of IL-6 and CRP in circulation [26].

This shows that IL-6 has importance in inflammation, obesity, and cardiac disease [27]. Increased visceral adipose tissue production of IL-6 explains the association between deposit of central fat and complications of cardio vascular diseases. Since VAT is linked to liver by portal vein, IL-6 produced by adipose tissue increases the VLDL secretion and hypertriglyceridaemia affecting the liver metabolism [28].

Studies show that IL-6 could have been included in insulin resistance and its complications[29]. The interleukin 6 acts via JAK/STAT pathway which regulates target gene transcription [30].Hence through this pathway there is interaction between cytokine and insulin signaling

pathway thereby impairing the effects of insulin. Although the exact mechanisms is not known , it could be due to an interaction between suppressor of cytokine signaling (SOCS)proteins and the insulin receptor [32,33] or tyrosine phosphatase activation [31].

CRP

CRP is an acute-phase reactant synthesized in the hepatic cells due to activation of IL-6 and TNF-α derived from adiposity. It shows several fundamental immunoregulatory functions, specifically, CRP shows PRRs activation. CRP also plays role in modulation of platelet activity, complement fixation, increasing leukocyte reactivity, and clearance of cellular debris from active inflammatory sites [34, 35].

INFLAMMATION - INSULIN RESISTANCE - T2DM

It was hypothesized that conditions like inflammation could have a major role in the diabetes pathophysiology[36]. It has also been explained that along with pro-inflammatory effects of cytokines, other factors like free fatty acids and reactive oxygen species in obesity acts by intracellular signaling pathways, which includes the IκB kinase, (IKK), nuclear factor (NF)-κB, c-Jun NH2-terminal kinase(JNK) and Activating Protein-1 (AP-1) signaling molecules. They acts with insulin signaling through threonine /serine inhibitory phosphorylation of IRS.

Hence the IL-6 and TNF-α decreases the actions of insulin [33,37,38,39]. Thus along with increased risk of cardiovascular diseases mediated through inflammation, the gradual rise of cytokines in the circulation may add to the effects of resistance in insulin activity and hyperglycemic state.

As duration and degree of hyperglycemia increases, the risk of chronic complication also increases like:

Microvasular effect:

- Retinopathy (nonproliferative/proliferative)
- Macular edema
- Eye disease
- Sensory and motor (mono- and polyneuropathy)
- Autonomic conditions
- Neuropathy
- Nephropathy

Macrovascular effect:

- Cerebrovascular disease
- Coronary heart disease
- Peripheral arterial disease

Others changes:

- Gastrointestinal (gastroparesis, diarrhea)
- Genitourinary (uropathy/sexual dysfunction)
- Dermatologic
- Infectious
- Glaucoma
- Cataracts
- Hearing loss
- Periodontal disease

Diabetic individuals usually get these types of complications only after 20 years of onset of diabetes. But in case of type 2 , with long period of asymptomatic phase of hyperglycemia, many people present with complications at the time of diagnosis [2].

DIABETES - INFLAMMATION - CARDIOVASCULAR RISK

The hypothesis proposes that hyperglycemia and insulin resistance which lead to increase in free fatty acid production, forms diacylglycerol leading to stimulation of protein kinase C (PKC).Additionally,PKC modifies the gene transcription of fibronectin, contractile proteins, type IV collagen and extracellular matrix proteins in neurons and endothelial cells. Drugs inhibiting PKC actions are being studied in clinical trials.

Endothelial dysfunction is a main feature of type 2 diabetes mellitus (DM). Endothelial dysfunction is also present in patients with insulin resistant stages prior to the development of overt hyperglycemia (IGT) and type 2 DM. Many articles quotes that endothelial dysfunction has been described in women with gestational diabetes, obesity and individuals with the metabolic syndrome. This suggest that insulin resistance is main key factor and endothelial dysfunction is not simply a result of hyperglycaemia. Early changes in the alteration of glycemic levels caused by impaired insulin secretion or obesity-related insulin resistance is the cause for structural and functional changes of the blood vessel leading to vascular complications of type 2 DM.

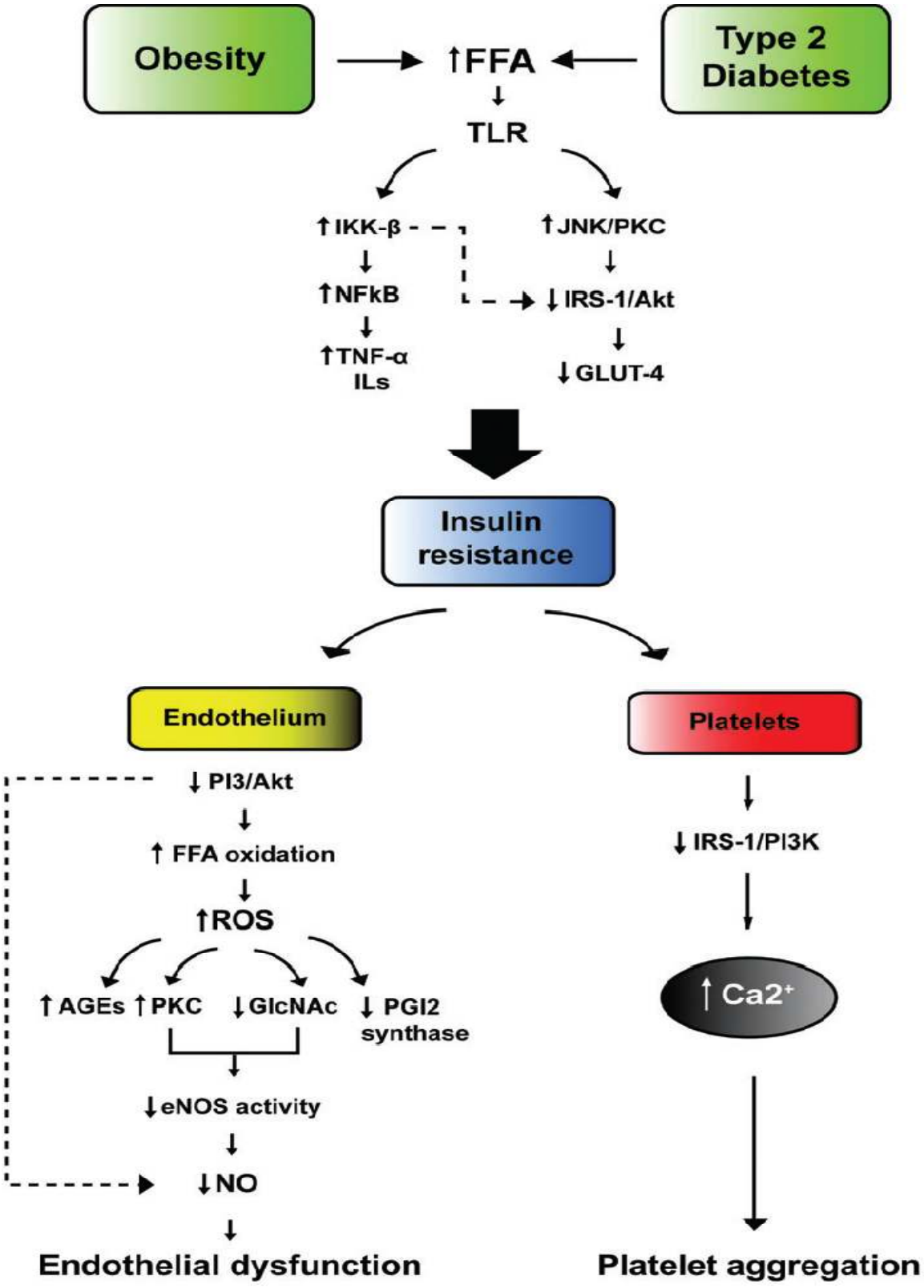
In diabetes and obese patients, there is rise in free fatty acid production which stimulates toll like receptor and continues the translocation of NF-kB and subsequently promotes the genes of inflammation like TNF-alpha and IL-6. Simultaneously JNK /protein kinase C decreases insulin receptor substrate-1 (IRS-1) phosphorylation and downstream Akt and PI3-kinase. Which further down-regulates the glucose transporter 4 and finally leads to resistance of Insulin. Impaired Insulin sensitivity in endothelium of the blood vessels promotes ROS formation, FFA oxidation, which subsequently stimulating the pathways like, PKC activation, AGE synthesis, and down-regulates PGI2. Finally eNOS activity is impaired leading to dysfunction of endothelium. In the

platelets impaired IRS1/PI3K pathway leads to accumulation of calcium and increases the aggregation of platelets [42, 43, 44.]

Hyperglycemia which alters the vascular endothelium is due to the impaired balance between accumulation of reactive oxygen species (ROS) and nitric oxide causing dysfunction of endothelium [43]. Increase in intracellular glucose levels leads to activation of PKC and production of ROS by the help pf NADPH oxidase and $p66^{Shc}$adaptor protein.

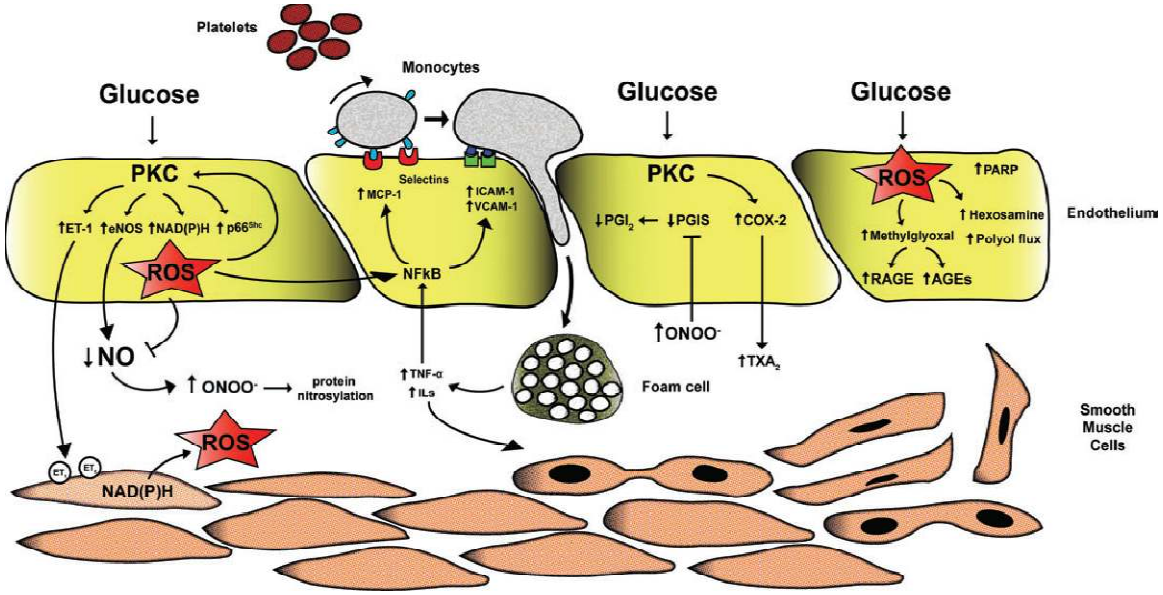
Increase in oxidative stress gradually impairs NO activity and reduces the protein nitrosylation. Protein nitrosylation usually decreases the effect of endothelial NO synthase and antioxidant enzymes [44]. PKC-dependent eNOS deregulation also reduces the NO availability; this reduced bioavail-ability of NO is a prominent indicator of cardiovascular outcomes [44].

Figure.4: Insulin resistance as a trigger of Artherothrombosis.



PKC causes enzyme up-regulation thus increases, uncoupling of endothelial nitric oxide syntheses (eNOS) and further leading to accumulation of free radicals. Simultaneously, insulin resistance and hyperglycemia decreases eNOS activity. Along with the lack of NO, glucose-induced activation of protein kinase C (PKC) produces increased production of endothelin-1(ET-1) causing constriction of blood vessel and platelet aggregation. Collection of superoxide anion also causes enhancement of pro-inflammatory genes monocyte chemoattractant protein (MCP-1), intracellular cell adhesion molecule (ICAM-1) and vascular cell adhesion molecule-1(VCAM-1) by the signaling of NFkB . The sequential process progresses to monocyte adhesion, rolling and diapedesis along with foam cells formation in the layer of sub-endothelium. Foam cell further increases cytokines of inflammation and keeps the proliferation of smooth muscle cells and vascular inflammation, thereby increasing the process of atherosclerosis. Furthermore, ROS enhances the production of glucose metabolite methylglyoxal leading to activation of AGE/RAGEsignalling and the pro-oxidanthexosamine and polyolpathway flux. Therefore there may be a common mechanism underlying both the development of endothelial dysfunction and insulin resistance [45- 51].

Figure .5: Insulin resistance and Endothelin Dysfunction.



CURRENT PHARMACOTHERAPY OF TYPE 2 DIABETES MELLITUS

Adequate glycemic control is necessary to address acute symptoms and to prevent, defer or reduce the severity of chronic microvascular and macrovascular complications. Choice of drug should address the pathophysiology, and combinations of differently acting agents are frequently required to provide additive efficacy. So, the current oral hypoglycemic agents available for the therapeutic uses are

The Biguanide Metformin often selected as initial oral ant diabetic drug therapy. It counters insulin resistance and lowers blood glucose through several insulin dependent and independent mechanisms. Sulfonylurea act on the pancreatic β cells to stimulate insulin secretion. The efficacy of Sulfonylurea depends on adequate remaining function of

the beta cells. Meglitinides ,also called as prandial insulin releasers, are rapid short acting insulin secretagogue taken before meals to boost insulin levels during digestion, thereby reducing prandial hyperglycemia and decreasing risk of interprandial hypoglycemia. Thiazolidinediones produce slow onset glucose lowering effect, attributed mainly to increased insulin sensitivity (especially increases peripheral glucose utilization) by activating nuclear receptor PPAR-γ .Gliptins increases prandial insulin secretion by inhibiting DPP-4 enzymes, which in turn increases plasma half life of insulinotropic incretin hormones. The newer agents such as (DPP-4 inhibitor) Sitagliptin, Vildagliptin, Saxagliptin, Linagliptin have been recently recommended by ADA in the therapeutic intervention of Diabetes Mellitus. α-glucosidase inhibitors slows the rate of carbohydrate digestion by competitively inhibiting the intestinal α-glucosidase enzymes.

Metformin

Belong s to the class of Biguanide. It is the most largely prescribed insulin sensitizer in the management of type 2 diabetes.

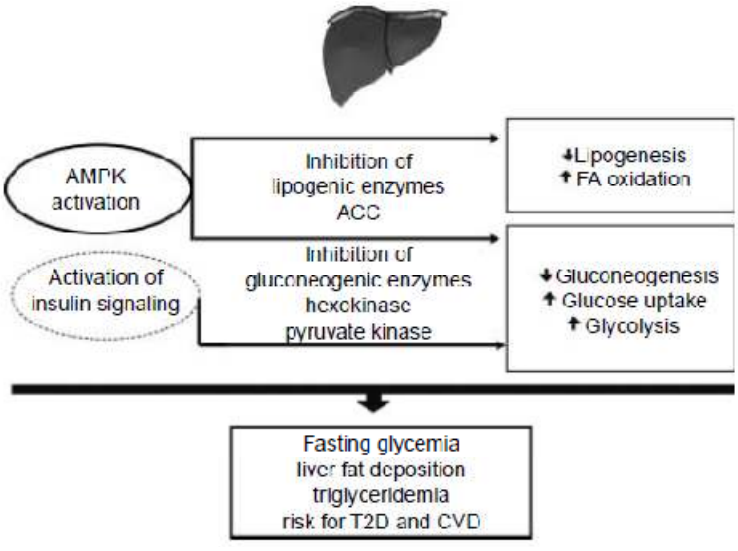
Primary effect of Metformin is to decrease liver glucose production by the stimulation of the AMP-activated protein kinase (AMPK) enzyme. It also have other mechanisms of action like impairing the production of gluconeogenesis in renal, decreasing in absorption of blood glucose from

the intestinal tract, direct stimulant for tissues glycolysis, and reduction in levels of plasma glucagon.

Action of Metformin does not depend on pancreatic beta cells function. These agents are commonly called as euglycemic agents. Because biguanide therapy does not cause hypoglycemia in type 2 diabetes patients.

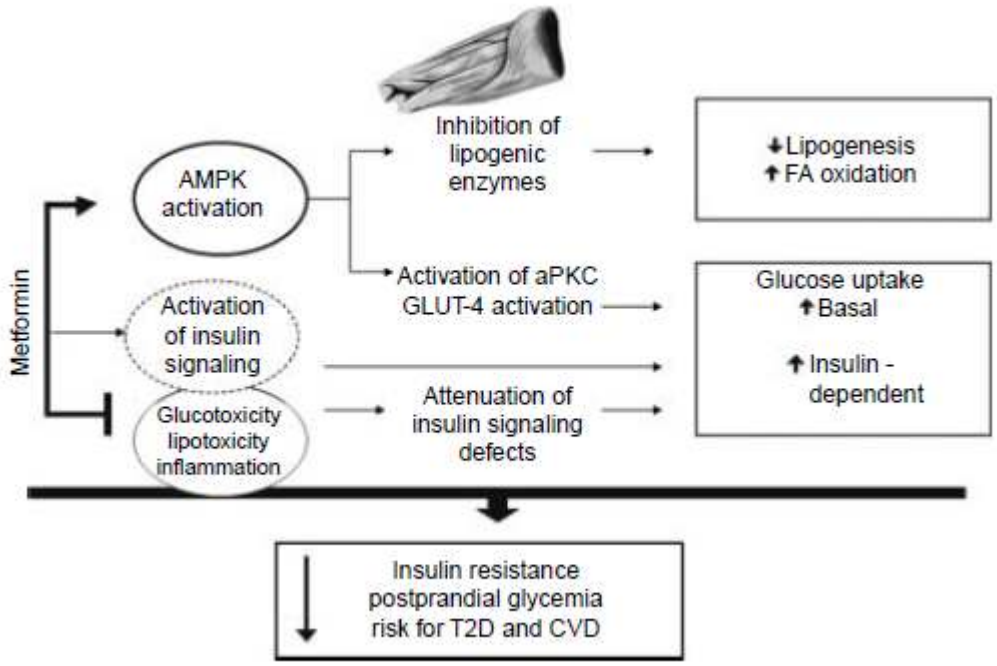
Metformin as pleotropic actions on several tissues. Insulin resistance and hyperinsulinemia have their effects on adipose tissue, liver, endothelium skeletal muscles and ovary. Biguanide was shown to modulate 5'-AMP-activated protein kinase (AMPK) dependent regulation, which in turn is upregulated by serine threonine protein kinase11 (SK 11). These signals are responsible for the suppression of genes encoding gluconeogenic and lipogenic liver enzymes (Acetyl coA carboxylase) there by inhibiting the hepatic gluconeogenesis and lipogenesis and increases the fatty acid oxidation [52, 53, 54].

Figure .6 : Metformin action on Liver.



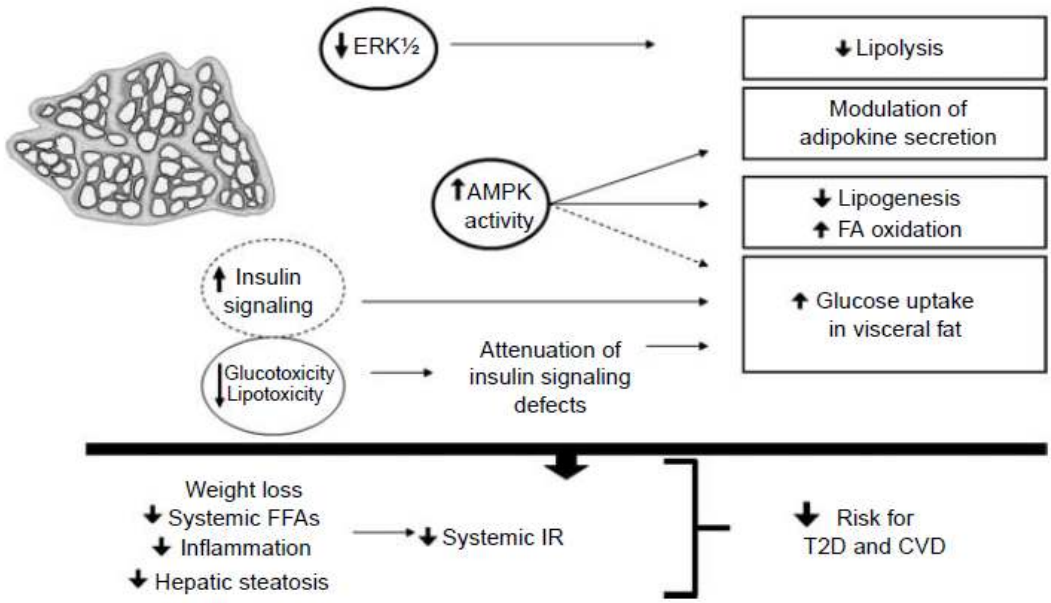
Increase in basal insulin stimulates the glucose uptake and this process is done by Metformin in the skeletal muscle. The glucose uptake from skeletal muscle appears to be influenced by activation of AMPK followed by aPKC and GLUT-4 activation. The Insulin stimulated glucose uptake may involve the increase in the signaling of Insulin through the decrease in inflammation, lipotoxicity and glucotoxicity. By AMPK activation, biguanide also inhibit lipid production while increasing the skeletal muscle fatty acid oxidation. Thus result of the above actions may bedue to decrease in the resistance of insulin which in turn attenuates the postprandial blood sugar levels [55, 56].

Figure.7: Metformin action on Skeletal Muscle.



Metformin may act through the inhibition of Extracellular-signal-regulated kinases (ERK1/2) phosphorylation which attenuates the TNF-α induced lipolysis in adipocytes; it also opposes expansion of adipose tissue by AMPK-dependent activation of oxidation of free fatty acid and attenuating the lipid production. This effect may contribute to decreased fat mass and antilipolytic action of metformin influence the sensitization of insulin by reducing the systemic FFA levels [57-62] .The Metformin's action decreases the glucotoxicity and lipotoxicity and may improve sensitivity of insulin in adipose tissue. In visceral adipose tissue glucose uptake is done through AMPK-dependent mechanism .Metformin may also have its influence on adipokine secretion through mitogen activated protein kinase or AMPK [63 – 69].

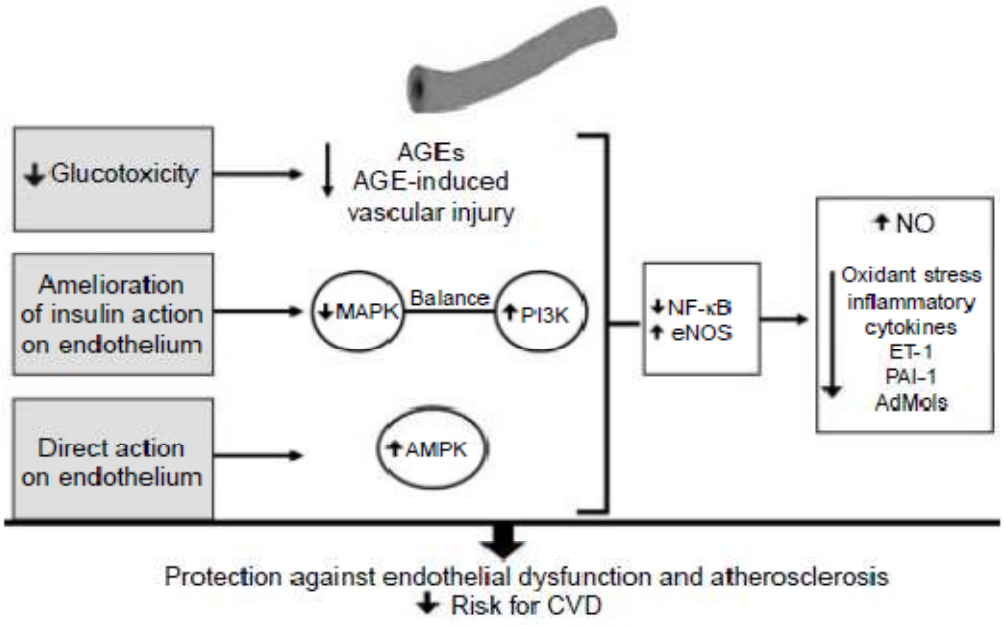
Figure.8: Metformin action on Adipose tissue.



METFORMIN ACTION ON ENDOTHELIUM

Metformin's effect on the endothelium of blood vessel is based on the decrease in glucotoxicity and decrease in levels of advanced glycated end products as well as decrease in hyperinsulinemia/insulin resistance[70]. At the molecular level, these mechanisms combine to prove a balance by decreasing the MAPK and increase in the PI3K-dependent cascades. They have promising effects directly on blood vessel endothelium, by activation of AMPK, apart from blood sugar lowering and sensitization of insulin. As a result, treatment with metformin decreases NF-kB, thus decreases production of endothelin-1 (ET-1), proinflammatory cytokines (IL-6, IL-8,IL-18,TNF-α), Plasminogen activator inhibitor (PAI-1), and adhesion molecules, while it increases eNOS and endothelial NO production [71]. Through these mechanisms Metformin can prevents the endothelial dysfunction and decrease in atherosclerotic injury. Due to its spectrum of metabolic actions, metformin is a useful adjuvant to modification of lifestyle in obese and overweight PCOS patients who has IGT or metabolic syndrome features.

Figure.9: Metformin action on Endothelium



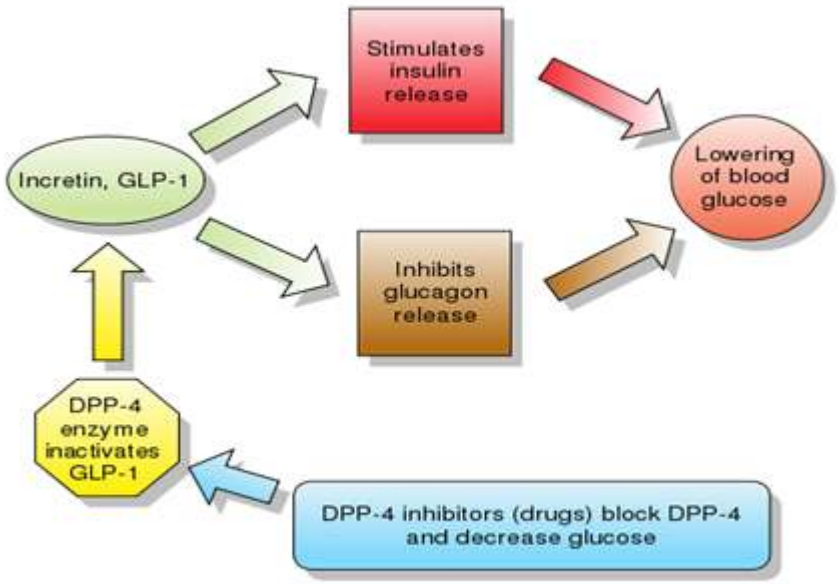
In women with T2D and PCOS Metformin is the first-line choice of treatment. Metformin's targets have been explored to add the ovary and the endothelium. Molecular studies have proven the mechanisms of Metformin actions via AMPK, a serine–threonine kinase which includes its action on the muscle, liver, endothelium, and the ovary. The direct endothelial actions biguanide appears to be useful in endothelial dysfunction [72].

DPP-4 Inhibitor

Sitagliptin, is a DPP-4 inhibitor, blocks the dipeptidyl peptidase enzyme that causes degradation of incretin which are produced with response to a meal, in turn increases incretin level and the insulin secretion and reduce the glucagon level, and thus it helps in improving the fasting and

postprandial hyperglycemia .Hence inhibitors of DPP4 helps in increasing the insulin secretion.

Figure.10: Mechanism of action of DDP-4 Inhibitors



Sitagliptin increases the bioavailability of incretin, glucagon-like peptide-1 and insulin tropic polypeptide dependent on glucose which implies it's anti-diabetic effect.

Studies shows that since DPP-IV is present as CD26 on cell membranes and known to cause pro-inflammatory signals, thus Sitagliptin was hypothesized to exert an anti-inflammatory action in a study 22 diabetic patients were randomized and given either Sitagliptin 100 mg daily or placebo for 12 week period. Baseline investigations like

Fasting blood samples were obtained before the starting of treatment and 12 wk of treatment. Results showed that HbA1C, mRNA expression in mononuclear cell of CD26, proinflammatory cytokine,TNF alpha, Toll like receptor (TLR)-4, fell significantly after 12 weeks of Sitagliptin therapy. Plasma concentration of C-reactive protein, IL-6 and free fatty acid levels fell significantly after 12 weeks of Sitagliptin therapy. It concludes that these effects show a promising action of anti-inflammatory effect and thus helps in inhibition of atherosclerosis [73].

The anti-inflammatory actions of Sitagliptin may influence to a potential antiatherogenic action of Sitagliptin. Regarding this concept, retrospective meta-analysis of Sitagliptin treatment based studies shown that there was a greater reduction of about 50% in cardiovascular events in the Sitagliptin group than the controls [74].

Reno protective effect of Sitagliptin was investigated in study using Cisplatin induced nephrotoxicity in mice. In that study 48 male balb-c mice were equally divided into 4 groups as, control, Sitagliptin group, Cisplatin group and Cisplatin plus Sitagliptin group. The mice were sacrificed after 72 h of Cisplatin injection the results showed that Sitagliptin significantly decrease the nephrotoxic effect of Cisplatin with increased activity of antioxidant enzymes, improved kidney function, renal histopathological scoring and decreased tissue level of TNF-α. It

can be concluded that Sitagliptin may have a protective role against Cisplatin induced acute nephrotoxicity via antioxidant and anti-inflammatory pathway. Thus from the study we came to know that Sitagliptin may exert antioxidant and anti-inflammatory role which will also help in preventing the atherosclerotic process [75].

Studies hypothesized that Sitagliptin, a DPP4-inhibitor, may also have a role in improving functions of endothelium in DM patients with coronary artery disease (CAD).

In that study, 40 patients with CAD and uncontrolled DM, were grouped for 6 months therapy of either add-on with Sitagliptin treatment or aggressive conventional treatment. Reactive hyperemia peripheral arterial tonometry index (RHI), was assessed for endothelial function

The change in RHI percent, was more in the Sitagliptin group than in the control group .Significant decrease in the high-sensitivity C-reactive protein level was seen in Sitagliptin treated group, but no such change was seen in the control group. It was concluded that Sitagliptin significantly improve endothelial function and inflammatory state in uncontrolled DM patients with coronary artery disease, apart from its blood glucose lowering effect. Hence it suggests that Sitagliptin has beneficial actions on the cardiovascular system in diabetes patients [76]. Recent researches had proved that Sitagliptin and Exendin-4 apart from

activating the phosphorylation of AMPK, it also inhibit the activation of MAPK including p38 and ERK [77- 80]. Stimulation of AMP-activated protein kinase(AMPK), in vascular cells found to possess anti-atherosclerotic effects [81-83] by increasing the Akt/endothelial NO synthase (eNOS)/NO signaling pathway,which futher causes suppression of p38 activation of nuclear factor-κB and following suppression of downstream inflammatory responses [83]. It also suppresses mitogen activated protein kinase (MAPK) which has beneficial effects in atherosclerosis through decreasing adhesion molecules and anti-inflammatory effects, as well as increases the stability of the carotid plaques [83] .

Acarbose

Acarbose a competitive blockers of glycosidase and it decrease postprandial increase in blood glucose by prolonging the time of absorption and digestion of starch and disaccharides .Glucose and fructose are the only monosaccharide that can be delivered from the intestinal lumen and into the bloodstream. In case of other substances like starches, oligosaccharides, and disaccharides that must be converted to monosaccharides before getting absorbed in the duodenum

The two enzymes, α glycosidase and pancreatic amylase, get bind to the brush border of the intestinal cells and facilitate the digestion process.

α- glycosidase inhibitors decrease gastro intestinal absorption of dextrin disaccharides and starch . Inhibition of α- glycosidase delays the absorption of carbohydrates from GI tract and decreases the rate of rise of postprandial plasma glucose. These drugs also increase the release of the glucoregulatory hormone GLP-1 into the circulation, which may contribute to their glucose-lowering effects[24]. Impaired glucose tolerance, destroys the endothelium of the arterial blood vessel which starts as a sequence of pro-atherogenic process are associated with the sudden increase in postprandial blood glucose levels [84, 85].

Mechanisms underlying Acarbose will provide the cardiovascular benefits by directly pointing towards the postprandial blood glucose level, which limits and reduces the smooth muscle of endothelial damage which again decreases the risk of diabetic complications.

Study of STOP-NIDDM showed in prediabetes that Acarbose delays the process of intima media thickening (IMT) in individuals compared to placebo, therapeutic management with Acarbose decreases approximately 50% ($P = 0.027$) [86] yearly rise in carotid IMT.

Hence, the end result from substudy of STOP-NIDDM is also confirmed in the results of another sequential study, in which therapeutic management with Acarbose revealed to preserve vasodilatation of the endothelium compared to treatment with placebo.

The primary end point of the meta-analysis of improvement in risk factor by the treatment with Acarbose (MeRIA), which showed the results of 7 trials done in type 2 Diabetes, was the time taken to produce a cardiovascular event. The results proved that Acarbose treatment causes promising benefits in cardiovascular disease and damage [87]. Therapeutic management with Acarbose, decreases the cardiovascular risk by 35% overall and the risk of MI specifically by 64% [87].

Diabetic patients are at high risk for the other components like dyslipidemia and hypertension leading to metabolic syndrome, these components of metabolic syndrome has been cause for raise in morbidity and mortality [88, 89] .Hence prevention of diabetes and cardiac disease will be the primary aim in the management of metabolic syndrome [90]. Hence the studies have shown that Acarbose treatment has been shown to have promising results for metabolic syndrome.

The usage of other oral antidiabetes agents like thiazolidinediones, sulphonylureas are associated with 2-5 kg weight gain [91] but a another RCT done with acarbose and glibenclamide as monotherapy has found no change in weight in patients with Acarbose treatment [92] .After 3 years

treatment with Acarbose , individuals had shown reduction in the mean value of around 1.2kg, compared with controls.

Percentage of around 20% to 60% of diabetic patients will be at high risk to develop hypertension [93]. Another study in fourty four type 2 diabetes patients found reduction in systolic, diastolic and mean blood pressure values along with good glycaemic control in Acarbose treatment[94]. In the same way another randomized study in obese diabetes patients found that treatment with Acarbose showed decrease in blood pressure by 6 month treatment, compared to Glibenclamide [95].

Decrease in postprandial hyperglycemia in diabetes patients after Acarbose treatment have also been shown to decrease the proinflammatory transcription factor (NFκ B) activity and nuclear localization [96] . This suggest a underlying mechanism of Acarbose in their anti-inflammatory effects .This would be associated with decrease in coagulation factors levels after treatment with Acarbose. This was shown by acarbose which reduces the fibrinogen level in type 2 diabetic patients[97], and decreases the serum CRP values in patients with IGT [98].

Studies have shown that Acarbose improves the insulin sensitivity in elderly diabetic patients [99]. Limiting factor of excess insulin is hypoglycemia [100,101] .This is the major reason for mortality and morbidity [102] but due to its different mode of action; Acarbose doesn't activate the secretion of Insulin and doesn't cause hypoglycemia on monotherapy [101].

Systemic availability of Acarbose is less than 2 % and so the toxic reaction is probability is very low and no drug interactions.[103, 104].

In a study, 20 diabetes patients with liver disease Acarbose therapy reduces the HbA1c after 8 weeks of therapy [105] and reduces postprandial blood sugar levels by about 50% [106] .

Diabetic related complications are more common in elderly patients. Study conducted in elderly diabetic patients found that treatment with Acarbose monotherapy showed reduced blood sugar levels, without causing hypoglycemia and there were no revelant clinical changes in vital signs within the period of treatment [107,108].

It was demonstrated that the safety of Acarbose in long-term in post marketing survillence and in placebo controlled trials showed that combination of acarbose plus other antidiabetic therapies proved a better safety profile [109] .

The compliance of Acarbose therapy is affected by the adverse effects like mild gastro intestinal events, and effects may be reduced by low dosing regimen in the starting of the treatment. But still Acarbose has a better safety profile, and so it is good for the treatment of type 2 diabetes and one of the very few oral hypoglycemic drugs approved by FDA for management of prediabetes.

Thus finally, the Acarbose has been shown to decrease the risk of cardiovascular events irrespective of patient's weight and age.

Sulphonylurea

Role of Sulfonylurea's is to rise insulin release from pancreas by two additional mechanisms of action- a decrease in serum glucagon levels and potassium channel closer in extra pancreatic tissue.

The sulfonylureas continue to be widely prescribed which are divided into first-generation and second-generation. They differ mainly in their potency and adverse effects. The first generation sulfonylurea are not used much now a days as the second-generation agents become generic and less cost .

These potent sulfonylurea like Glipizide, Glyburide, and Glimepiride—should be used cautiously in cardiovascular disease patients or in patients of elderly age group, in whom hypoglycemia would be especially dangerous

Studies done in animals proved that sulfonylurea shows their actions by getting attached to sulfonylurea receptors (SUR), which futher connected to ATPdependent k^+channels. Receptor SUR1 binding in pancreatic beta cells shows insulinotropic effects [110-113].

Other sulfonylurea's also binds and have effect on receptors SUR2A/B present over the coronary smooth muscle and myocardium

thereby preventing ischemic preconditioning which is said have a protective role [113] .Interference with ischemic preconditioning which is proven to have adaptive role (a phenomenon where repeated exposure to mild or moderate ischemia protects the myocardium against damage during subsequent episodes of severe ischemia.) may be the cause for these adverse cardiac effects of sulfonylurea [113].

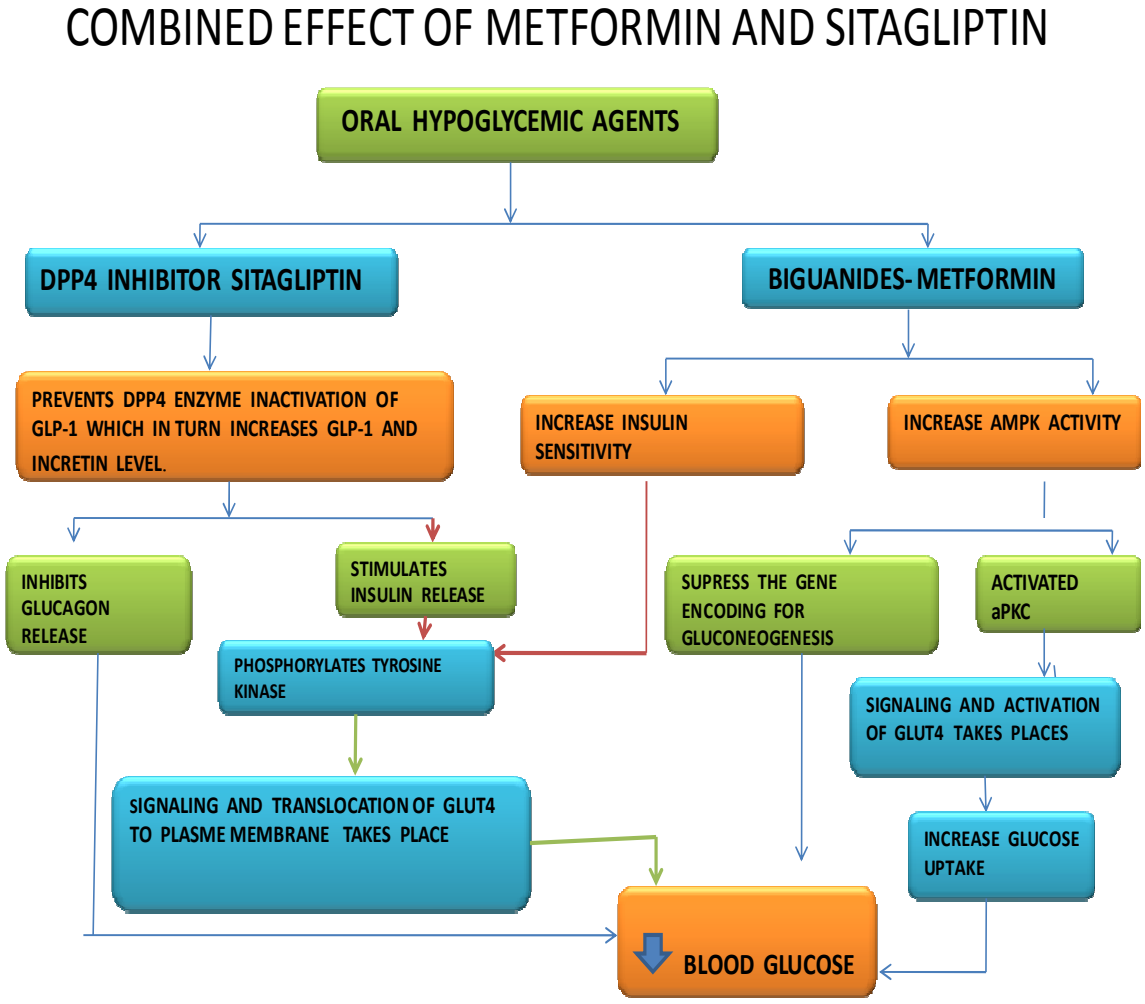
The second generation sulfonylurea's like Glipizide or Gliclazide shows little binding or affinity over SUR2 and have shown a favorable outcomes in cardiovascular disorders, in their initial period of therapeutic management [114]. Studies which were done retrospectively have shown that Glimepride and Glibenclamide which have low affinity for SUR 1 selectivity ,were associated with more events in cardiovascular disorders like angina, myocardial infarction, peripheral vascular disease and stroke.[114] .

ANTI-INFLAMMATORY EFFECTS OF ORAL HYPOGLYCEMIC AGENTS AND THEIR PRESENT STATUS.

Previous study by Andrew et al, has shown that Obese diabetic patients treated with metformin had lower levels of hsCRP ,expression of TNF-α and TLR 2/4, than their counterparts receiving placebo [115] . The results from the previous studies proved that Acarbose treatment causes

promising benefits in cardiovascular disease and damage [87] .Another study done with Acarbose has proven that it decrease the proinflammatory transcription factor (NFκ B) activity and nuclear localization [96] .This suggest a underlying mechanism by which the anti-inflammatory effects of Acarbose could have been mediated. This was shown by Acarbose which reduces the level of fibrinogen in type 2 diabetic patients [97] ,and decreases the serum C-reactive protein levels in individuals with IGT [98] .Prevoius studies proved that Sitagliptin apart from stimulating the AMPK phosporylation it also inhibit the activation of MAPK including p38 and ERK [77 – 80] .Sitagliptin could arrest the progression of atherosclerosis possibly by AMPK activation and suppressing the MAPK, leading to decreases in adhesion molecules and inflammatory cytokine. Previous studies showed fall in Plasma concentration of C-reactive protein, IL-6 and free fatty acid levels after 12 weeks of Sitagliptin therapy. It concludes that these effects show a promising action of anti-inflammatory effect and thus helps in inhibition of atherosclerosis [73].

Figure.11: Synergistic action of Metformin and Sitagliptin



Theoretically a synergism occurs in mechanism of action in Sitagliptin and Metformin via GLUT4 in reducing the plasma glucose.

Since both Metformin and Sitagliptin have their effect in reducing the blood glucose level and inflammatory markers, this combination will be helpful in the reduction of micro and macro vascular complications of Type 2 Diabetes Mellitus

Based on this background information , this study targeted the oral hypoglycemic agents like Acarbose and Sitagliptin to look for their anti-inflammatory action when it is given as an add on therapy in patients of type 2 DM who were already on Metformin Sulfonylurea combination for more than one year of treatment and whose HbA1c is >7.5%.

MATERIALS AND METHODS

STUDY DESIGN :

A Prospective Open Labelled Comparitive Randomised clinical controlled study.

DATA COLLECTION METHODS:

Clinical examination and Lab investigation

STUDY POPULATION:

Patients who had attended the Out Patient departments of Endocrinology (Dialectology), General Medicine and Master health checkup unit of PSGIMSR Hospitals.

SAMPLE SIZE: 30 Convenient sample.(Pilot study)

SAMPLING METHOD: Simple Random Sampling.

INCLUSION CRITERIA:

- Type 2 Diabetes Mellitus
- HbA1C >7.5 %
- Age: 25-65 yrs

EXCLUSION CRITERIA:

- Type 1 DM, DM due to genetic defects, GDM

- H/o Cardiac, Renal diseases and old CVA.
- Altered Liver Function Test.
- H/O Alcohol intake, Smoking.
- H/o Pancreatitis
- Patients on Statin group of drugs, Oral Steroid therapy , Fibrate therapy.

DURATION OF STUDY: 3 Months

METHODOLOGY

This study was a pilot prospective open labeled randomized controlled trial .The study population was Type 2 diabetic patients attending Endocrinology and Medicine OPD PSGIMS&R Hospitals Coimbatore. We included all type 2 diabetic patients in a age group of around 25-65 years, who were on conventional treatment of Metformin and Sulfonylurea combination drugs and HbA1C level was $\geq$ 7.5 % .we mainly targeted the patients who were still not under control even after their usage of conventional treatment.

Patients with diabetes other than type 2, history of cardiac, renal and cerebrovascular diseases, patients on treatment with statins, fibrates and steroids, history of altered liver function and pancreatitis, known history of alcohol intake and smoking were excluded from the study.

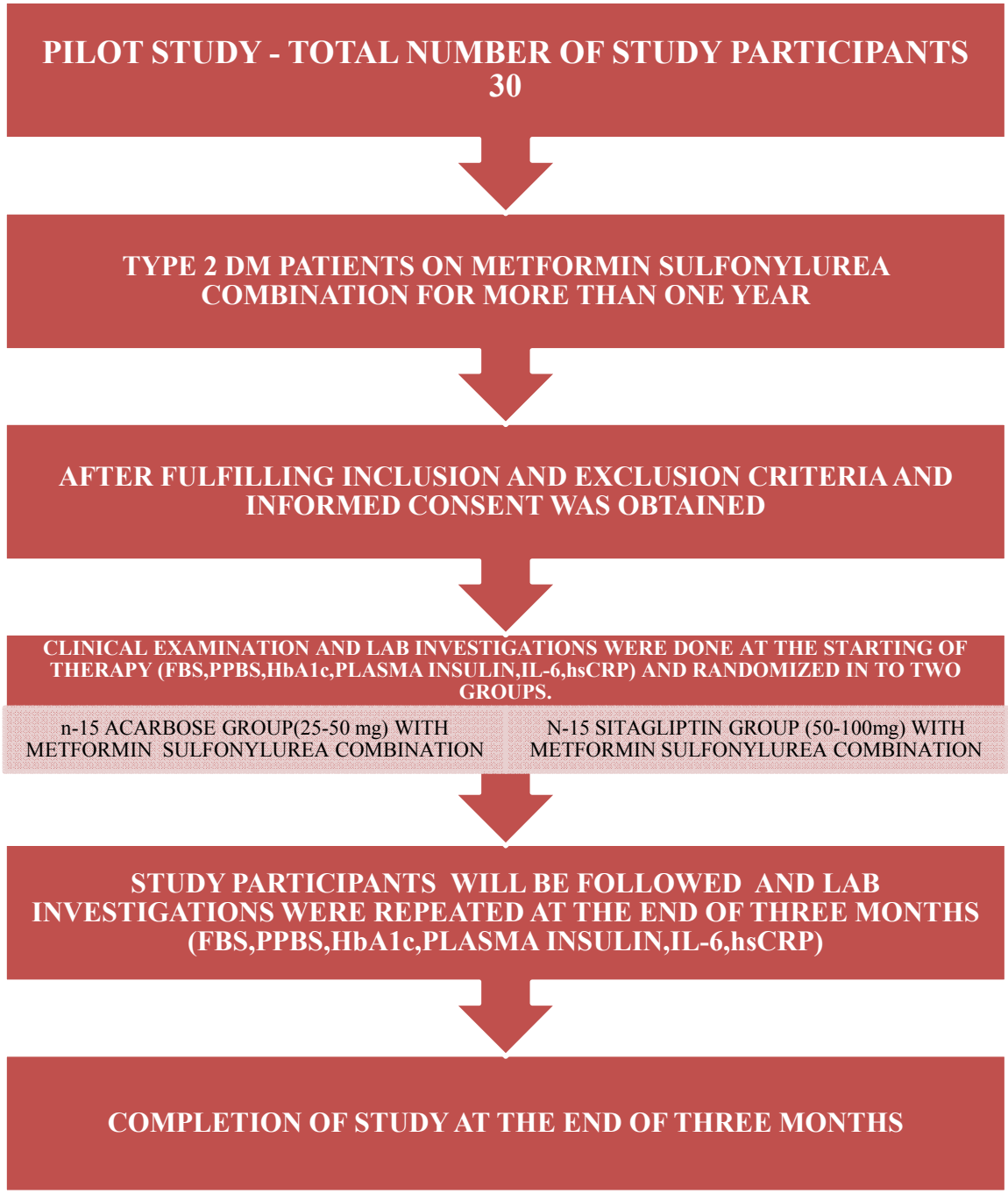
The study protocol was approved by the Institute Human Ethics Committee (IHEC) preceding the start of the study. The details and the purpose of the study protocol were explained to each participant and to their attenders individually and clarified their doubts before getting the informed consent forms. The informed consents forms provided to the participants were either in English or in Tamil. The participants, who gave written informed consent, came under the inclusion criteria were enrolled for the study. The copy of consent forms were attached in the annexure. The patients who declined to give informed consent were also excluded from the study.

According to the protocol 30 type 2 diabetic patients who fits under our inclusion criteria's were recruited after getting informed consent. Basic demographic data's like name, age and anthropometric measurements of Height, weight, body mass index were recorded. History of the patient, any co morbidities was noted.

Then the clinical examination and the base line investigations (Fasting blood glucose, postprandial blood glucose, HbA1C, hsCRP, IL6,Plasma Insulin) were done before starting the treatment. Later those 30 participants were randomly divided into two groups with 15 patients per group. One group was on Sitagliptin, Metformin and Sulphonylurea combination and the other group was on Acarbose, Metformin and

sulphonyl urea combination. Sitagliptin was started on 50mg OD and then titrated to 100mg according to the patient's blood glucose level by the physician. In the same way, Acarbose was started on 25mg BD initially and later it was titrated to 50mg BD or 25mg TDS according to the patient's blood glucose level .The study participants were followed for 3 months and they were advised to come for review after two weeks to see whether the dosage of the drugs were enough or they were in need for titration of the drug dosage. In between the three months period of study, the participant's FBS, PPBS levels were noted in the case file. The patients were asked to come to receive the drugs (Sitagliptin, Acarbose) according to their convenience. Finally at the end of three months study participants were asked to come for review and the clinical examination and the investigations (FBS, PPBS, HbA1C, IL6, hsCRP and Plasma insulin) were done again and the study was completed. Over view of study design is given below.

Figure. 12: Flow Chart representing the Methodology.



This study was to observe the anti-inflammatory effects of Sitagliptin and Acarbose when its given as third drug to patients on the drug combination of Metformin and Sulfonylurea more than 1 year duration, HbA1C above 7.5 .

This study targets mainly the inflammatory marker and their relationship with the clinical, biochemical parameters by checking their level before and after the therapeutic management using the following standard techniques.

Plasma glucose

Patient's blood samples were collected for fasting and post prandial blood sample and sent to biochemistry laboratory.

Method:

Enzymatic reference method with hexokinase.

Principle:

Hexokinase (HK) catalyzes the phosphorylation of glucose by ATP to form glucose-6 phosphate and ADP. To follow the reaction, a second enzyme, glucose-6 phosphate dehydrogenase (G6PDH) is used to catalyze oxidation of glucose-6 phosphate by NAD^+ to form NADH.

Reaction:

$$\text{D-glucose} + \text{ATP} \xrightarrow{\text{HK}} \text{D-glucose-6 phosphate} + \text{ADP}$$

$$\text{D-glucose-6 phosphate} + NAD^+ \xrightarrow{\text{G6PDH}} \text{D-6 phosphogluconate} + NADH + H^+$$

The concentration of NADH formed is directly proportional to the glucose concentration. It is determined by measuring the increase in absorbance at 340nm.

Glycated haemoglobin: (HbA1c)

Here again the patient's blood samples were collected in blood collection center and sent to biochemistry laboratory for analyzing HbA1C values.

Method:

Turbidimetric inhibition immunoassay

Principle:

Total Hb and HbA1c concentrations are determined after hemolysis of the anti-coagulated whole blood specimen. Total Hb is measured colorimetrically. HbA1c is determined immunoturbidimetrically. The ratio of both concentrations yields the final percent HbA1c result.

The anti-coagulated whole blood specimen is hemolysed automatically with HbA1c hemolysis reagent in the predilution cuvette. Erythrocytes are lysed by low osmotic pressure. The released Hb is proteolytically degraded to pepsin, to make the beta-N terminal structures more accessible for the immunoassay. Additionally, the heme portions are oxidized for the Hb assay.

Total Hb is dertermined in the hemolysate using a cyanide-free colorimetric method based on the formation of a brownish-green chromophore (alkaline hematin D-575) in alkaline detergent solution. The solor intensity is proportional to the Hb concentration in the sample and is determined by monitoring the increase in absorbance at 552nm. The test result is calculated using a fixed factor determined from the primary calibrator chlorohemin.

HbA1c is measured using monoclonal antibodies attached to latex particles. The antibodies bind the beta-N terminal fragments of HbA1c. Remaining free antibodies are agglutinated with a synthetic polymer carrying multiple copies of the beta-N terminal structure of HbA1c. The change in turbidity is inversely related to the amount of bound glycopeptides and is measured turbidimetrically at 552nm.

The final result is expressed as percent HbA1c and is calculated from the HbA1c/Hb ratio as follows.

Protocol 1: According to IFCC

HbA1c (%) = (HbA1c/Hb)*100

Protocol 1: According to DCCT/NGSP

HbA1c (%) = (HbA1c/Hb)*87.6+2.27

Plasma insulin

Patients blood samples were collected in fasting and and sent to biochemistry laboratory for analyzing plasma insulin.

Method

It was done in cobas e411 auto analyser which is based on Electrochemiluminescence immunoassay (ECLIA)

Test principle

Sandwhich principle. Total duration of assay: 18minutes.

1st incubation: insulin from 20µl sample, a biotinylated monoclonal insulin specific antibody, and a monoclonal insulin-specific antibody labeled with a ruthenium complex from a sandwich complex.

2nd incubation: After addition of streptavidin-coated particles,the complex becomes bound to the solid phase via interaction of biotin and streptavidin.

The reaction mixture is aspirated into measuring cell where the microparticles are magnetically captured onto the surface of electrode. Unbound substances are then removed with procell.Application of a voltage to the electrode then induce chemiluminescent emission which is measured by a photomultiper.

Results are determined via a calibration curve which is instrument – specifically generated by 2-point calibration and a master curve provided via the reagent barcode.

High Sensitive - CRP

Sample for High sensitivity C - reactive protein (hs-CRP) was collected in red topped vacutainer which is a tube without any anticoagulant. The blood samples for Interleukin -6 (IL-6) and Interleukin -18 (IL-18) were collected in yellow topped vacutainer which had acid citrate dextrose as anticoagulant.

The high sensitivity CRP was tested in the Clinical Biochemistry lab using the Cobas Integra C - reactive protein (Latex). This machine utilizes the principle of Particle enhanced turbidimetric assay. Human CRP agglutinates with latex particles coated with monoclonal anti-CRP antibodies. The precipitate is determined turbidimetrically at 552nm.Sample collected was centrifuged and the serum was used for analyses. The analyzer automatically calculates the analyte concentration of each sample. The value <0.05mg/dl was considered to be normal.

Interleukin-6

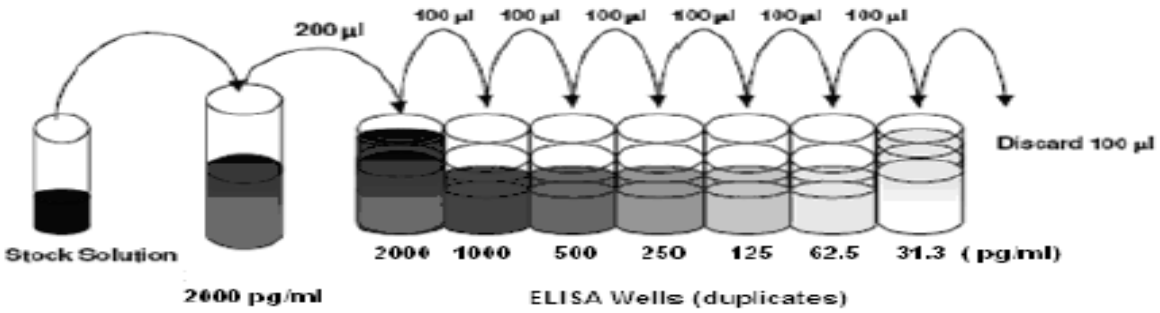
The Interleukin-6 was estimated in the Pharmacology laboratory using Krishgen Biosystems imported from California USA. The IL-6 enzyme immunoassays are programmed for quantification of human interleukin-6(IL-6). Samples and other requirements used for quantification are coated in microtiter plate which is previously coated with first monoclonal Ab, anti IL-6 in the presence of second antibody

linked to streptavidin Horseradish peroxidase (Streptavidin-HRP). After when the incubation period is over these wells are washed with the help of a colored substrate the enzyme activity is detected. This color is proportional to the IL-6 concentration in the serum sample.

Serum was obtained by allowing the blood to clot for 30 minutes and by centrifuging it for 10 mins at 1000 x g. then they were stored. Then the serum was separated carefully from clot in a separate plastic tubes and stored at < - 20 degree C.

The kit contains a plate with ready to use 96 wells. The reagents were prepared for assaying IL-6. 5 ml of 20X wash buffer was added to 95 ml and 1 ml of 5X Assay diluents to 4 ml of distilled water. 25 microliters of Detection Antibody to 9975µl of 1X Assay Diluents to get final volume to 10 ml and 50µl of streptavidin-HRP to 9950µl of 1X Assay Diluents to make final volume to 10 ml were added. Recombinant protein was diluted by adding 20µl of standard solution in 10 ml of 1X Assay Diluents and top standard solution 2000pg/ml was prepared. Six two-fold serial dilutions were prepared from the top standard solution 2000pg/ml such that concentrations are 1000pg/ml, 500pg/ml, 250pg/ml, 125pg/ml, 62.5pg/ml, 31.3pg/ml and 1X assay diluents serve as zero standards .

Figure.13: Picture of IL-6 processing in ELISA wells.



100µl/well of standards and samples were added to the plate. After sealing it was incubated at 37 degree C for 1 hour and 30 minutes. The wells were washed at least for 4 times using the wash buffer 1X and the plate was turned upside-down and tapped firmly onto a clean absorbent paper.100µl of diluted Detection Antibody was added to each well and the plate was sealed and incubated at 37 degree c for 1 hour and 30 minutes. Again the wells were washed using wash buffer 1X and 100µl of diluted streptavidin-HRP was added to each well after which the plate was sealed and incubated at 37 degree c for 30 minutes. The wells were washed again four times and this time the wells were soaked in wash solution for 30 seconds to 60 seconds in between each wash to minimize the background error. Finally, freshly prepared TMB substrate 100µl was added to each well and incubated in the dark for 15-30 minutes. The wells showing positive reaction turned blue in color after which the reaction was stopped by adding 100µl of stop solution.

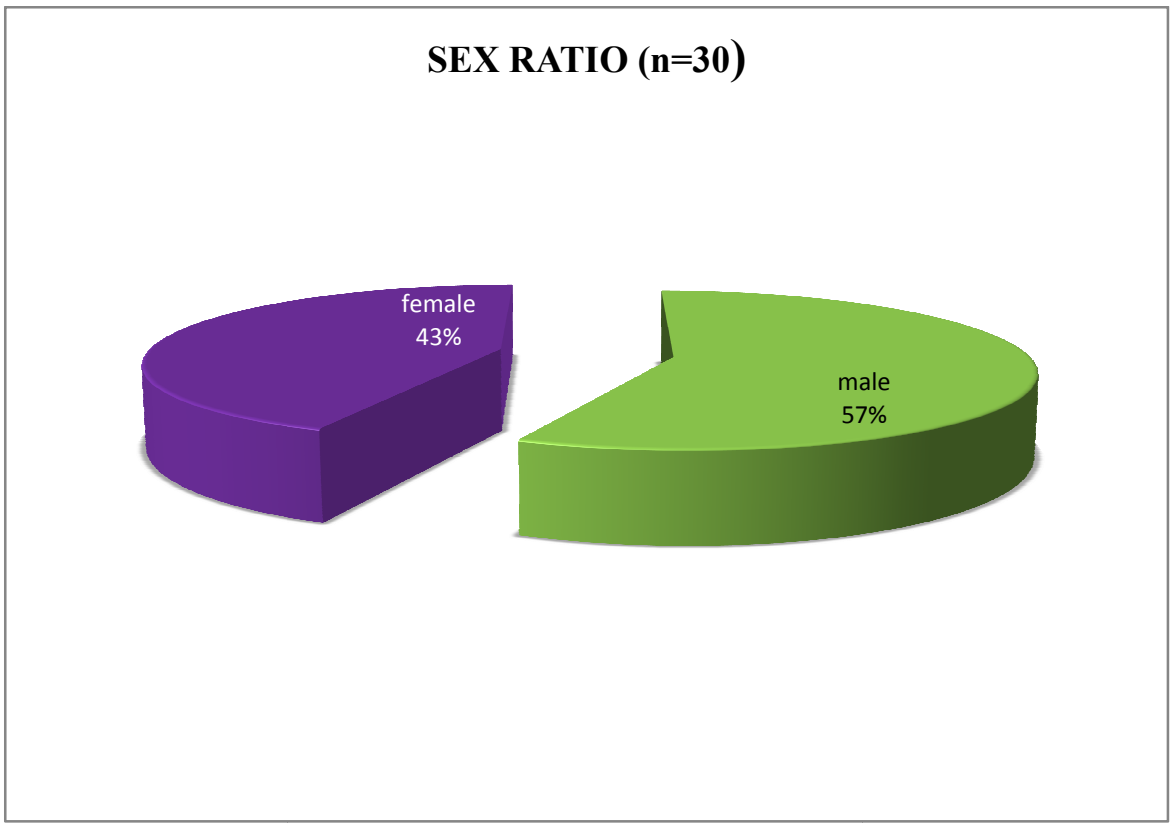
The plate was then inserted into an ELISA reader and the absorbance read at 450 nm within 30 minutes of adding stop solution. ELISA reader is a Bio-Rad system which is attached to a computer which has curve fitting software. The unknown concentrations of interleukin-6 were then identified by plotting the graph.

STATISTICAL ANALYSIS

Data were analysed using SPSS soft ware version 19.0. Between group values were interpreted using independent sample T and paired T test for before and after values in both Acarbose and Sitagliptin groups.

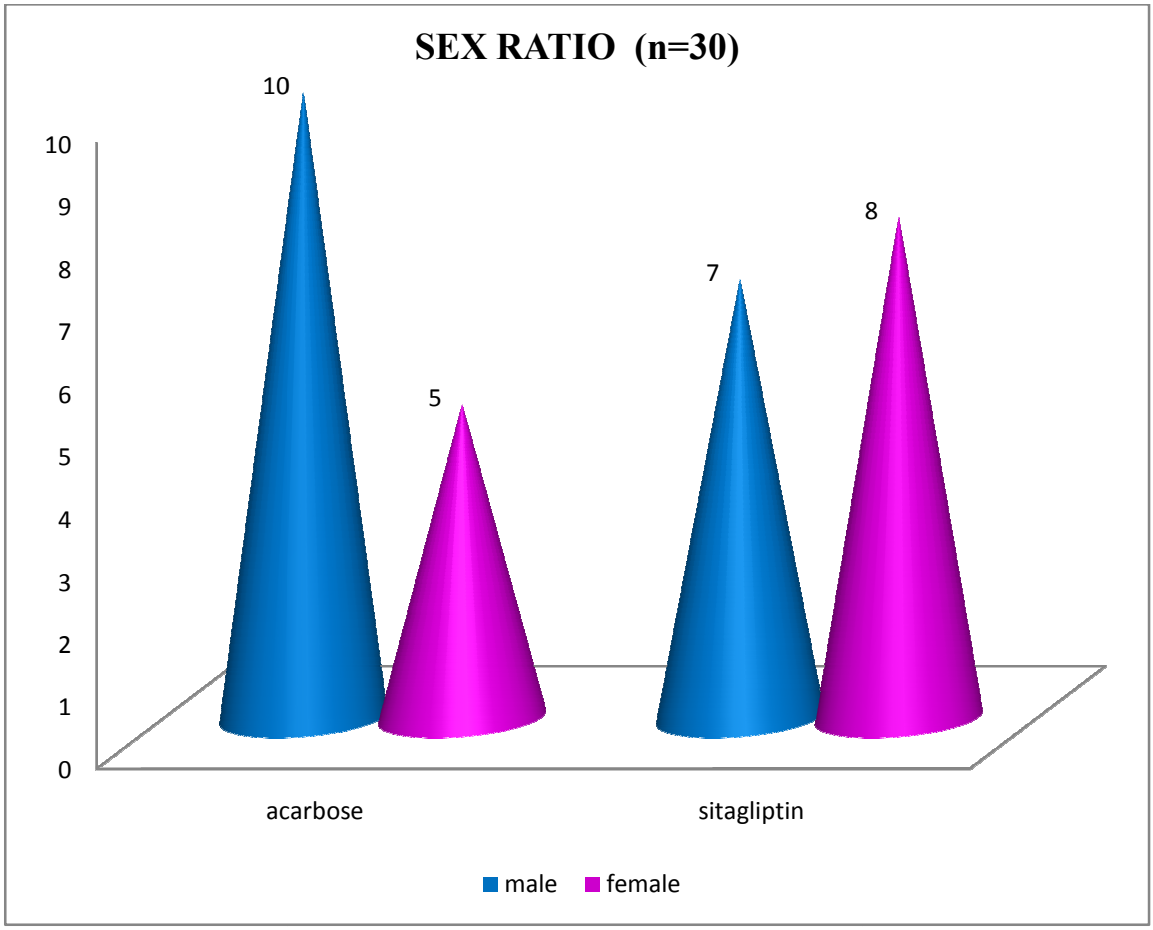
RESULTS

Figure.14: Picture representing Sex ratio



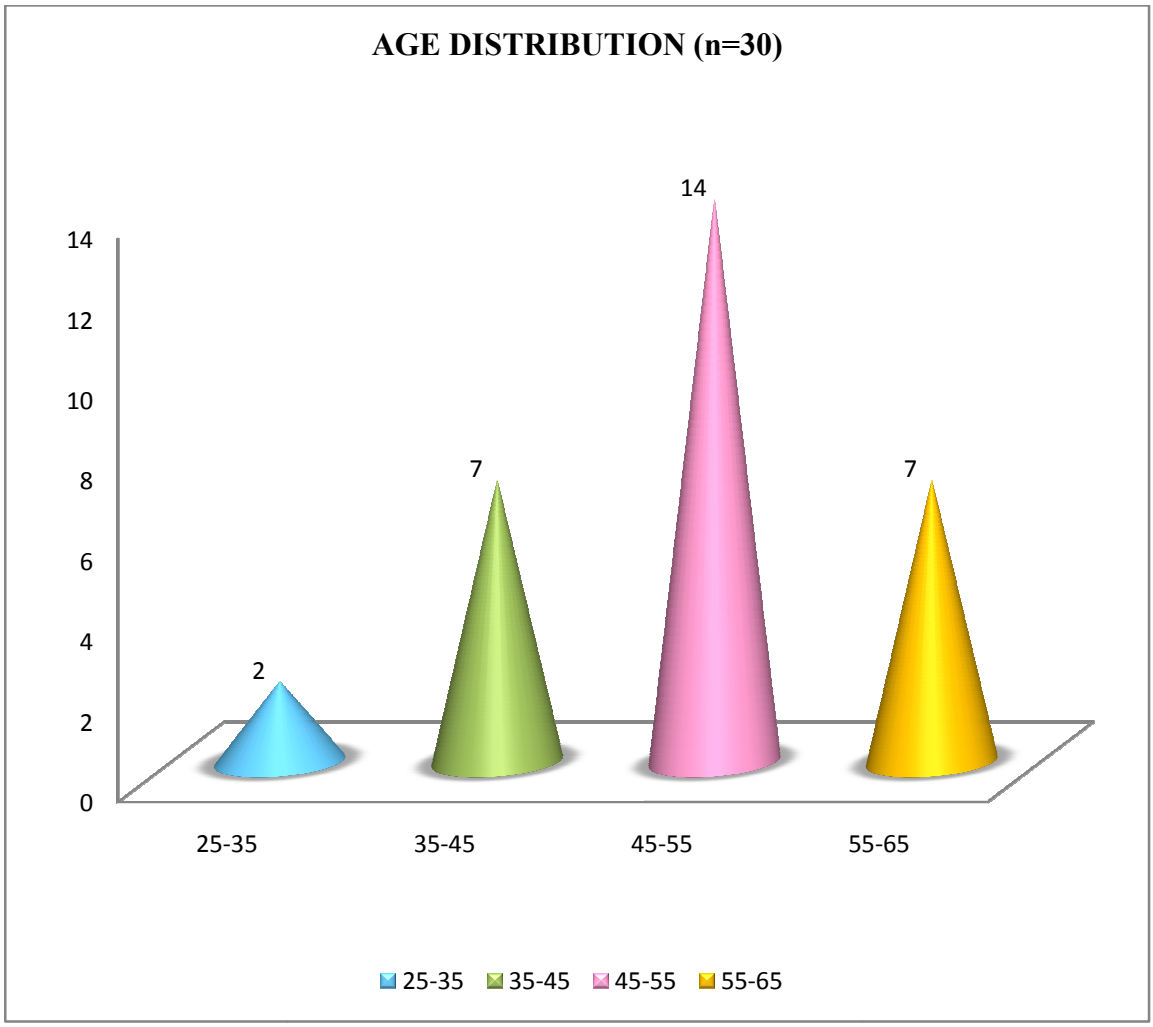
In this study total number of patients recruited was 30 in that 57% of patients were male and 43% of diabetic patients were female.

Figure. 15: Sex ratio distribution in Acarbose and Sitagliptin.



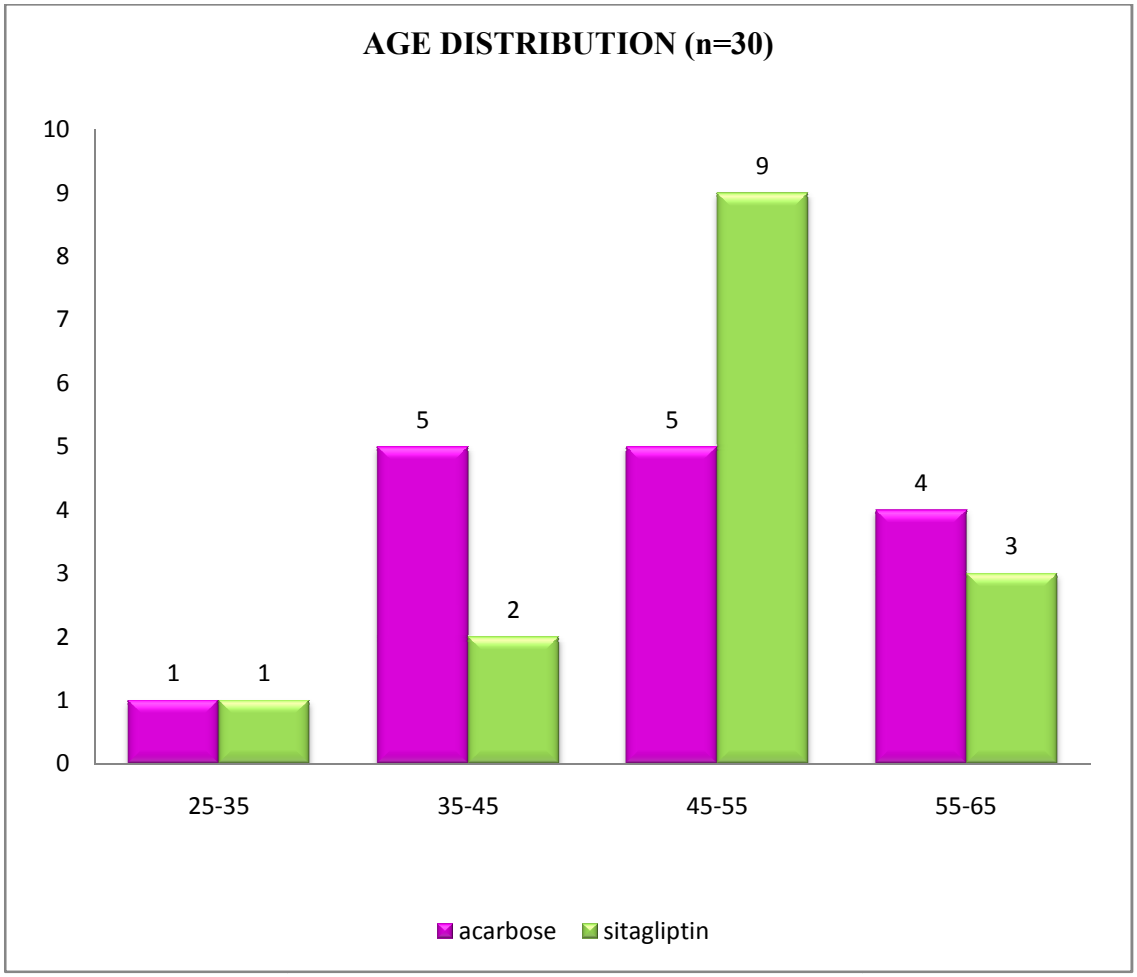
In Acarbose group (n=15), 67 % patients were male and 33% patients were female. In Sitagliptin group (n=15) 47 % patients were male and 53 % patients were female.

Figure. 16: Age distribution among the study participants



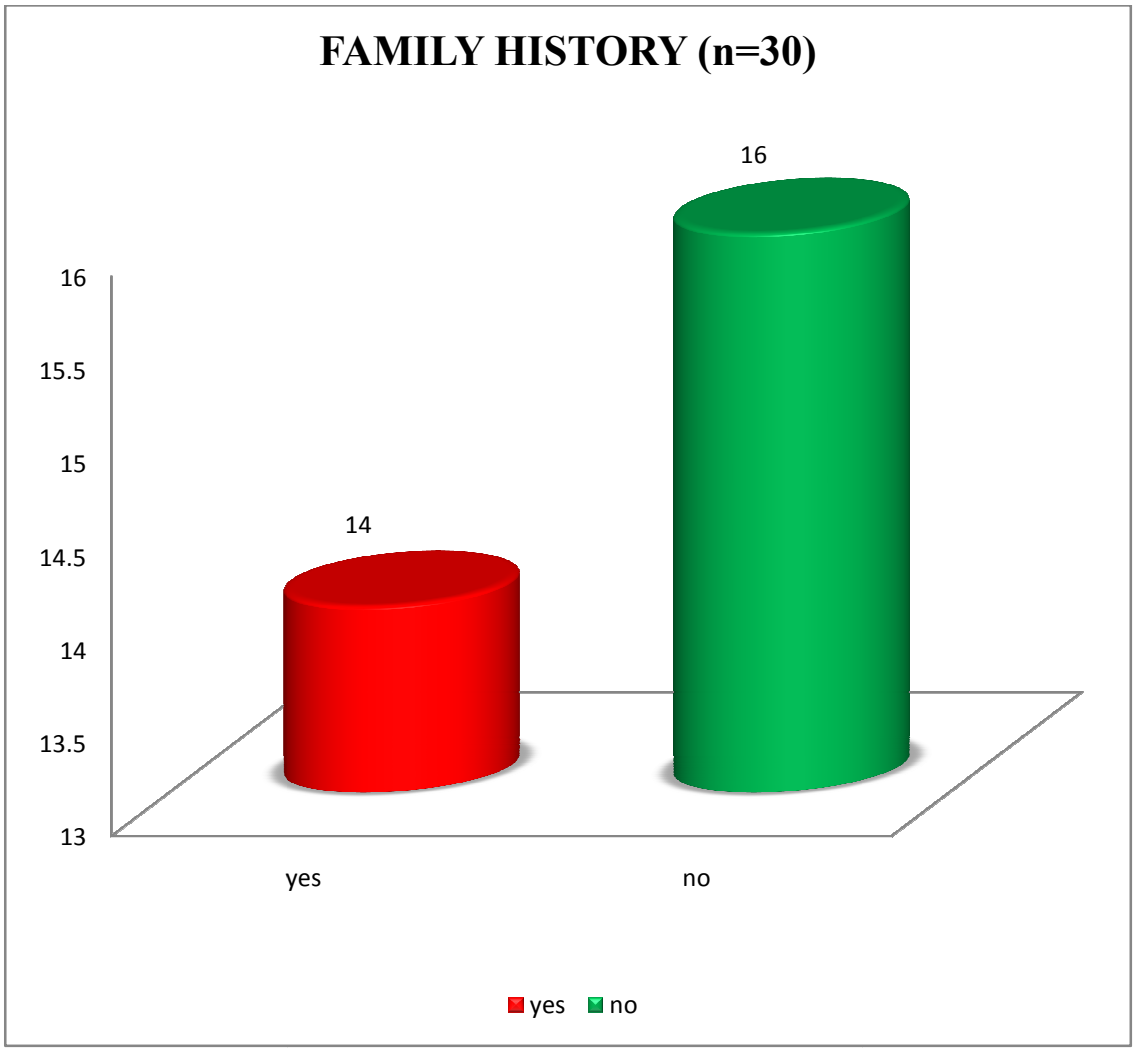
Among the total number of 30 patients 7% of patients were in 25yrs to 35 yrs of age group, 23% were in between 35 to 45 yrs of age, 47% of patients were in between 45 to 55 yrs of age group and 24% were in 55 to 65 years of age.

Figure. 17: Picture of Age distribution in Acarbose and Sitagliptin group.



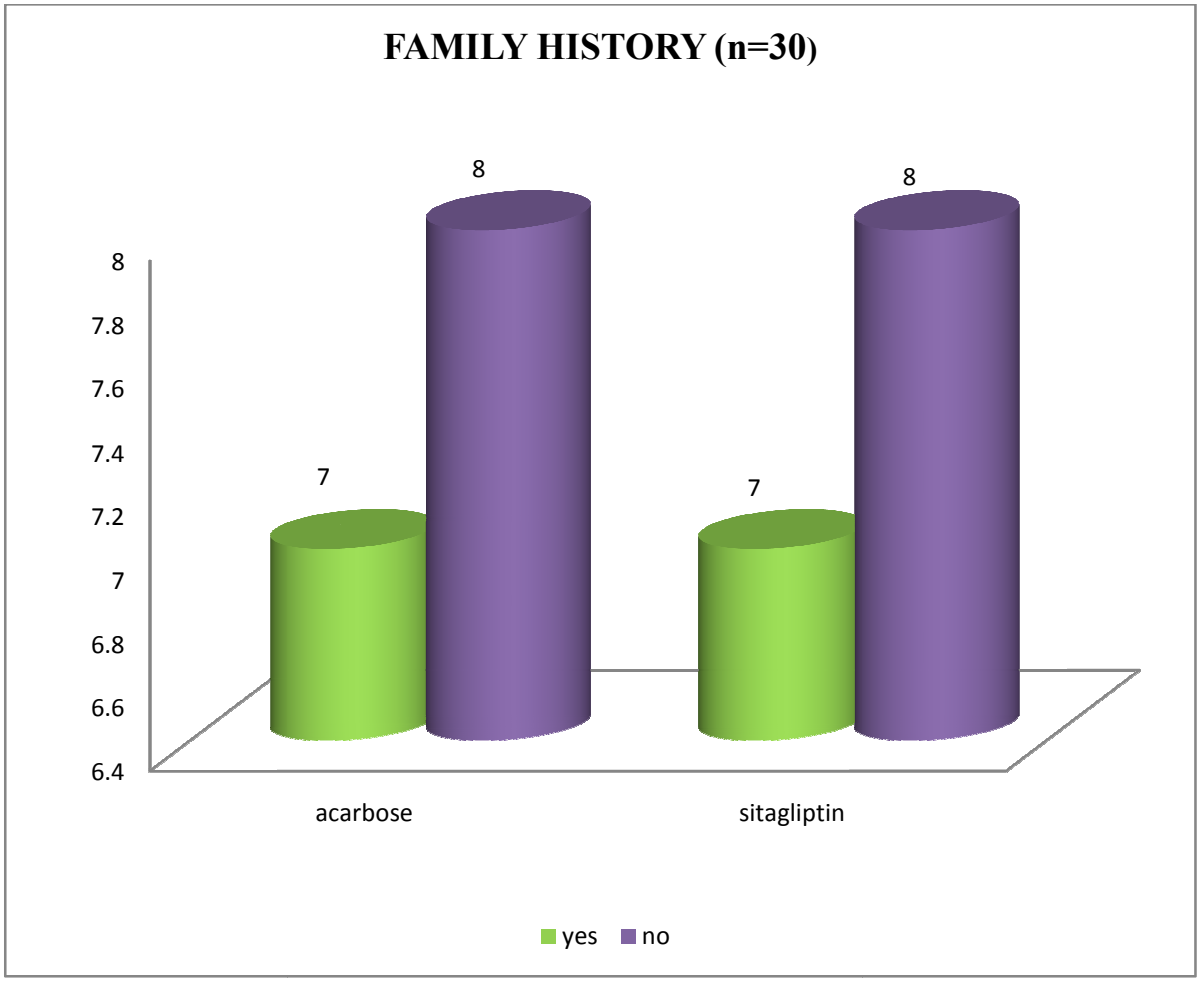
This bar diagram shows the age distribution separately in both Acarbose and Sitagliptin group. In that 47% study participants comes under 45- 55 yrs of age. Among them 33% belongs to Acarbose group and 60% patients belongs to Sitagliptin group.

Figure.18: Family history of Diabetes in Study population



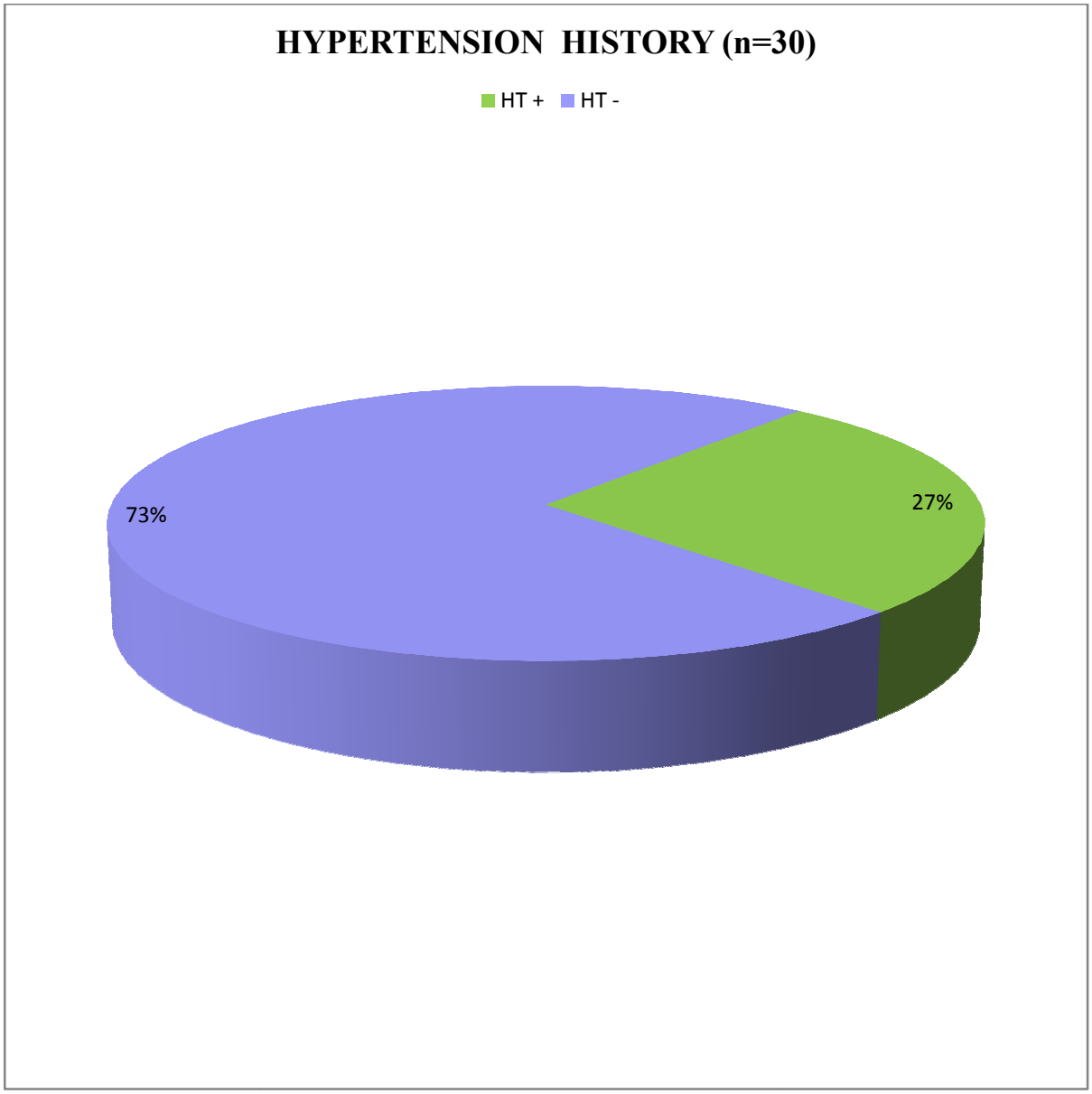
This diagram explain the total number of patients with family history of diabetes in this study (n=30), 47% patients had family history of diabetes mellitus and 53% patients had no family history of diabetes.

Figure.19: Family history of Diabetes in both Acrabose and Sitagliptin group.



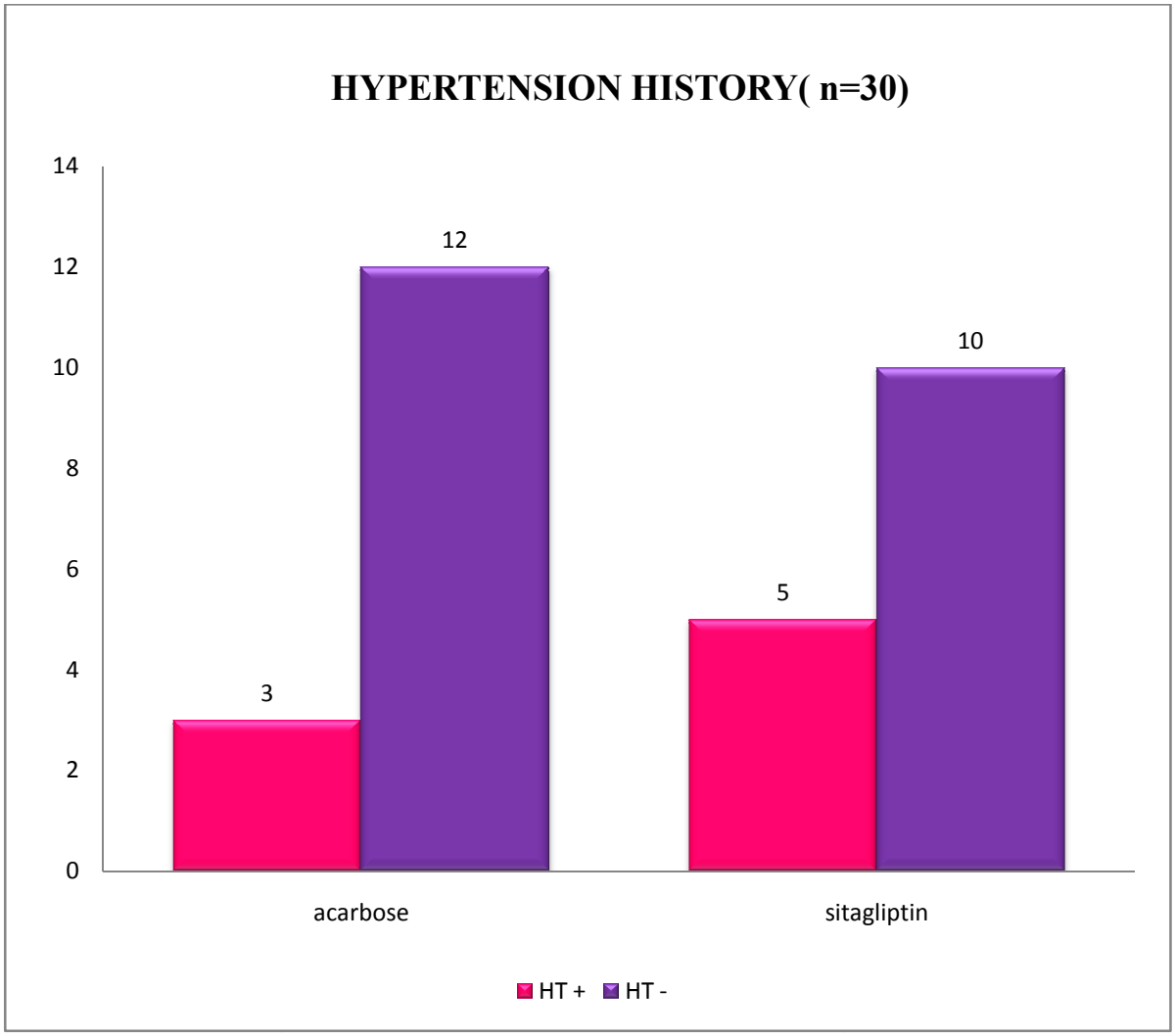
Regarding the family history of DM this diagram shows equal number of 46 % in each Acarbose and Sitagliptin group having positive family history and 53% in each Acarbose and Sitagliptin have no family history of diabetes .

Figure.20: History of Hypertension among the Study Population.



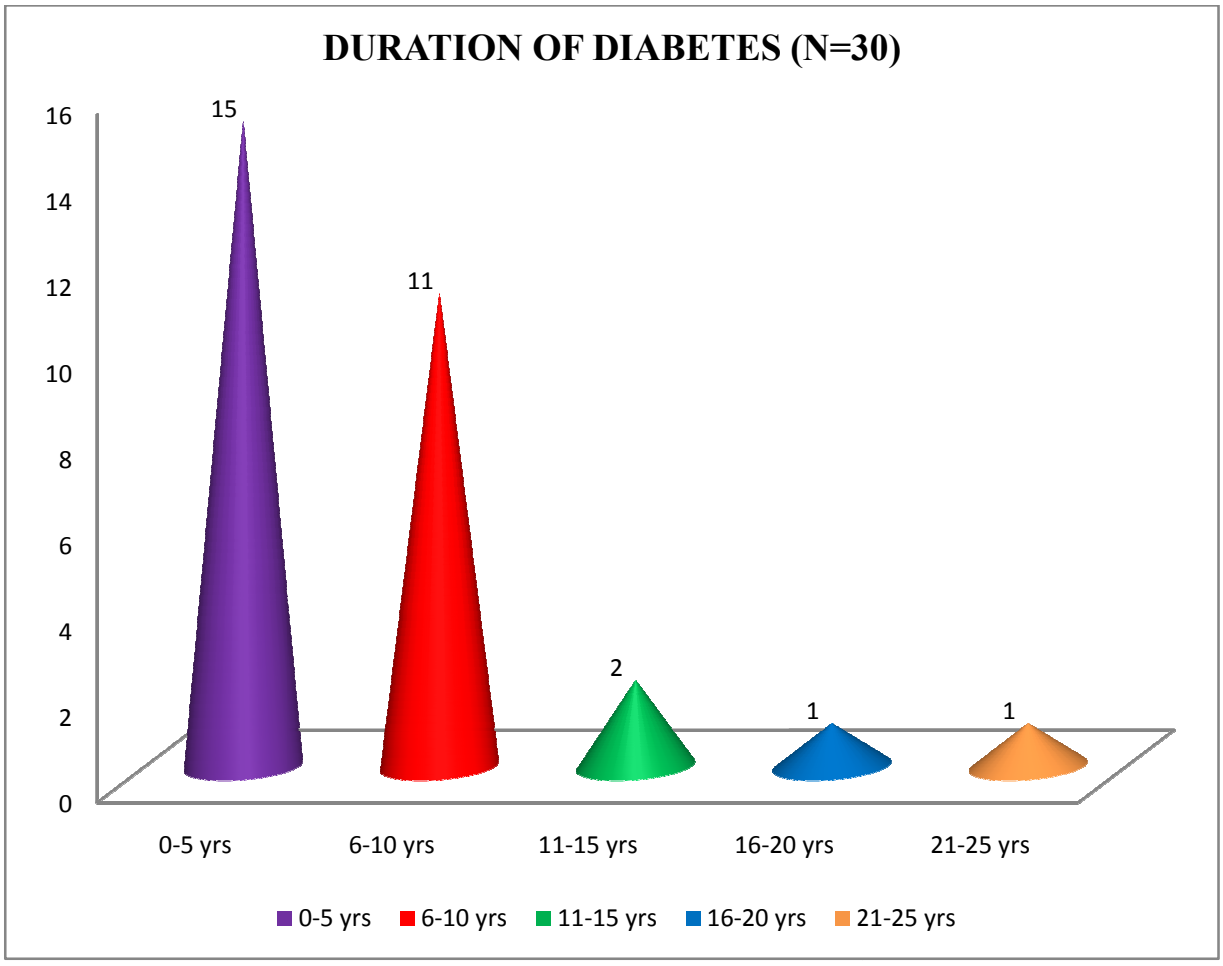
This Pie chart explains the history of hypertension associated with diabetes. 27% of patients had history of hypertension in this study and the remaining 73% had no history of hypertension.

Figure.21: History of Hypertension in Acarbose and Sitagliptin group.



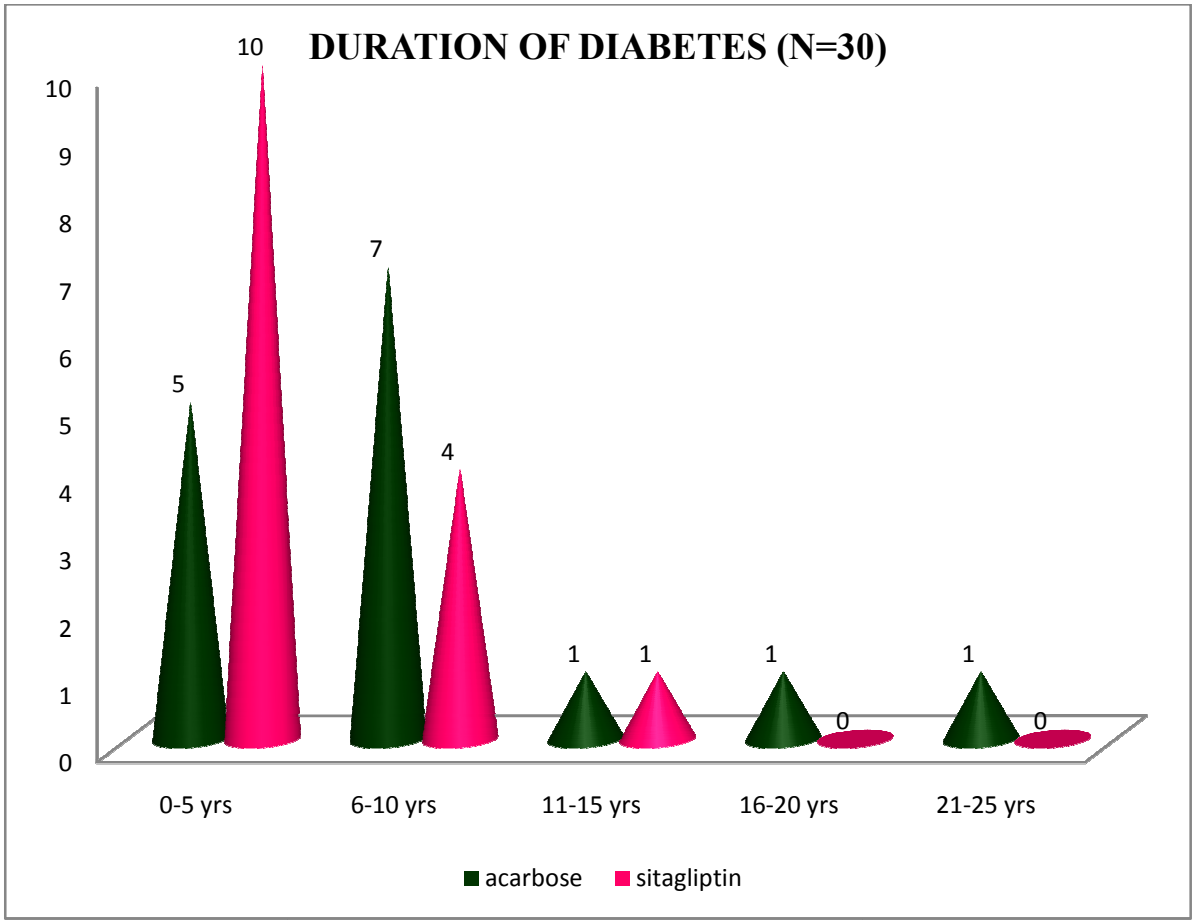
When comparing the association of hypertension in this study 20% had positive family history in Acarbose group and 34% patients had positive family history in Sitagliptin group. History of other associated diseases, like 10% of study patients had history of hypothyroid and they were on regular treatment.

Figure.22: Duration of Diabetes in the Study Population



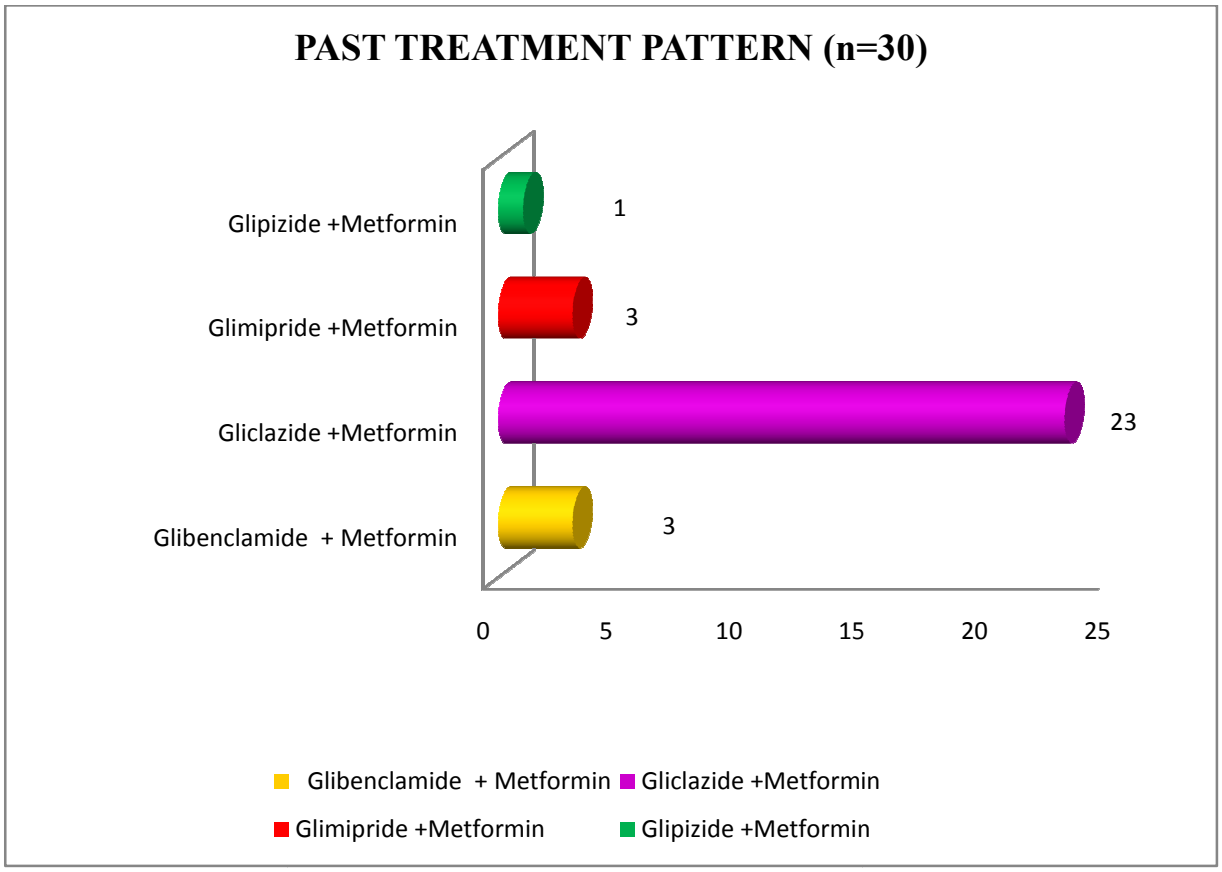
Regarding the duration of diabetes, in this study 50% were in between 0-5 years of diabetes, 37% of patients were in between 6-10 yrs ,7% of patients were in between 11-15 yrs , 4% of patients were in between 16-20 years and another 4% of patients were in between 21-25 years of duration of Diabetes Mellitus .

Figure.23: Duration of Diabetes in Acrabose and Sitagliptin group



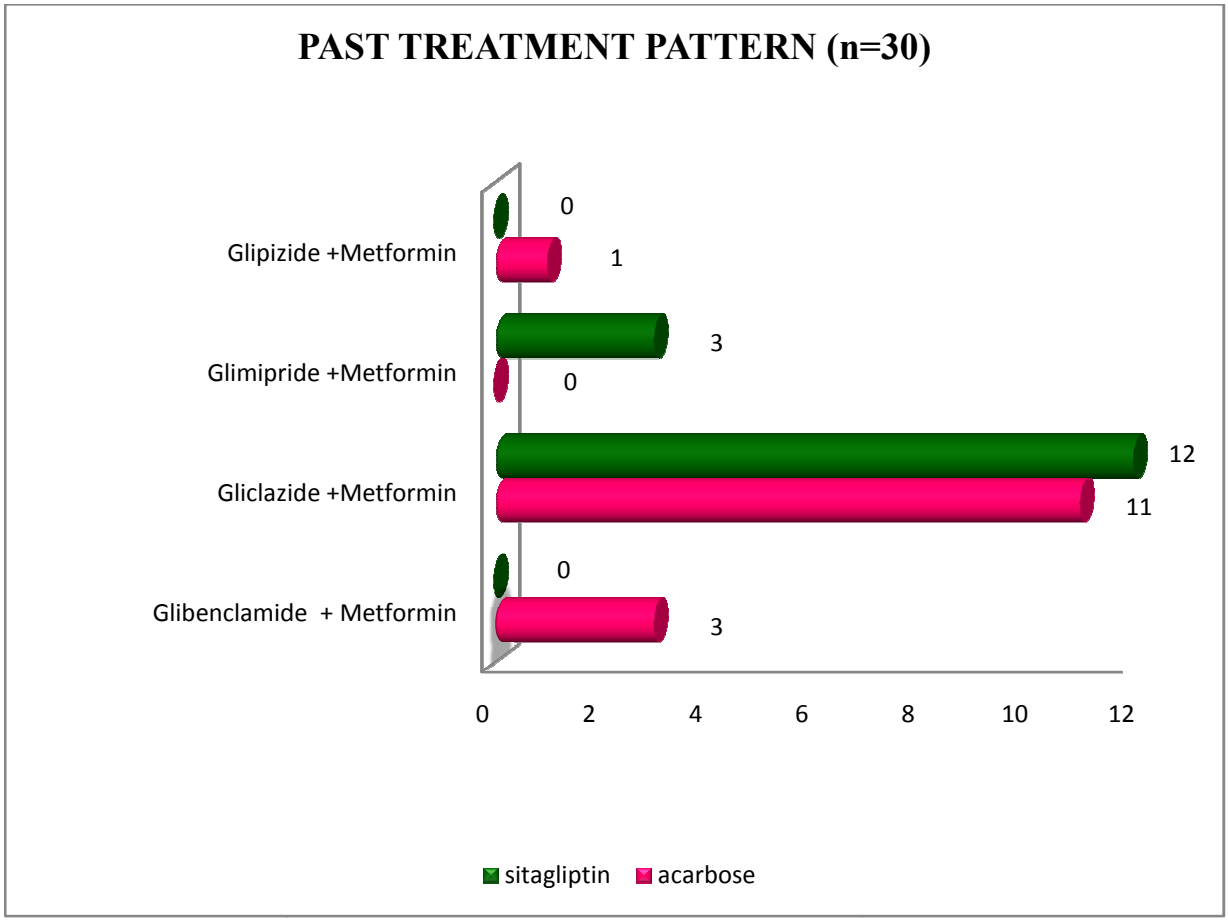
This diagram shows the duration of diabetes separately in both Acarbose and Sitagliptin group. Maximum of 33% of study population lie in-between 0-5 years duration of diabetes in Acarbose group and 67% of study participants lie in between 0-5 years in Sitagliptin group. This mainly implied the chronicity and progression of disease pattern in these study participants.

Figure.24: Past treatment pattern in the Study Population.



This diagram explains mainly the past treatment pattern of this study participants in which 77% were on Gliclazide and Metformin combination ,remaining 10% of patients were on Glibenclamide ,Glimipride and metformin combination and 3 % of patients were on Glipizide and Metformin combination.

Figure.25: Past treatment pattern in Acarbose and Sitagliptin group.



This diagram again explains the past treatment pattern in each Acarbose and Sitagliptin group separately. As seen in the before diagram (Fig.24) 77% of patients were on Gliclazide and metformin treatment of the total population. . Among the Gliclazide and metformin treatment patients 73% were in Acarbose group and 80% of patients were in Sitagliptin group.

Figure.26: Body mass index of the study population

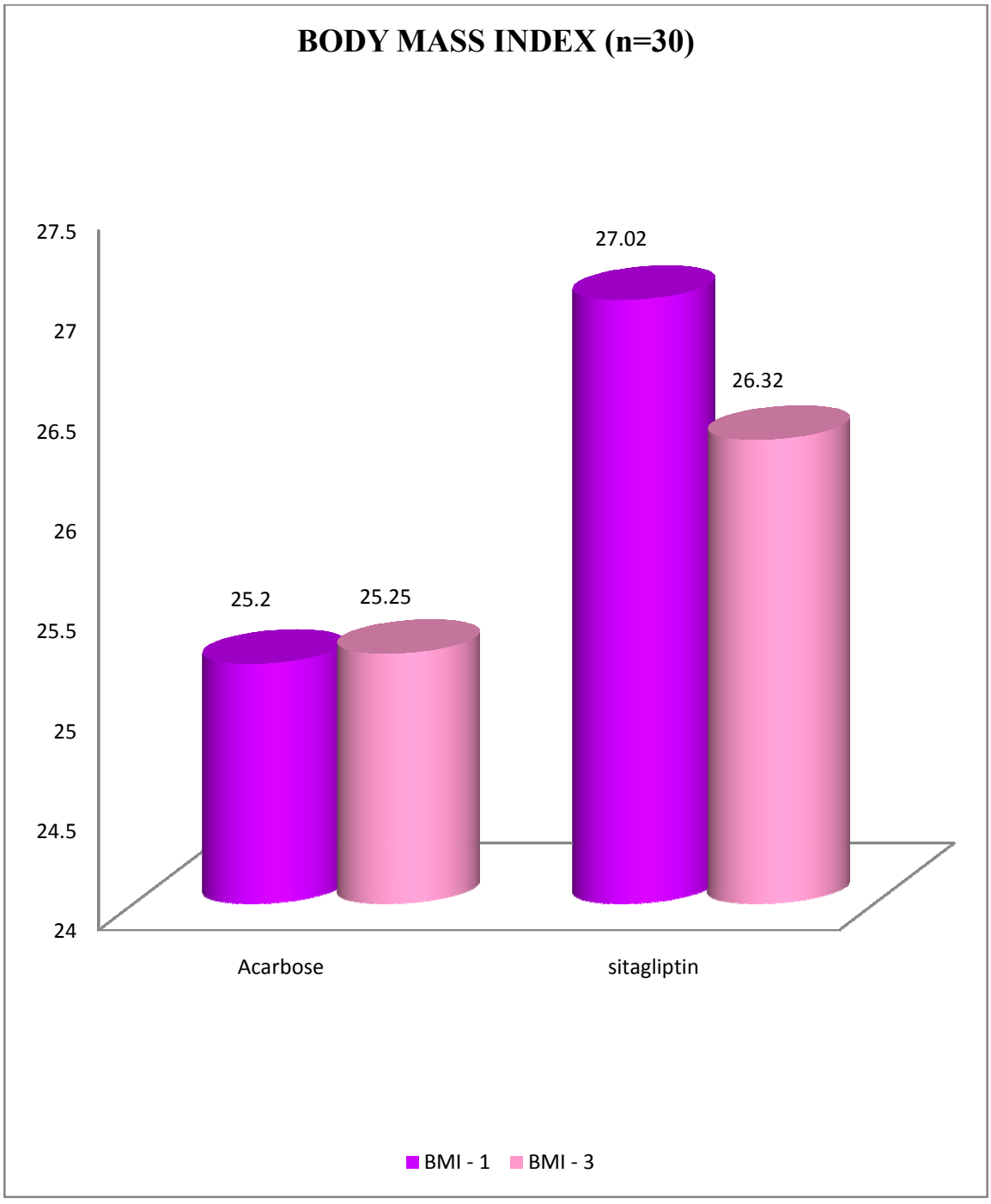


Table.1 Body Mass Index in Acarbose and Sitagliptin groups.

	Acarbose(Mean +SD)	**Sitagliptin (Mean + SD)**
BMI-1	25.20 ± 2.84	27.02 ±4.64
BMI-3	25.25 ±2.83	26.32 ±4.81
Mean difference	-.056 ± 0.79	0.69 ±1.04
p value	0.796	0.033

Results of Body Mass Index in Acarbose and Sitagliptin groups showed no statistical significance even though there is greater reduction in Sitagliptin group in which mean value decreased from 27.02 ± 4.64 SD to 26.32 ± 4.81 SD (p value 0.033) with 95% confidence interval.

Figure.27: Fasting Blood Sugar status of the Study Population

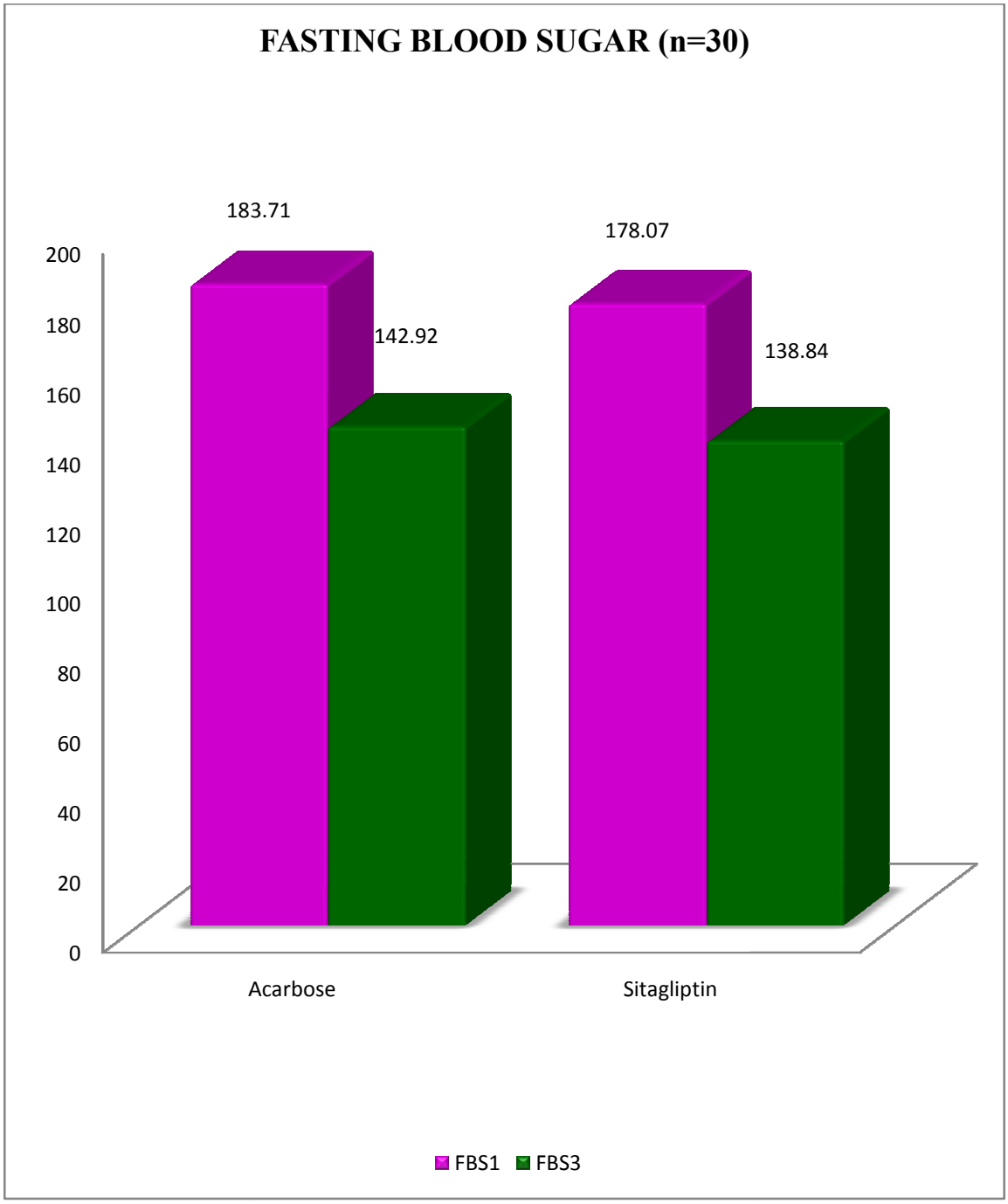


Table.2: FBS in Acarbose and Sitagliptin groups.

	Acarbose(Mean +SD)	Sitagliptin(Mean + SD)
FBS-1	183.71 ± 44.99	178.07 ± 46.73
FBS-3	142.92 ±33.60	138.84 ±25.13
Mean difference	40.78 ±47.75	39.23 ± 52.50
p value	0.007	0.020
95 % CI	13.21 – 68.35	7.50 -70.95

Results of FBS in this study showed reduction in mean value in both Acarbose and Sitagliptin group ranging from 183.71 ± 44.99 SD to 142.92 ±33.60 SD, 178.07 ± 46.73 SD to 138.84 ±25.13 SD respectively. This was of statistical significance with p value 0.007, 0.020 respectively. There was no significant difference seen in the reduction of FBS on comparing Acarbose and Sitagliptin group (p value = 0.725).

Figure.28: Post Prandial Blood Sugar in study population

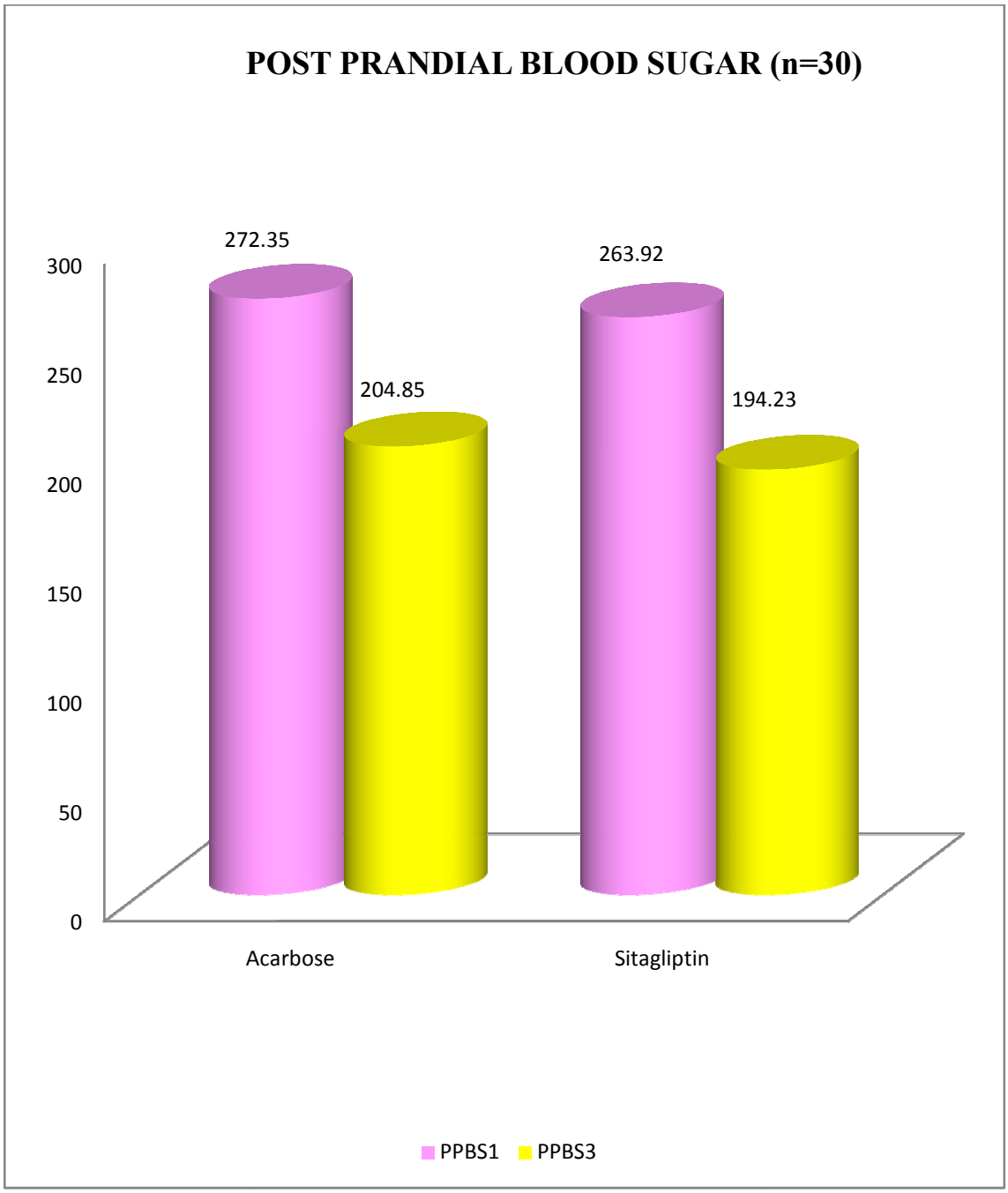


Table .3: PPBS in Acarbose and Sitagliptin groups.

	Acarbose(Mean +SD)	Sitagliptin(Mean + SD)
PPBS -1	272.35 ± 64.08	263.92 ± 101.60
PPBS – 3	204.85 ± 57.97	194.23 ± 44.50
Mean difference	67.50 ± 76.83	69.69 ± 98.3
p value	0.006	0.025
95 % CI	23.13 – 111.86	10.24 -129.14

Results of PPBS in this study showed reduction in mean value in both Acarbose and Sitagliptin group ranging from 272.35 ± 64.08 SD to 204.85 ± 57.97 SD , 263.92 ± 101.60 SD to 194.23 ± 44.50 SD respectively. This was of statistical significance with p value 0.006, 0.025 respectively. But there was no significant difference seen in the reduction of PPBS on comparing Acarbose and Sitagliptin group (p value = 0.600).

Figure.29:HbA1c status of the study population.

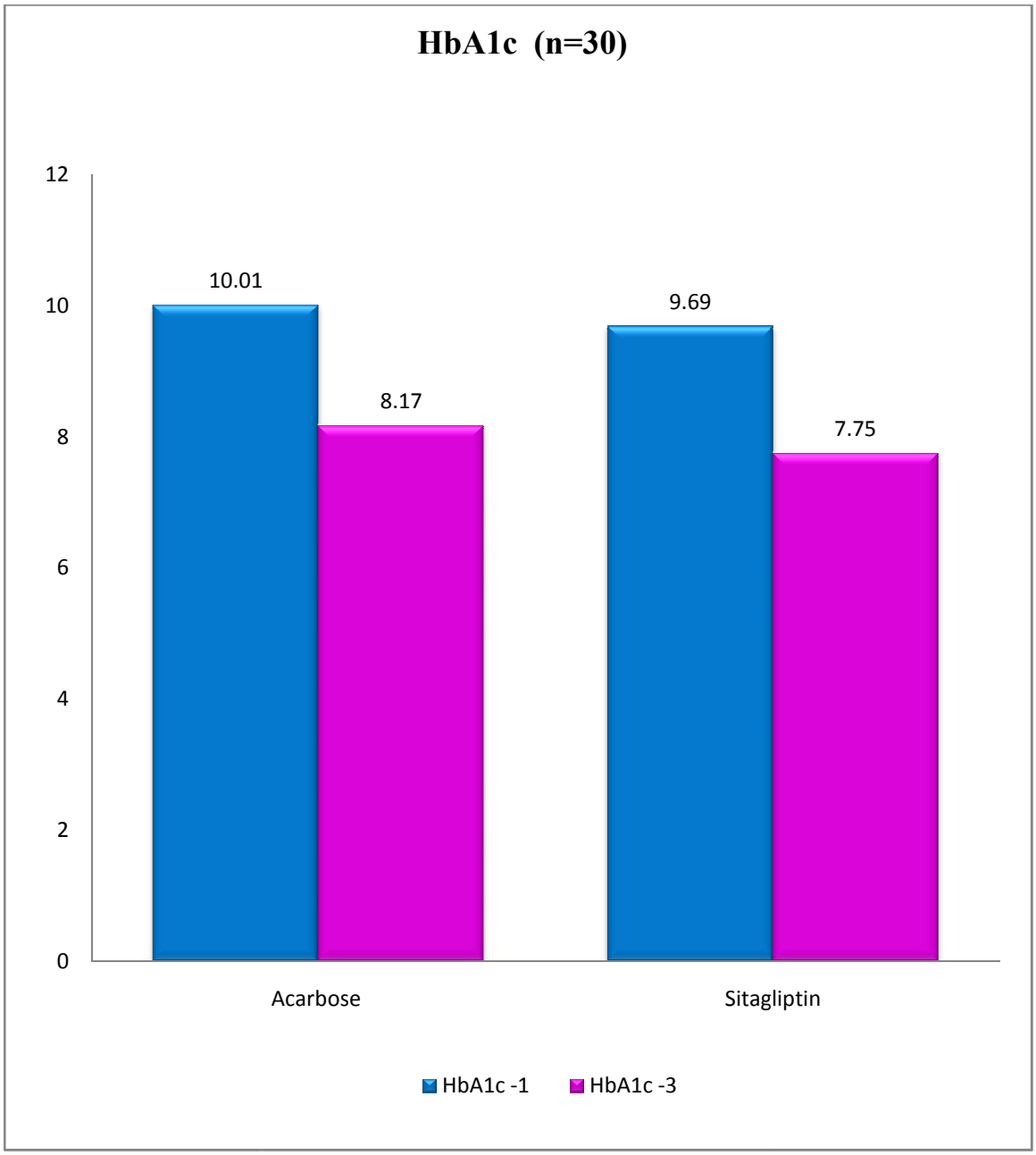


Table.4: HbA1c status in Acarbose and Sitagliptin groups.

	Acarbose(Mean +SD)	Sitagliptin(Mean + SD)
HbA1c-1	10.01 ± 1.51	9.69 ± 1.53
HbA1c – 3	8.17 ± 1.28	7.75 ± 1.32
Mean difference	1.83 ± 1.67	1.94 ± 1.99
p value	0.001	0.004
95 % CI	1.018 – 2.80	1.043 – 3.14

Results of HbA1c in this study showed reduction in mean value in both Acarbose and Sitagliptin group ranging from 10.01 ± 1.51 SD to 8.17 ± 1.28 SD , 9.69 ±1.53 SD to 7.75 ± 1.32 SD respectively. This was of statistical significance with p value of 0.001, 0.004 respectively. But there was no significant difference seen in the reduction of HbA1c on comparing the after values of Acarbose and Sitagliptin group (p value =0.403).

Figure.30: Plasma Insulin status of the Study population

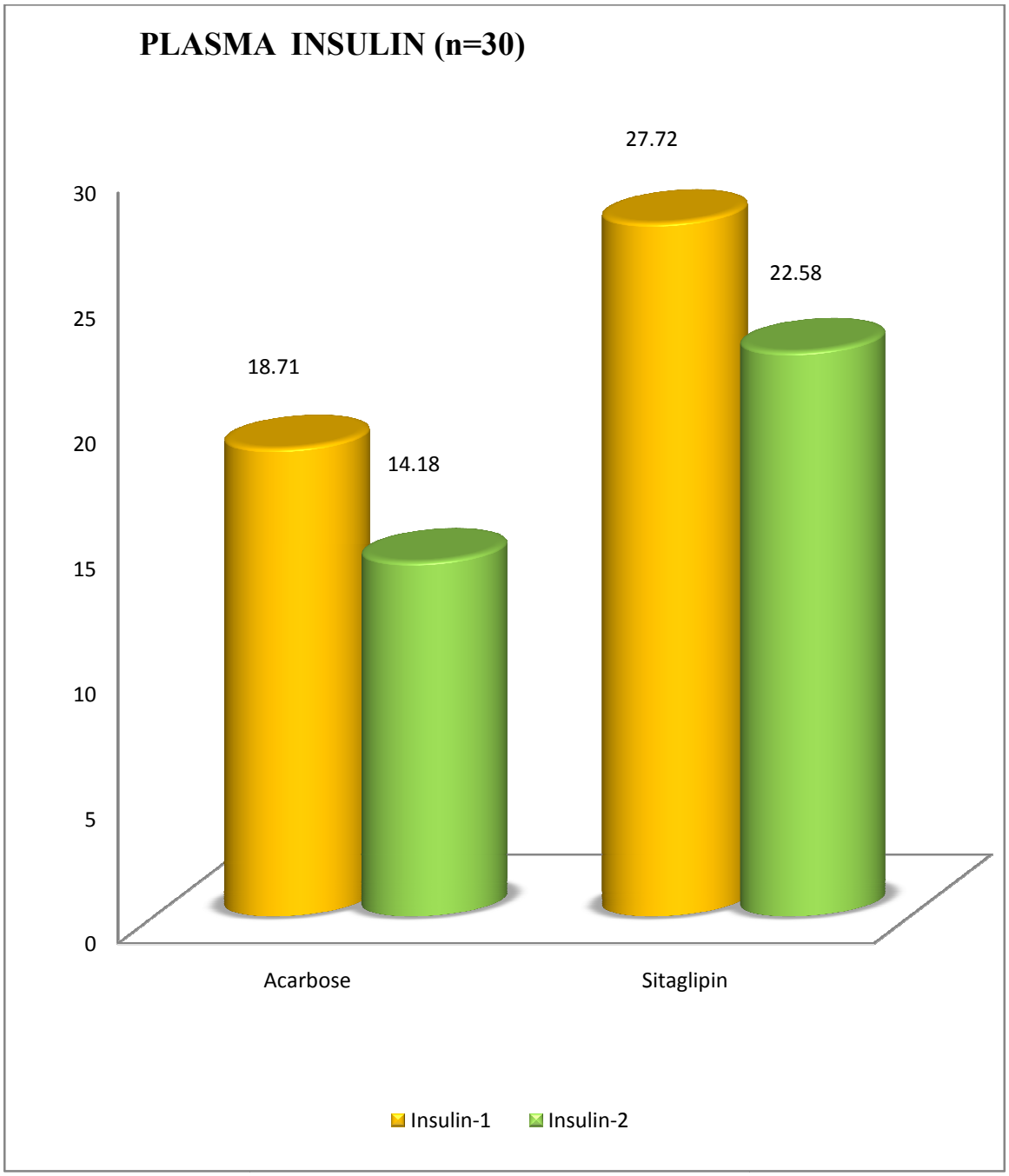


Table. 5: Plasma Insulin status in Acarbose and Sitagliptin groups.

	Acarbose(Mean +SD)	Sitagliptin(Mean + SD)
Insulin -1	18.71 ± 16.42	27.72 ± 25.98
Insulin – 3	14.18 ± 6.71	22.58 ± 17.68
Mean difference	4.52 ± 16.60	5.13 ± 17.9
p value	0.327	0.324

Results of Plasma Insulin in this study showed reduction in mean value in both Acarbose and Sitagliptin group ranging from 18.71 ± 16.42 SD to 14.18 ± 6.71 SD , 27.72 ±25.98 to 22.58 ± 17.68 SD respectively. This was of no statistical significance because of p value = 0.327, 0.324 respectively. There was also no significant difference seen in the reduction of Plasma Insulin on comparing the after values of Acarbose and Sitagliptin group (p value =0.110).

Figure.31: Insulin Resistance of the study population

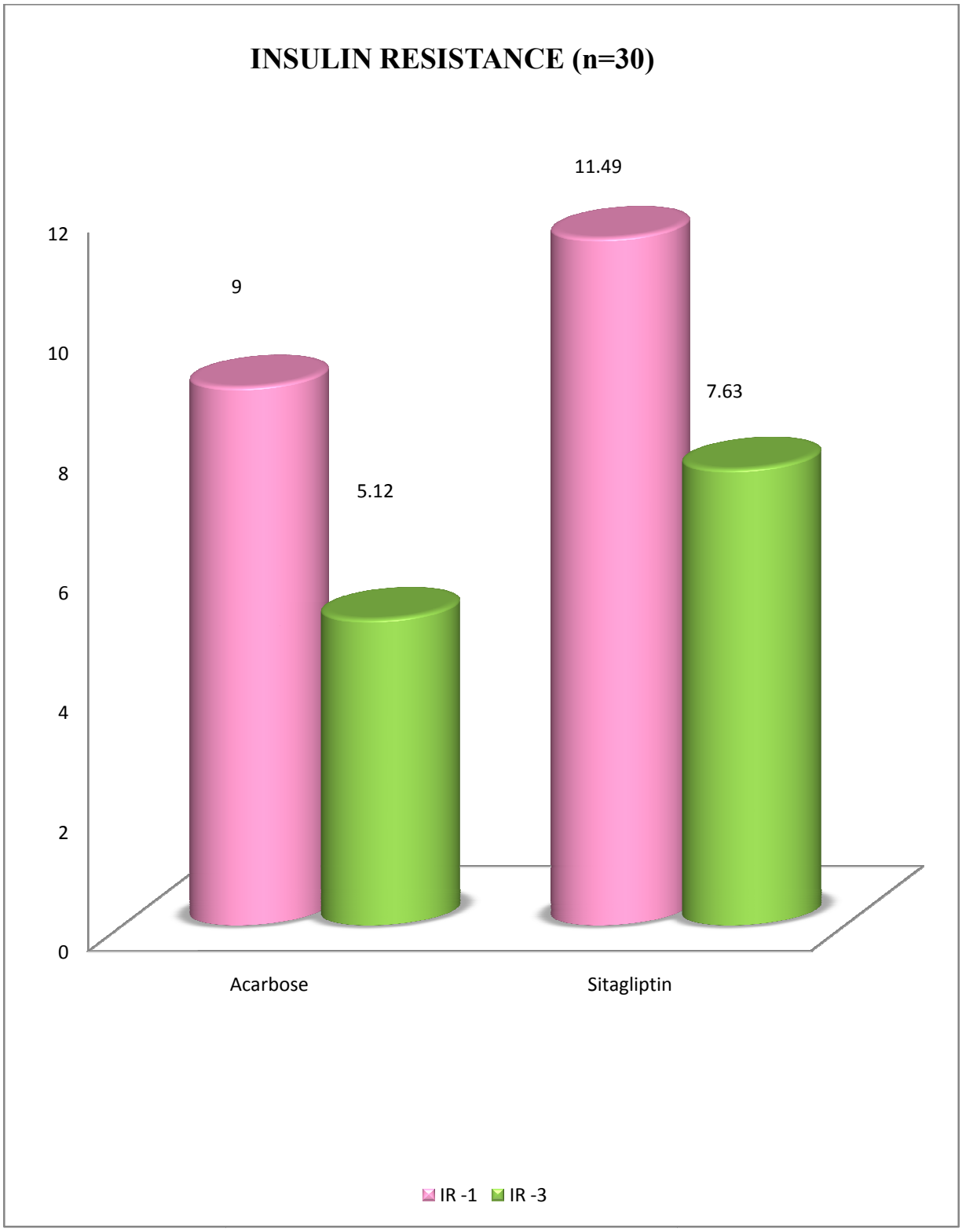


Table. 6: Insulin Resistance status in Acarbose and Sitagliptin groups.

	Acarbose(Mean +SD)	Sitagliptin(Mean + SD)
IR-1	9.00 ± 8.38	11.49 ± 9.42
IR – 3	5.12 ± 3.14	7.63 ± 5.78
Mean difference	3.88 ± 8.19	3.86 ± 7.84
p value	0.100	0.101

Results of IR in this study showed reduction in mean value in both Acarbose and Sitagliptin group ranging from 9.00 ± 8.38 SD to 5.12 ± 3.14 SD , 11.49 ±9.42 SD to 7.63 ± 5.78 SD respectively. This was of no statistical significance because of p value = 0.100 , 0.101 respectively. There was also no significant difference seen in the reduction of IR on comparing the end values of Acarbose and Sitagliptin group (p value =0.170).

Figure. 32: hsCRP status of the study population

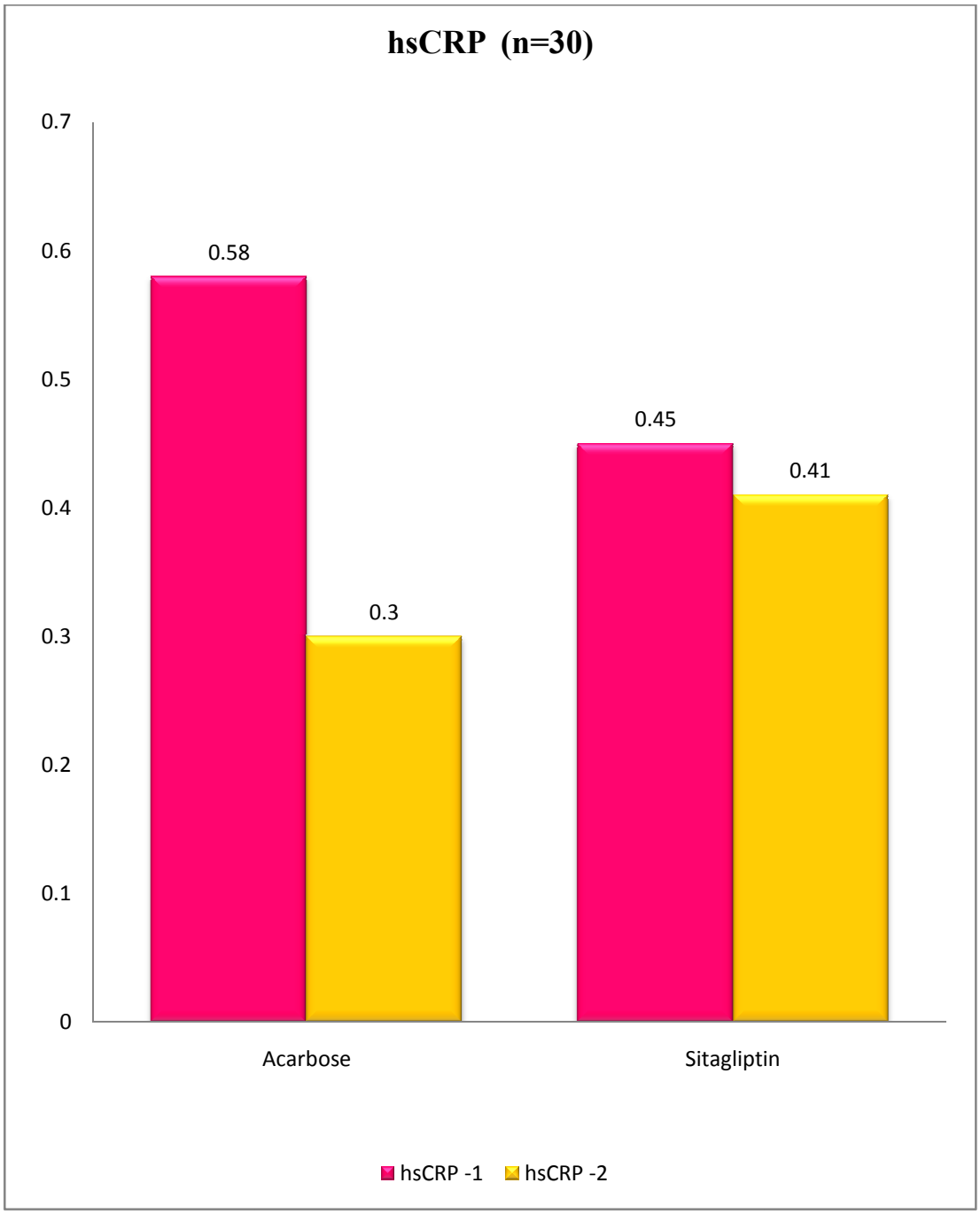


Table. 7: hcCRP status in Acarbose and Sitagliptin groups.

	Acarbose(Mean +SD)	Sitagliptin(Mean + SD)
hs CRP-1	0.58 ±0.93	0.45 ± 0.43
hs CRP – 3	0.30±0.3	0.41 ± 0.39
Mean difference	0.27 ± 0.89	0.48 ± 0.40
p value	0.265	0.671

Results of hsCRP in this study showed reduction in mean value in both Acarbose and Sitagliptin group ranging from 0.58 ± 0.93 SD to 0.30 ± 0.30 SD, 0.45 ± 0.43 SD to 0.41 ± 0.39 SD respectively. This was of no statistical significance because of p value = 0.265 , 0.671 respectively. There was also no significant difference seen in the reduction of hsCRP on comparing the end results Acarbose and Sitagliptin group (p value =0.451).

Figure.33: IL-6 status of study population

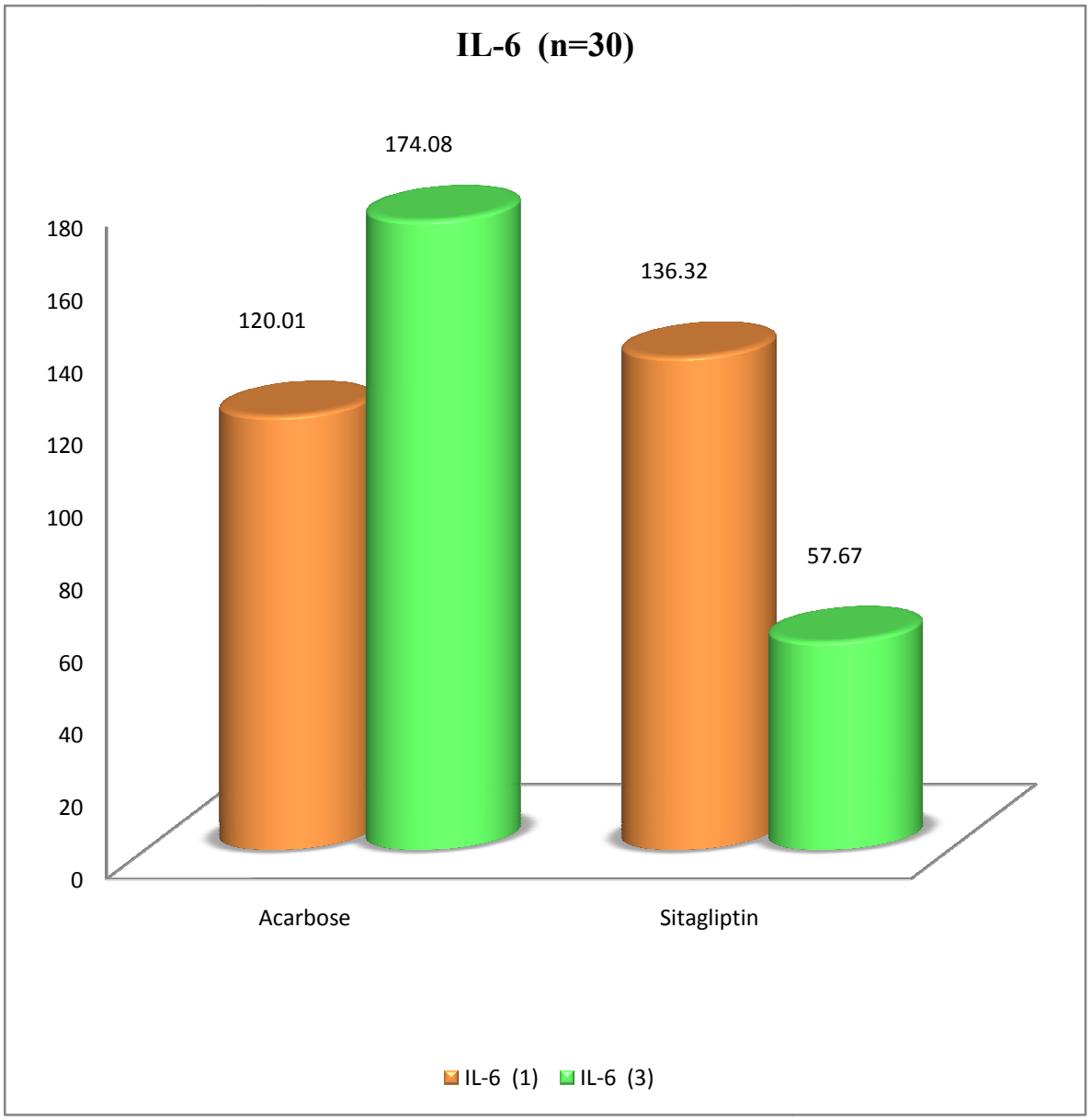


Table.8: IL-6 status in Acarbose and Sitagliptin groups.

	Acarbose(Mean ±SD)	Sitagliptin(Mean ± SD)
IL-6(1)	120.01 ± 203.3	136.32 ± 241.81
IL-6 (3)	174.08 ± 492.1	57.67 ± 78.55
Mean difference	-54.07 ±36.7	78.64 ±269.97
P value	0.591	0.314

Results of IL-6 in this study showed reduction in mean value of Sitagliptin group ranging from 136.32 ± 241.81 SD to 57.679 ± 78.55 SD (p value =0.314). But in case of Acarbose group there was little increase in IL-6 levels with the mean value ranging from 120.01 ± 203.3 SD to 174.08 ± 492.1 SD .(p value=0.591). There was also no significant difference seen in the reduction of hsCRP on comparing the after values of Acarbose and Sitagliptin group (p value =0.451).

DISCUSSION

It is a known one that, the risk of cardiovascular disorder is high in both type I and type II Diabetes Mellitus .But, the period of onset is different in both types. In case of type I DM risk of CVD arise after some decades but in type II DM, it sets very early in the asymptomatic period of hyperglycemia and even by the time of diagnosis these patients are at potential risk. This is mainly due to the Insulin Resistance, which emerges at an early stage of the start of pathogenesis in the type II Diabetes Mellitus. Many studies have proven that Insulin resistance was the main culprit which cause impairment in insulin signalling pathway and there by leading to glucotoxicity, lipogenesis, increase in free fatty acid oxidation and inflammation.

Inflammation which is one of the factors responsible for endothelial dysfunction and IR also leads to increase in platelet aggregation and all these process collectively lead to atherogenesis and cardiovascular disease ultimately. Therapeutic management is the only option to intervene these processes, and to prevent or delay the risk of macrovascular complications. TNF-α, IL-6, hsCRP are the inflammatory markers involved in the process of atherogenesis. Studies have proven that the increase in the inflammatory markers would predict the risk of cardiovascular complications at the earliest.

Few OHA's had been proved earlier to reduce the inflammatory markers along with hypoglycemic effects thereby being cardio protective. This study was aimed at comparing the inflammatory reduction property of Sitagliptin in comparison with Acarbose which was proved to elicit anti inflammatory property as an add –on drug therapy with Metformin and Sulfonylurea combination.

In this study totally 30 type 2 diabetes patients around the age group of 25-65 years, on Metformin and Sulfonylurea combination ,HbA1c value >7.5 were recruited. After getting informed consent and randomized into two groups in which one group was added on Acarbose and the other group was added with Sitagliptin along with Metformin, Sulfonylurea combinations.

Previous study by Andrew et al, has shown that Obese diabetic patients treated with metformin had lower levels of hsCRP ,expression of TNF-α and TLR 2/4, than their counterparts receiving placebo [115] .Studies have shown that Sulfonylureas didn't have any cardio protective effect because they actually prevent the cardiac preconditioning thereby preventing the damage to myocardium[84] But still Sulfonylurea effectively controls the blood glucose levels and had shown effective outcomes in the clinical parameters. Another study Eberhard Standl et al, proved that Acarbose could reduce the low grade inflammatory markers like hsCRP and NF-kB activation in type 2 diabetes patients [117,118]. More over

Studies done with Sitagliptin have also proven their anti-inflammatory effect in type 2 diabetes patients [73]. Recently there is an ongoing study on Sitagliptin as initial monotherapy for the therapeutic management of type 2 diabetes mellitus in pediatric participants. When this study proves the safety and efficacy of Sitagliptin as initial therapy along with their additional beneficial actions of anti-inflammation in pediatric type 2 diabetes patients, then it will be of great use in preventing or delaying the progression of atherogenesis in young patients.

With this back ground information Sitagliptin and Acarbose were given as an add on therapy with Metformin and Sulfonylurea combination. Thus this study aimed to compare the influence of anti-inflammatory effects of Sitagliptin and Acarbose in patients who were already on Metformin Sulfonylurea combination as the mode of treatment.

The study also aimed to compare the before and after values of these inflammatory markers in each group separately. Baseline inflammatory values were noted during the starting of treatment and those 30 patients were followed for 3 months and finally the inflammatory markers were repeated again at the end of the third month. During the study period three patients withdrew from the study due to their poor compliance.

In this study the results of BMI showed greater reduction in the Sitagliptin group with mean value from 27.02 ±4.64 SD to 26.32 ± 4.81SD, p value = 0.033 compared to the Acarbose group whose BMI value ranges from 25.20 ±2.84 SD to 25.25 ± 2.83 SD, p=0.796 (Table 1), (Fig.26). But still there is no much of statistical significance in the reduction of BMI values when compared between Acarbose and Sitagliptin groups.The study conducted in USA in the year 2012 by Antoine Makdissi et al, has proven that Sitagliptin potentially reduces the BMI in type 2 Diabetes Mellitus patients . This study results were consistent with his study.

Study conducted by Michael J Theodorakis has shown reduction in fasting glucose levels after Acarbose therapy [116] .and another study showed that in Sitagliptin therapy, the fasting blood glucose was reduced after 12 weeks of therapeutic management [73].

Regarding the results of fasting blood glucose in the Acarbose treated group, the mean value was 183.71 ± 44.99 SD at the starting of the study. It was reduced at the end of the three months to the mean value of 142.92± 33.60 SD (Fig.27). The p value was 0.007 which was statistically significant with 95% confidence interval. (Table.2)

In the Sitagliptin group mean value of fasting blood glucose at the starting of the study was 178.07±46.73 SD and it was found to be reduced at the end of the three months with the mean value of 138.84±25.13 SD

(Fig 27). The p value was 0.020 which was statistically significant with 95% confidence interval.(Table.2)

Fasting blood glucose at the end of the three months was compared between Acarbose and Sitagliptin group. It was found that Acarbose group reduces the FBS levels to the mean value of 142.92 ±33.60 SD compared to the Sitagliptin group which reduced the FBS levels to the mean value of 138.84±25.13 SD. Although the mean value was reduced greater in Acarbose group but it was not statistically significant because p value was found to be 0.725.

Studies have proven that, there was reduction in the postprandial blood glucose when added Acarbose additionally with other oral hypoglycemic agents [116].

In this study results of postprandial blood glucose in the Acarbose group, at the starting of the treatment was with the mean value 272.35±64.08 SD and at the end of the three months the PPBS was reduced to the mean value of 204.85± 57.97 SD(Fig.28). The p value was 0.006 which was statistically significant with 95% confidence interval (Table.3).

In the Sitagliptin group the mean value of postprandial blood glucose level at the starting of therapy is 263.92 ±101.60 SD it was reduced to the mean value of 194.23±44.50 SD at the end of the three

months (Fig 28) . The p value was 0.025 which was statistically significant. (Table.3).

PPBS levels at the end of three months were compared between Acarbose and Sitagliptin group. It was found that PPBS levels of Sitagliptin group was reduced to the mean value of 194.23±44.50 SD compared to the Acarbose group ,whose mean value of PPBS at the end of the three months was found to be 204.85±57.97 SD. But finally there was no statistical significance in the reduction of PPBS between both the groups (Fig 28)

Many international studies showed the anti-inflammatory effects of Sitagliptin, with 0.7% reduction in the HbA1c value [73] .In the same way in this study in Acarbose and Sitagliptin treated group the mean value of HbA1C at the beginning of the study period was found to be 10.01±1.51 SD and 9.69±1.53 SD respectively. The mean value of HbA1c was reduced in both the groups at the end of the three months to the mean value of 8.17±1.28 SD and 7.75±1.32 SD and p values were 0.001 and 0.004 respectively with statistical significance (Fig.29) (Table.4). But in this study, when compared the mean values of HbA1C levels at the end of the three months in Acarbose and Sitagliptin groups , it was found that Sitagliptin group showed greater reduction with the mean value of 7.75±1.32 SD compared to the Acarbose group whose mean value was

found to be 8.17±1.28 SD (Fig.29) . Those results were not statistically significant.(p value = 0.403).

Next parameter which was analysed in this study was regarding the plasma insulin levels and the Insulin resistance. At the starting of the study, the mean value of Plasma insulin in Acarbose and Sitagliptin group were found to be 18.71±16.42 SD and 27.72±25.98 SD respectively.(Table.5) (Fig.30). The results of plasma insulin in Acarbose and Sitagliptin groups at the end of the three months were found to be reduced to 14.18±6.71SD and 22.58±17.68 SD with p value=0.327 and 0.324 respectively. Though the mean values in both the groups were reduced, it was not statistically significant. Whereas for Insulin Resistance in Acarbose and Sitagliptin group mean value was found to be 9.00±8.38 SD and 11.49 ±9.42 SD respectively in the starting of treatment (Table.6) (Fig.31).

At the end of the three months in Acarbose and Sitagliptin group the mean value of Insulin Resistance were reduced to 5.12±3.14 SD and 7.63 ±5.78 SD respectively (Fig. 31) . Though there was reduction in the mean value in both the groups it was not statistically significant. (p value =0.100 and 0.101 respectively)

Other studies have proven that Sitagliptin reduces the mean Insulin Resistance [73] but not shows greater reduction in the fasting Insulin level. Acarbose has proven to reduce the Plasma Insulin level which was done

in ealier studies by Michael J Theodorakis et al [28]. This study has also compared the Plasma Insulin and Insulin Resistance values at the end of the three months between the Acarbose and Sitagliptin group ,the mean values were reduced to 14.18±6.71 SD and 22.58±17.68 SD respectively for plasma insulin and 5.12±3.14 SD and 7.63±5.78 SD respectively for Insulin resistance (Fig.30,31). But these results were not statistically significant. (p value = 0.110 for Plasma Insulin and 0.170 for Insulin Resistance.

Many studies are in search of the anti-inflammatory effects of Acarbose and DPP-4 inhibitors and their potential to reduce the risk of cardiovascular diseases. Other studies done on the potential of Acarbose to reduce the cardiovascular events which showed the reduction in the serum hsCRP levels after the treatment with Acarbose [117,118] .Another study formulated by Husam Ghanim et al, which showed the anti-inflammatory effects of sitagliptin have proven that there was reduction in the serum levels of hsCRP and IL-6 after three months treatment of Sitagliptin therapy [73] .

In this study mean levels of hsCRP at the starting of study in Acarbose and Sitagliptin groups were found to be 0.58 ±0.93 SD and 0.45 ± 0.43 SD respectively (Table.7) (Fig.32). At the end of the three months levels of hsCRP was reduced in both Acarbose and Sitagliptin group to the mean value of 0.30±0.3 SD (p value= 0.265) and 0.41 ± 0.39 SD (p

value= 0.671) respectively(Fig 32). Though there is greater reduction in mean values of hsCRP levels which were consistent with the results of previous studies [117, 118, and 73] these results were not statistically significant in this study.

When the values of hsCRP were compared between Acarbose and Sitagliptin groups,at the end of the three months there were no statistical significance reduction in those hsCRP values (p value=0.451).

Next inflammatory marker which this study analysed was the IL-6 levels. The mean values in the starting of treatment were 120.01 $\pm$ 203.3 SD in Acarbose group and 136.32 $\pm$ 241.81 SD in Sitagliptin group (Table.8) (Fig.33). At the end of the three months the mean values of IL-6 in Sitagliptin group was found to be reduced to 57.69 $\pm$ 78.55 SD but in Acarbose group it was found to be increased to the mean value of 174.08 $\pm$ 492.1 SD. When the before and after values of Sitagliptin group was analyzed, it showed reduction in the mean values of IL-6 levels at the end of the three months (Fig 33). But there was no statistical significance.

When comparing the after values of IL-6 in Acarbose and Sitagliptin group, greater reduction was seen in the Sitagliptin group after 3 months of therapy. Thus the findings in the study was consistent with the previous studies which showed the anti- inflammatory of Sitagliptin therapy [73] But this is again not statistically significant (p value = 0.408).

This study has shown the potential role of Sitagliptin in reducing the inflammatory markers apart from reducing the blood glucose level when was given as an add on therapy with Metformin and Sulfonylurea combination, as we know the fact that Metformin was already known to have anti-inflammatory action. The study which was done in the year 2011 has proven that initial therapy with Metformin and Sitagliptin combination showed superior glycemic improvement and weight loss compared with Metformin monotherapy [119] .Hence fixed dose combination of Sitagliptin and Metformin may be helpful in preventing or delaying the progress of atherogenesis when given at initial therapy in the management of type 2 DM.

Acarbose also reduced the hsCRP levels when given as an add on therapy with Metformin which was already known to have anti-inflammatory action. But there is no such reduction in the IL-6 values, Hence further studies with more sample size and a long term follow up of minimum of 6-9months period is a must to observe the progression of anti-inflammatory action in these groups.

CONCLUSION

In this study Sitagliptin combination reduced the mean value of FBS, PPBS, HbA1c, Plasma Insulin , Insulin Resistance, hsCRP and IL-6 , which were similar to the results of previous studies [73,76 ,77,78].

Regarding the results of the Anti-inflammatory markers in Sitagliptin treated group with metformin sulphonylureas combination showed a definite reduction in the mean values of both hsCRP, IL-6 levels after the three months treatment, but still it is not statistically significant. This may be due to less sample size (pilot study), the results obtained were had wide range of values and the patients were followed only for 3 months. Hence, long term follows up and more sample size may be mandatory in future to get the statistically significant reduction in the inflammatory markers, but Sitagliptin has a definite role in reducing chronic inflammation. Reduction in the mean values of hsCRP and IL-6 level in Sitagliptin with Metformin Sulfonylurea combination showed the anti-inflammatory action since few reference proved the anti-inflammatory property of Metformin [71,72,115]. This study explained the synergism when Sitagliptin given along with Metformin Sulfonylurea combination. Based on this study findings fixed dose combination of Sitagliptin and Metformin may be helpful in preventing the progress of diabetes.

In Acarbose combination, there was a reduction in the mean values of FBS, PPBS, HbA1c, Plasma Insulin, Insulin Resistance, hsCRP but not

IL-6. When the results of inflammatory markers in Acarbose group were analyzed .and compared with previous studies [96, 97, 98], Acarbose also reduces the inflammatory markers moderately in this study. This study showed a great mean value reduction in hsCRP levels but not in IL-6, may be because of small sample size (pilot study), poor compliance of patients, and presence of infections during the course of study period, and the patients were followed only for 3 months. Hence long term follow up and large sample size may be required to see the progression in the reduction of inflammatory markers and the role of these drugs in the insulin resistant states.

This study demonstrated the additive anti inflammatory effect of Sitagliptin, as this group of drugs is now in trend and commonly been prescribed either as base line therapy or based on the HbA1C values. When such anti inflammatory property is pronounced with Sitagliptin, it can be sure that addition of Sitagliptin with Metformin will be a beneficial combination at the initial therapy to the patients in preventing the progression of diabetic related cardio vascular complications [119].In future, if there are any clinical studies expressing positive role for Sitagliptin as monotheraphy either for prediabetes or Type II diabetic state, the patients will have a good benefit in terms of better clinical outcome with reduction in the chronic inflammatory status.

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ABBREVIATIONS

AGE	-	Advanced Glycated End products
AMPK	-	Adenosine Mono Phosphate
AP -1	-	Activated protein -1
ADA	-	American Diabetic Association
CAD	-	Coronary Artery Disease
CAMP	-	Cyclic Adenosine Mono Phosphate
DPP -4	-	Dipeptidyl Peptidase -4
DM	-	Diabetes Mellitus
ET -1	-	Endothelin -1
ERK	-	Extracellular signal Regulated Kinase
eNOs	-	Endothelin Nitric Oxide Synthatase
FBS	-	Fasting Blood Sugar
FFA	-	Free Fatty Acid
GLUT	-	Glucose Transporter
GPCR	-	G-Protein Coupled Receptor
G -6 – P	-	Glucose-6- Phosphate
G -1 –P	-	Glucose -1- Phosphate
GLP	-	Glucagon like Peptide
HbA1c	-	Gylcated Hemoglobin A1C
HDL	-	High Density Lipoprotein
hsCRP	-	High Sensitive C- Reactive Protein
IFG	-	Impaired Fasting Glucose

IGT - Impaired Glucose Tolerance

IL -6 - Interleukin -6

JNK - c- Jun NH_2 Kinase

MAP - Mitogen Activated Protein Kinase

NFkB - Nuclear Factor k B

NO - Nitric Oxide

OHA - Oral Hypoglycemic Agents

PAI - Plasminogen Activator Inhibitor

PCOS - Poly Cystic Ovarian Syndrome

PGI2 - Prostacycilin

PI3 –kinase - Phosphoinositol -3 Kinase

PPAR –γ - - Peroxisomal Proliferative Activated Receptor

ROS - Reactive Oxygen Species

SUR - Sulfonylurea Receptor

TLR - Toll like Receptor

TNF –α - Tumour Necrosing Factor -α

VAT - Visceral Adipose Tissue

VLDL - Visceral Low Density Lipoprotein

WAT - White Adipose Tissue.

CASE PROFORMA

IDENTIFICATION NO:

AGE/SEX:

ADDRESS:

HEIGHT: WEIGHT: BMI:

HISTORY:

BEFORE DRUG THERAPY	DURING DRUG THERAPY ATER 2 WEEKS	AFTER DRUG THERAPY FOR 3MONTHS DURATION
FBS	FBS	FBS
PPBS	PPBS	PPBS
HbA1C		HbA1C
PLASMA INSULIN		PLASMA INSULIN
IL6		IL6
CRP		CRP

அ. உமா மகேஸ்வரி ஆகிய நான் பி.எஸ்.ஜி மருத்துவக் கல்லூரியின் மருந்தியல் துறையின்கீழ் முதல் ஆண்டு மருந்தியல் மேற்படிப்பு படித்துக் கொண்டு இருக்கிறேன். நான் "சர்க்கரை நோயாளிகளில் சர்க்கரை நோய்க்கு பயன்படுத்தும் மருந்துகளின் அழற்சிநீக்கு தன்மையை அறியும் ஆராய்ச்சியை மேற்கொள்ள உள்ளேன்.

என் ஆய்வு வழிகாட்டி : கே. புவனேஸ்வரி

ஆய்வு மேற்கொள்வதற்கான அடிப்படை:-

சர்க்கரை நோயினால் வரும் இருதயக் கோளாரு மற்றும் பல விளைவுகளை அழற்சி மார்க்கர்களின் மூலம் அறியலாம். மெட்பார்மின், சல்பனையில்யூரியா, அகார்போஸ், சிட்டாகிளப்டின் போன்ற மருந்துகளுக்கு அழற்சி நீக்கு தன்மை உள்ளதால் சர்க்கரை நோயினால் வரும் விளைவுகளை கட்டுப்படுத்தலாம். இவற்றின் அடிப்படையில் இவ்ஆராய்ச்சி மேற்கொள்ளபட உள்ளது.

ஆய்வுக்குட்படுபவரின் ஒப்புதல்:-

நான் இந்த ஆராய்ச்சியின் நோக்கம் மற்றும் அதன் பயன்பாட்டினைப் பற்றி தெளிவாகவும், விளக்கமாகவும் தெரியப்படுத்தப்பட்டுள்ளேன்.

இவ்ஆய்விற்காக 30 சர்க்கரை நோயாளிகள் (வயது 25 – 65) பி.எஸ்.ஜி மருத்துவமனையில் இருந்து தேர்ந்தெடுத்து அவர்கள் எல்லோருக்கும் முதலில் உடல்நலம் சரிபார்த்த பிறகு அவர்களிடமிருந்து சுமார் 5-10ml வரை இரத்தம் இரண்டு முறை அதாவது ஆய்வின் ஆரம்பத்திலும், பிறகு 3 மாதங்களுக்கு பின்பும் எடுக்கப்படும் என்பதை எனக்கு தெளிவாகத் தெரியப்படுத்தபட்டுள்ளது.

இந்த ஆய்விற்காக கொடுக்கப்படும் அகார்போஸ் (ரூ.28) (அல்லது) சிட்டாகிளிப்டின் (ரூ.40) போன்ற மருந்துகள் நான் ஏற்க்கனவே எடுத்துக் கொள்ளும் மருந்துகளுடன் சேர்த்து இலவசமாக 3மாதங்களுக்கு மட்டுமே கொடுக்கப்படும் என்பதையும், அதன்பிறகு நான் விருப்பப்பட்டால் இந்த மருந்துகளை நான் என்செலவில் எடுத்துக் கொள்ளலாம் என்றும் அல்லது சர்க்கரை நோய்க்காக இருக்கும் மாற்று மருந்துகளை என் வசதிக்கேற்ப எடுத்துக்கொள்ளலாம் என்பதையும் தெளிவாக தெரியப்படுத்தப்பட்டுள்ளேன்.

மேலும் இவ்ஆய்வில் எடுக்கும் மருந்துகளால் சிலசமயங்களில் மட்டும் ஏற்படும் பக்கவிளைவுகளான சர்க்கரை அளவு குறைதல், தலைசுற்றல், வயிற்றுபோருமல், ஜீரனகோலாரு போன்றவை ஏற்படலாம் என்பதையும் இவை

அனைத்தும் சரிபடுத்தக்கூடியவை என்பதையும் எனக்குத் தெளிவாக தெரியப்படுத்தப்பட்டுள்ளது.

இந்த ஆய்வில் கிடைக்கும் தகவல்கள் 5வருடங்கள் பாதுகாக்கப்படும். இவை வேறு எந்த ஆய்விற்கும் பயன்படுத்தப்பட மாட்டாது. எந்த நிலையிலும் என்னைப் பற்றிய தகவல்கள் யாருக்கும் தெரிவிக்கப்பட மாட்டாது என்பதை நான் நன்கு அறிவேன்.

இந்த ஆய்வில் பங்கேற்க ஒப்புக்கொள்வதால் எந்த விதமான பலனும் எனக்குக் கிடையாது, எந்த நேரத்தில் வேண்டுமானாலும் ஆய்விலிருந்து விலகிக் கொள்ளும் உரிமை எனக்கு உண்டு. ஆய்விலிருந்து விலகிக் கொள்வதால் எனக்கு அளிக்கப்படும் சிகிச்சையில் எந்தவித மாற்றமும் இருக்காது என்பதை எனக்கு தெளிவாகத் தெரியப்படுத்தப்பட்டுள்ளது.

மேலும் இந்த ஆய்வில் பங்கு கொள்வது என் சொந்த விருப்பம். இதில் எந்தவிதக் கட்டாயமும் இல்லை. இந்த ஆராய்ச்சியில் பங்கு கொள்ளவும், இந்த ஆராய்ச்சியின் மருத்துவ ரீதியான குறிப்புகளை வரும் காலத்திலும் உபயோகப்படுத்திக் கொள்ளவும் முழு மனதுடன் சம்மதிக்கிறேன்.

ஆய்வுக்குட்படுபவரின்

பெயர் :

முகவரி :

கையொப்பம் :

தேதி :

Study Volunteer ID:
Study Volunteer Name:

Version 2.3.2. Effective: 26th August, 2011

PSG Institute of Medical Science and Research, Coimbatore
Institutional Human Ethics Committee
INFORMED CONSENT FORMAT FOR RESEARCH PROJECTS

(strike off items that are not applicable)

I ,DR.UMA MAHESWARI, am carrying out a study on the topic: TO COMPARE THE ANTI-INFLAMMATORY EFFECT OF ORALHYPOGLYCEMIC DRUGS IN TYPE 2 DIABETES MELLITUS

as part of my research project being carried out under the aegis of the Department of: PHARMACOLOGY

(*Applicable to students only*): My / our research guide is: DR.K.BHUVANESWARI

The justification for this study is: Decrease in the Inflammatory markers will be helpful in preventing the complications of Diabetes mellitus.Therefore in future combination of these drugs can help to reduce the cardiovascular events.

The objectives of this study are:

To compare the anti-inflammatory effect of Metformin Sulfonylurea and Sitagliptin combination with Metformin Sulfonylurea and Acarbose combination in Type2DM patients by using Anti-inflammatory markers (IL6,hs CRP)

Sample size/Location: 30 patients from PSG Hospital

Studyparticipants :Chronic diabetes mellitus patients(minimum of 5 years) in PSG hospital..age group : 25 years -65 years

We request you to kindly cooperate with us in this study. We propose collect background information and other relevant details related to this study. We will be carrying out:

Data collected will be stored for a period of five years. We will / will not use the data as part of another study.

Clinical examination (Specify details and purpose): general examination will be done before starting the clinical trial to know the health status of the study participant .

Blood sample collection: Specify quantity of blood being drawn: 5-10 ml.

No. of times it will be collected: 2

Whether blood sample collection is part of routine procedure or for research (study) purpose:Research purpose

Purpose: To check for inflammatory markers(hsCRP AND IL-6) , FBS,PPBS,HbA1C,plasma insulin.

Whether blood sample collected will be stored after study period:NO it will be destroyed

Whether blood sample collected will be sold: No

Medications like Sitagliptin(RS 40) or Acarbose(RS 28 one strip) will be given as a add on therapy to the already existing standard drugs free of cost for 3 months duration only. We inform that you may continue the tablets if you are satisfied with those drugs otherwise you may continue the already existing standard drugs alone or you can go for other alternatives.

Medication given, if any, duration, side effects, purpose, benefits:
Sitagliptin and Acarbose wil be given as add on drugs with Metformin Sulfonylurea combinations for 3 months duration.
Side effects : GI disturbances ,Hypoglycemia (rarely),,Pancreatitis,Hepatotoxicity on chronic use(very rarely).
Purpose : To reduce blood glucose level
Benefits : To attain normoglycemic state ,to reduce lipid level and weight reduction.

Whether medication given is part of routine procedure: Yes (If not, state reasons for giving this medication)

Whether alternatives are available for medication given: Yes

Benefits from this study: Complications of diabetes mellitus will be prevented or delayed by reducing the inflammatory markers.

Risks involved by participating in this study: nil

How the **results** will be used: by comparing the effects of these two add on drugs ,it will be useful in the therapeutic management of DM in preventing the cardiovascular events.

If you are uncomfortable in answering any of our questions during the course of the interview / biological sample collection, **you have the right to withdraw from the interview / study at anytime.** You have the freedom to withdraw from the study at any point of time. Kindly be assured that your refusal to participate or withdrawal at any stage, if you so decide, will not result in any form of compromise or discrimination in the services offered nor would it attract any penalty. You will continue to have access to the regular services offered to a patient. You will **NOT** be paid any remuneration for the time you spend with us for this interview / study. The information provided by you will be kept in strict confidence. Under no circumstances shall we reveal the identity of the respondent or their families to anyone. The information that we collect shall be used for approved research purposes only. You will be informed about any significant new findings - including adverse events, if any, – whether directly related to you or to other participants of this study, developed during the course of this research which may relate to your willingness to continue participation.

Consent: The above information regarding the study, has been read by me/ read to me, and has been explained to me by the investigator/s. Having understood the same, I hereby give my consent to them to interview me. I am affixing my signature / left thumb impression to indicate my consent and willingness to participate in this study (i.e., willingly abide by the project requirements).

Signature / Left thumb impression of the Study Volunteer / Legal Representative:

Signature of the Interviewer with date: Witness: