

“COMPARISON OF PLASMA ADIPONECTIN
LEVELS BETWEEN NORMAL WEIGHT AND
OVERWEIGHT TYPE 2 DIABETIC
INDIVIDUALS – A CASE CONTROL STUDY”

By

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Dissertation submitted to the
KLE University, Belgaum, Karnataka

In Partial Fulfillment
of the requirements for the degree of

M. D. MEDICINE

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MAY - 2009

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I hereby declare that this dissertation entitled
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CASE CONTROL STUDY”** is a bonafide and genuine
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LIST OF ABBREVIATIONS USED

ACRP30	-	Adipolyte compliment related pattern
BMI	-	Body mass index
CAD	-	Coronary artery disease
cAD	-	Collagenous domain
C1q	-	Complement 1 q
CT	-	Computed tomography
CUPS	-	Chennai Urban Population Study
dl	-	Deci litre
FBS	-	Fasting blood sugar
FFA	-	Free fatty acids
gAD	-	Globular domain
GBP28	-	Gelatin binding protein of 28 kilo Dalton
GIR	-	Glucose infusion rate
HbA _{1C}	-	Glycated haemoglobin
HC	-	Hip circumference
HDL	-	High density lipoprotein
HIV	-	Human immunodeficiency virus
Hs-CRP	-	High sensitive C – reactive protein
IDF	-	International Diabetic Federation
IRS 1	-	Insulin receptor substrate 1
n	-	Number of patients
NF Beta	-	Nuclear Factor beta
PAI-1	-	Plasminogen activated insulin type 1
PKA	-	Protein kinase A

PPARY	-	Peroxisome proliferation activated receptor gama
P1-3K	-	Phosphatidyl Inositol 3 Kinase
TNF	-	Tumor necrosis factor alpha
TZD	-	Thiozolidinedione
VDVL	-	Very low density lipoprotein
WC	-	Waist circumference
WHO	-	World Health Organization
WHR	-	Waist Hip Ratio

ABSTRACT

Background and Objectives

Adiponectin is the most abundant and the most lately discovered adipocytokine. It has been labeled as the molecule of this decade due to its protective role in disease like diabetes and coronary artery disease. The objectives of the study were to determine correlation between the plasma adiponectin level in normal weight and overweight type-2 diabetic individuals and to correlate the relation between plasma adiponectin and other anthropometric measurements of obesity.

Methods

The present case control study was conducted during the period of Jan 2007 to May 2008 on 90 age matched male subjects attending KLES Dr. Prabhakar Kore Hospital and Medical Research Centre, Belgaum. The subjects were stratified into four groups based on BMI. Only well controlled (HbA1c < 7.5%) diabetics with normal ECG and not on Thiazolidinedione were included. All were studied with respect to anthropometry and Plasma adiponectin (Measured by ELISA, both globular and full-length).

Results

It was found that Mean adiponectin is higher in non-diabetics compared to diabetics. Among the four groups adiponectin levels were lower in the two overweight groups versus non-overweight groups ($p < 0.001$). There was a statistically significant ($p < 0.001$) inverse relation between the range of BMI values and Plasma Adiponectin amongst diabetics. Based on WHR, adiponectin

levels were significantly lower ($p < 0.001$) in the centrally obese subjects compared to normal-WHR subjects, whether diabetic or not. The correlation coefficient of reduced adiponectin is higher with WHR (+0.9209 and +0.9690 for diabetics and non-diabetics respectively) than with BMI (+0.7494 and +0.6924 for diabetics and non-diabetics respectively).

Conclusion and interpretation

Both diabetes and obesity have an inverse correlation with Adiponectin. Plasma adiponectin levels correlates better with WHR than BMI and probably predicts insulin resistance and development of atherosclerosis.

Keywords

Adiponectin; Obesity; Diabetes; Waist Hip Ratio; Body Mass Index

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Introduction



Objectives



Review of Literature



Methodology



Results



Discussion



Conclusion



Summary



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Annexure – I



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INTRODUCTION

Adiponectin is the most abundant and the most lately discovered adipocytokine, which has been labeled as the molecule of this decade because of its properties of preventing deadly diseases like diabetes, coronary artery disease and obesity. National statistics shows that 12.5% of Indian adult population (more than 20 years) is suffering from diabetes. Globally, every fourth diabetic of the world is an Indian (International Diabetic Federation Figures). Asian Indians have a unique phenotype characterized by increased abdominal obesity and visceral fat despite low body mass index (BMI). Though studies have indicated adiponectin to be associated with diabetes and obesity in the west, there are virtually no studies relating it with diabetes and obesity in the unique Asian Indians phenotype.

This study had explored, if there is any association between excess weight and serum adiponectin in the Asian type-2 diabetic individuals. It had also correlated adiponectin levels in diabetic individuals who are not over weight by BMI but are metabolically obese as measured by waist hip ratio, thereby emphasizing on the body fat distribution as an important marker of obesity.

This research has also emphasized on the importance of weight reduction in raising adiponectin levels in Indian population until adiponectin itself can be used as a therapeutic modality. It is also a baseline study to perform large scale studies on the therapeutic intervention of adiponectin.

OBJECTIVES

The objectives of the present study were;

Primary objective

To determine correlation between the plasma adiponectin level in normal weight (BMI less than 23) and over weight type-2 diabetic individuals

Secondary objective

To correlate the relation between plasma adiponectin and other anthropometric measurements of obesity.

REVIEW OF LITERATURE

EPIDEMIOLOGY OF DIABETES AND OBESITY

Type 2 diabetes, currently affecting 5 to 10% of most populations and has become the most frequently encountered metabolic disorder in the world; its prevalence is growing more rapidly among developing nations, primarily due to the rapid demographic and epidemiological transitions occurring in these countries as a consequence of urbanization, industrialization and globalization. Studies have projected that the global prevalence of Type 2 diabetes will rise from 171 million in the year 2000 to 366 million by the year 2030 and these figures are higher than earlier estimate. The International Diabetes Federation (IDF) has independently obtained similar estimates.

Indeed, diabetes has reached epidemic proportions in developing countries, due to the associated factors such as poverty, illiteracy, and lack of access to health care, the social, economic, and human costs of diabetes are likely to be quite formidable in developing countries. Thus there is little doubt that the prevalence of diabetes is rising in astronomical proportions during the last two decades, particularly in India.

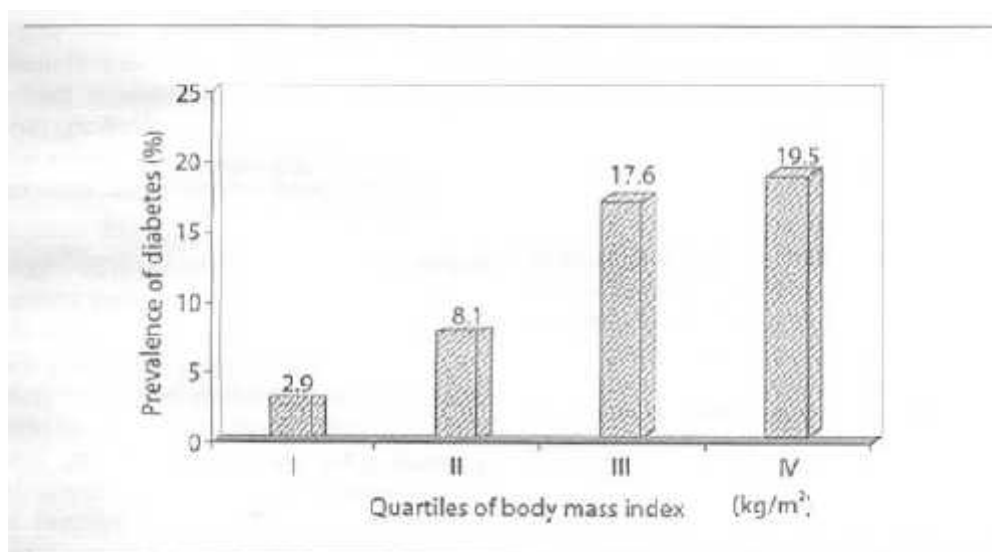


Figure No. 1: Prevalence of diabetes in quartiles of body mass index

Obesity defined as “excess of body fat relative to weight”, is the sixth most important risk factor contributing to the overall burden of disease worldwide. Overweight refers to an excess body weight compared to set standards, which may come from muscles (lean body mass), bone, fat (adipose tissues), some time tumors and/or body water. It is observed that women have more body fat than men. Most health care providers agree that men with more than 25% of total body fat and women with more than 30% total body fat should be considered obese. The obesity epidemic that started in the middle of last century has now established itself in North America and Western Europe, their appears no abatement. O’Dowd has predicted that over 12 million adults in England will be obese by the year 2010. In developing countries too, the epidemic has taken deep roots and in many urban populations of low and middle income countries of Asia, Latin America and Africa there is paradoxically a dual

burden of obesity and malnutrition, often within the same families. Obesity develops because of a mis-match between energy intake and expenditure that results from behavior (feeding behavior and time spent in activities) and physiology (resting metabolism and expenditure when active). Both of these traits are affected by environmental and genetic factors. In addition, the availability of abundant energy rich processed foods in last few decades has contributed to the sharp rise of prevalence of obesity worldwide.

INDIAN SCENARIO

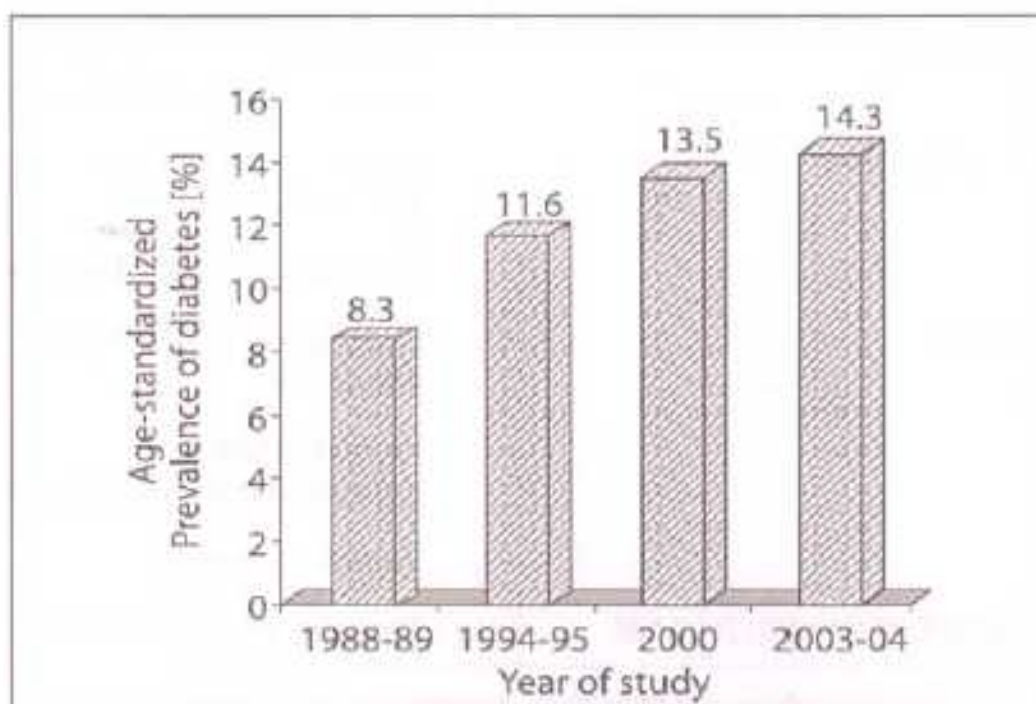


Figure No. 2: Secular trend in the prevalence of diabetes in Chennai

With its population over one billion, India leads the world with its largest number of diabetic subjects. The prevalence of diabetes in the subcontinent is growing rapidly in both urban and rural areas. In the rural population, the

prevalence is four to five times lower than in the urban areas. In 1972, the prevalence of diabetes in urban areas was 2.1% and it has rapidly climbed to 12% to 16 % representing a 600 to 800% increase in prevalence rates over 30 year period. Age standardized prevalence of diabetes at different time periods in Chennai, South India one of the few regions of the country where repeated surveys have been conducted. It can be seen that within a span of 14 years, the prevalence of diabetes increased by 72.3%. A study conducted in the rural Indian population reported nearly a three fold increase in age and sex adjusted prevalence of diabetes (from 2.2% to 6.36%) in 2003 when compared with a similar study done 14 years earlier. In the peri urban population the prevalence is found to be midway between the rural and urban populations (5.9%). Studies in India have also consistently shown that with affluence, the prevalence of diabetes and related disorders tend to increase .Thus subjects in the higher socio-economic group had a higher risk of developing diabetes. This is in marked contrast to that seen in the developed countries, where an inverse relation between socio-economic status and diabetes is noted that the prevalence of diabetes is higher in the lower socio-economic groups. A recent review, which analyzed the trends in dietary transitions in India, revealed a rapid increase in diet-related non-communicable diseases, including diabetes. In the Chennai Urban Population Study (CUPS), the age standardized prevalence of diabetes was significantly higher the middle compared to the low-income group (12.4% versus 6.5%) which clearly demonstrates that that with affluence there is a marked increase in the prevalence rates of diabetes.

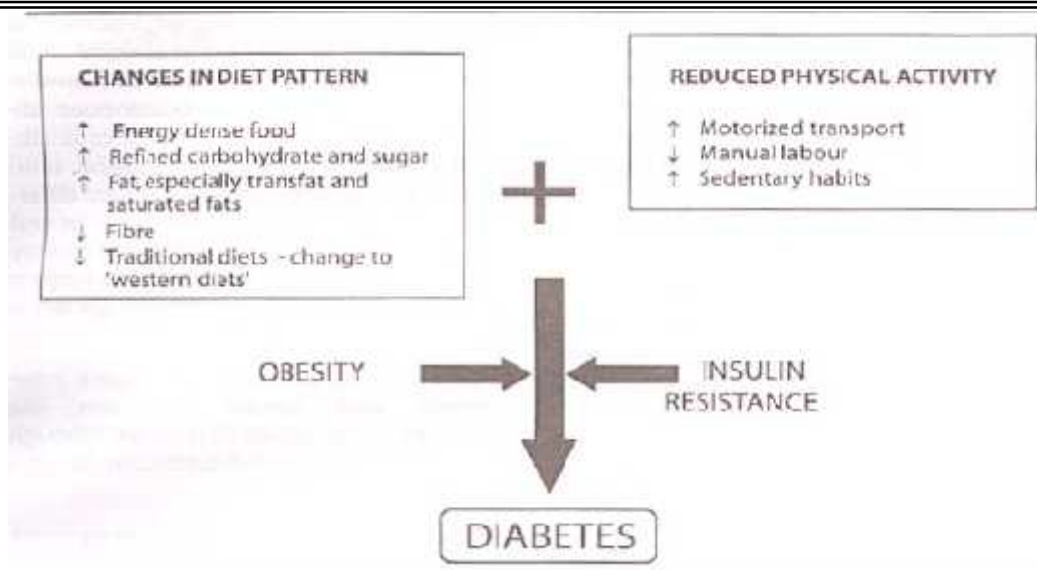


Figure No. 3: Changes due to transition from an active rural to sedentary urban socioeconomic milieu

India is currently experiencing a rapid epidemiological transition which has resulted in increased life expectancy and decrease mortality due to communicable diseases. As a consequence of industrialization and urbanization there has also been an increase in the standard of living leading to nutritional transition with consumption of diets that are energy dense and high in fat and sugar content. Moreover with changes in occupation from predominantly agriculture based manual labor jobs to sedentary office type jobs; there is a perceptible decrease in physical activity. This is the basis for the rapid weight gain and obesity seen in several parts of the subcontinent. Several reports suggest that for any given body mass index (BMI), Indians tend to have increased waist circumference. Further, Indians also tend to have excess body fat, abdominal and truncal obesity. For any given waist circumference; they have increased body fat accumulation and for any given body fat, they have increased insulin resistance. These features have been referred to as the “Asian Indian phenotype or Paradox”.



Figure No. 4: Difference in body fat inspite of same BMI between an Indian and a European physician

Source: Yajnik CS, Yudkin JS. The Y-Y paradox. Lancet 2004; 363:163.

There is paucity of nation wide data on the prevalence of obesity. However, studies in different states of India provide some data on the magnitude of the health threat due to this problem. The published data on obesity from different studies shows that the prevalence of obesity ranges from 10% to 50%. However the only nation wide study is the national health survey on women and this revealed a low prevalence rate. According to the Nutritional Foundation of India, the prevalence of obesity is one percent for males and four percent for females in slums while the corresponding figures for the middle socio-economic class was 32.2% and 50% respectively. In the Chennai Urban Population Study (CUPS), over 35% of the males in the middle-income group were obese compared to 13% in the low-income group. The corresponding figures for

females were 33% and 24% respectively. Abdominal obesity among the middle income was 47.4% compared to 19.2% in the low income group.

The rising prevalence of obesity has several health consequences as obesity is a predecessor for many related conditions like diabetes, dyslipidemia, hypertension and coronary heart disease.

THE ADIPOCYTE AND ADIPOSE TISSUE

Adipose tissue is composed of the lipid-storing adipose cell and a stromal/vascular compartment in which preadipocytes reside. Adipose mass increases by enlargement of adipose cells through lipid deposition, as well as by an increase in the number of adipocytes. The process by which adipose cells are derived from a mesenchymal preadipocyte involves an orchestrated series of differentiation steps mediated by a cascade of specific transcription factors. One of the key transcription factors is peroxisome proliferator-activated receptor (PPAR), a nuclear receptor that binds the thiazolidinedione class of insulin-sensitizing drugs used in the treatment of type 2 diabetes.

Recent research has shown that adipose tissue is not simply an inert storage depot for lipids but is also an important endocrine organ that plays a key role in the integration of endocrine, metabolic, and inflammatory signals for the control of energy homeostasis. The adipocyte has been shown to secrete a variety of bioactive proteins into the circulation. These secretory proteins, which have been collectively named adipocytokines,¹ include leptin,² tumor necrosis factor (TNF)-alpha,³ plasminogen activator inhibitor type 1 (PAI-1),⁴ adiponectin,⁵ resistin,⁶ and adiponectin.⁷ Adiponectin, the gene product of the adipose most abundant

gene transcript one (apM1),⁷ is a novel and important member of the adipocytokine family.

Synthesis of Adiponectin

Adiponectin cDNA was first isolated by large scale random sequencing of the human adipose tissue cDNA library.⁷ It is a collagen-like protein that is exclusively synthesized in white adipose tissue, is induced during adipocyte differentiation, and circulates at relatively high (microgram/milliliter) concentrations in the serum. Both murine and human forms of adiponectin have been isolated independently by several groups, and various descriptive names have been given to the same compound by different investigators: adipocyte complement-related protein of 30 kilodalton (Acrp30),⁸ Adipo Q⁹ and gelatin binding protein of 28 kilodalton (GBP28).¹⁰ The former two are murine analogs and the latter the human counterpart.

Properties of Adiponectin

Adiponectin has been postulated to play an important role in the modulation of glucose and lipid metabolism in insulin sensitive tissues in both humans and animals. Decreased circulating adiponectin levels have been demonstrated in genetic and diet-induced murine models of obesity,¹¹ as well as in diet induced forms of human obesity.¹² Low adiponectin levels have also been strongly implicated in the development of insulin resistance in mouse models of both obesity and lipoatrophy.¹¹ In humans, plasma levels of adiponectin are significantly lower in insulin-resistant states including type 2 diabetes¹³ and can be increased upon administration of the insulin-sensitizing thiazolidinedione

(TZD) class of compounds.^{14,15,16,17} Plasma adiponectin levels in diabetic subjects with coronary artery disease (CAD) are lower than in diabetic patients without CAD, suggesting that adiponectin may have anti-atherogenic properties.¹⁸ In studies done on human aortic endothelial cells, adiponectin has been shown to dose-dependently decrease the surface expression of vascular adhesion molecules known to modulate endothelial inflammatory responses.¹⁹ It also inhibits proliferation of vascular smooth muscle cells,²⁰ and concentrates within the vascular intima of catheter-injured vessels.²¹ In clinical studies, low adiponectin levels have been associated with an atherogenic lipid profile.^{18,22} The association of low adiponectin levels with obesity, insulin resistance, CAD, and dyslipidemia indicates that this novel protein may be an important new marker of the metabolic syndrome.

Structure and processing of Adiponectin

A description of the cDNA encoding adiponectin was first reported in 1995.⁸ Adiponectin is a protein of 247 amino acids consisting of four domains, an amino-terminal signal sequence, a variable region, a collagenous domain (cAd), and a carboxy-terminal globular domain (gAd)⁸ (Figure. No. 4). On the basis of both its primary amino acid sequence and its subunit domain structure, adiponectin is most similar to C1q, a member of the complement-related family of proteins. However, X-ray crystallography of the globular fragment of adiponectin also reveals a striking structural homology to TNF-alpha, suggesting an evolutionary link between the TNF-alpha family members and adiponectin.²³ Once synthesized, mammalian adiponectin undergoes posttranslational hydroxylation and glycosylation yielding eight isoforms.²⁴ Six of the adiponectin

isoforms are glycosylated. O-linked glycosylation sites have been mapped to four lysine residues, 68, 71, 80, and 104, and one proline residue, 94, located within the collagenous domain.²⁴

Structure of Monomeric adiponectin

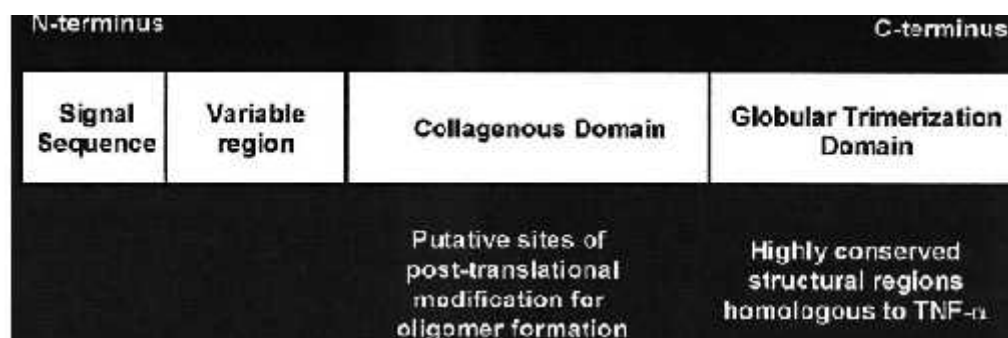


Figure No. 5: The domain structure of Acrp30: Signal sequence, species-specific variable region, collagenous domain and globular trimerization domain.

In addition, there is evidence that some of the O-linked glycans contain unique and adipocyte-specific disialic acid residues, a newly recognized class of sialyl groups in glycoproteins.²⁵ Functional analysis of full-length glycosylated mammalian adiponectin has revealed that it is significantly more potent as an insulin sensitizer than the recombinant nonglycosylated bacterial product. These observations suggest that posttranslational modifications of adiponectin may be necessary for optimal biological activity. The basic building block of adiponectin is a tightly associated trimer, which is formed by association between three monomers at the globular domains. Monomeric (30-kDa) adiponectin has not been observed in the circulation and appears to be confined to the adipocyte. Four to six trimers associate through their collagenous domains to form higher order

structures, or oligomers, which circulate in plasma at concentrations of 5 to 30 g/ml.^{8,12,23} (Figure No. 5).

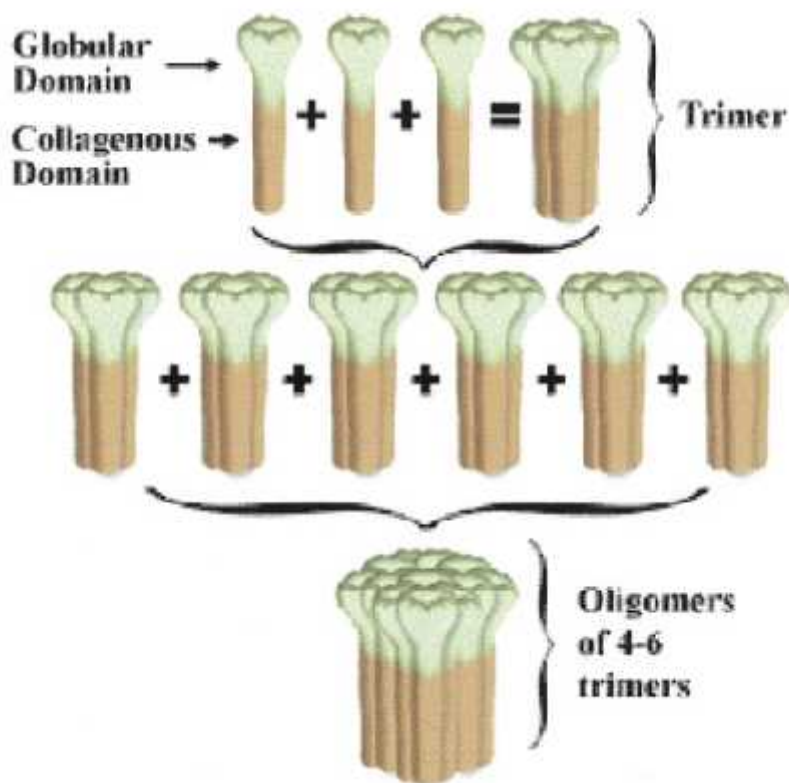


Figure No. 6: Model for assembly of adiponectin complexes. Three monomers form a trimer through associations between their globular domains. Four to six trimers associate noncovalently through their collagenous domains to form high-molecular-weight oligomers, which circulate in the plasma.

Without the collagenous domain, the globular domain of adiponectin still trimerizes but does not associate into higher-order structures.²³ Although the precise molecular mechanisms underlying the tight association of adiponectin trimers are not known, it is likely that interactions involving both the globular

and the collagenous domains are important for ensuring the stability and activity of the multimeric forms.

Measurement of Adiponectin

The current methods available for measuring adiponectin in plasma include a radioimmunoassay (Linco, St Charles, MO) that measures the multimeric form and an enzyme-linked immunosorbent assay (B-Bridge International, San Jose, CA) that recognizes the denatured monomer form. Circulating levels detected with either method appear to be similar.

The pharmacological effects of adiponectin

The pharmacological effects have been studied at animal, tissue, and cellular levels using a variety of recombinant adiponectin products. Studies investigating the bioactivity of full length adiponectin versus that of the globular domain alone have produced mixed results. The globular head domain of adiponectin has been shown to be more potent than the full-length form in ameliorating hyperglycemia and hyperinsulinemia in diet-induced and genetic forms of murine obesity¹¹ and in decreasing elevated plasma free fatty acids in mice fed a high-fat meal or given intravenous intralipid injections.²⁶ These results are in contrast to those of other studies,²⁷ whereby injection of bacterially produced globular adiponectin into mouse models of type 1 and 2 diabetes did not induce a decrease in serum glucose, although the full-length form did. It is possible that adiponectin exists as variable protein complexes that exert different effects in various tissues.

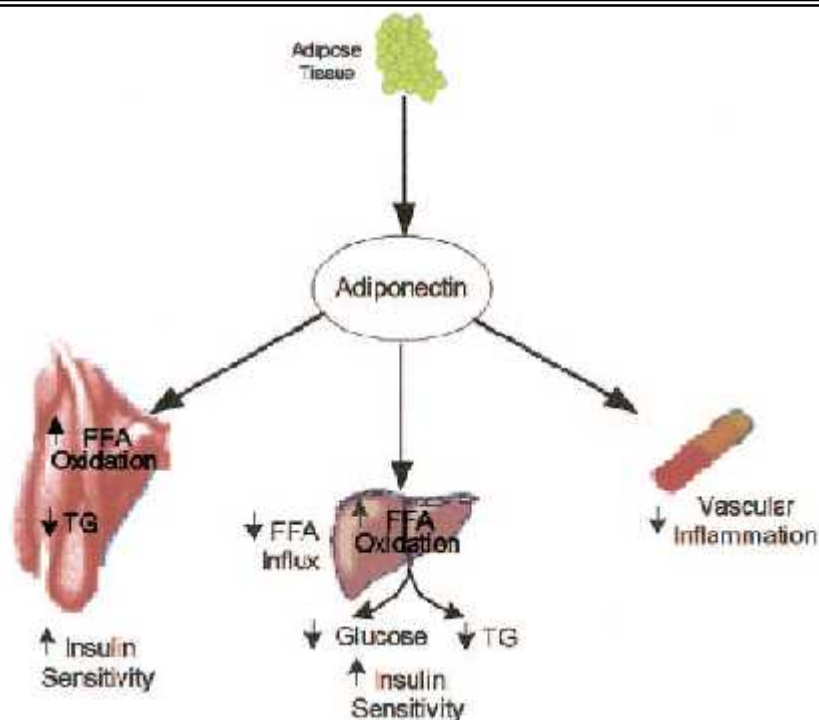


Figure No. 7: Hypothetical model for the actions of adiponectin.

In skeletal muscle, adiponectin increases tyrosine phosphorylation of the insulin receptor. This effect may contribute to increased insulin sensitivity. It also increases fatty acid oxidation, probably by activation 5'-AMP kinase, with resultant decreased intramyocellular steatosis. In the liver, the decreased free fatty acid influx and increased fatty acid oxidation contribute to reduced hepatic glucose output and VLDL (Very Low Density Lipoprotein) triglyceride synthesis. In vascular endothelium, adiponectin decreases monocyte adhesion to endothelium, adiponectin decreases monocyte adhesion to endothelium, suppresses macrophage to foam cell transformation and inhibits vascular smooth muscle cell proliferation and migration.

The mechanisms of action of adiponectin

The mechanisms of action are largely unknown and controversial. Adiponectin administration to rodents has been shown to increase insulin-induced tyrosine phosphorylation of the insulin receptor in skeletal muscle in association with increased whole-body insulin sensitivity.¹¹ These results were also validated in a recent study conducted in humans.²⁸ Stimulation of glucose utilization and fatty acid oxidation in skeletal muscle and liver by adiponectin may also occur through activation of 5-AMP kinase. 5-AMP-activated protein kinase is believed to play a crucial role in the regulation of energy expenditure and glucose and lipid metabolism. The tissue-specific effect of adiponectin on 5-AMP kinase has recently been demonstrated in mice. In these studies, both the globular and fulllength forms of adiponectin activated 5-AMP kinase in skeletal muscle, but only the full-length form stimulated phosphorylation and activation of AMP kinase in the liver.²⁹ In skeletal muscle of mice, adiponectin has been shown to increase expression of the genes encoding proteins involved in fatty acid transport and oxidation, such as CD36, acyl-CoA oxidase, and uncoupling protein, resulting in enhanced fat combustion and energy dissipation.¹¹ In the liver, low doses of adiponectin decreased the expression of proteins involved in fatty acid transport, such as CD36, leading to reduced fatty acid influx into the liver and hepatic triglyceride content.¹¹ Improved hepatic insulin sensitivity occurred, leading the investigators to postulate that the primary effects of adiponectin on muscle are to augment uptake and combustion of free fatty acids (FFAs), whereas decreased liver triglyceride content results from secondary reductions in serum FFA and triglyceride levels. In a separate experiment by the

same group,³⁰ amelioration of insulin resistance cell degranulation, and diabetes occurred in globular adiponectin transgenic (gAd Tg) crossed with leptindeficient ob/ob mice. Again, these findings were associated with increased skeletal muscle fatty acid oxidation. This finding is in contrast to that reported by another group in which, in the basal state, adiponectin exerted an insulin-sensitizing effect on hepatocytes with suppression of hepatic glucose output without a sustained attenuation of triglyceride accumulation in this tissue.²⁷ A unified theme for the method and site of adiponectin action thus remains to be determined (Figure No. 7).

Factors influencing Adiponectin levels

Although adiponectin is secreted only from adipose tissue, its levels are paradoxically lower in obese than in lean humans.¹² This is in contrast to most other adipocytokines, whose levels are increased in obesity in proportion to an increased total body fat mass. It is possible that although adiponectin expression is activated during adipogenesis, a feedback inhibition on its production may occur during the development of obesity. For example, adipocyte expression and secretion of adiponectin has been shown to be reduced by TNF- alpha.³¹ Therefore it may be reasonable to surmise that increased TNF- alpha and possibly other adipocytokines that are expressed in increased amounts in the obese state may at least be partially responsible for the decreased adiponectin production in obesity. Levels are also lower in diabetic patients compared with nondiabetic subjects,¹⁸ and are particularly low in subjects with CAD.¹⁸ Decreased levels are found in men compared with women,¹² and this may be androgen induced.³³ The incidence of cardiovascular death has been found to be higher in patients with

renal failure who have decreased adiponectin levels (hypoadiponectinemia).³⁴ Decreased adiponectin levels were found to be closely related to the degree of insulin resistance and hyperinsulinemia in a study conducted on Pima Indians and Caucasians individuals with a wide range of glucose tolerance.¹³ Ethnicity seems to play a role, since one study showed significantly higher plasma concentrations of adiponectin in Caucasians compared with BMI-matched Indo-Asians.³⁵ Several studies have reported a significant negative correlation between circulating adiponectin and triglyceride levels and a positive correlation between adiponectin and HDL cholesterol levels in type 2 diabetes¹⁸. Others have also demonstrated that plasma adiponectin concentrations were not only inversely related to triglyceride levels, atherogenic index (total: HDL cholesterol) and apolipoproteins (apos) B and E, but also positively correlated to serum HDL cholesterol and apo A-1 in nondiabetic female patients.²² These declines in adiponectin in hypertriglyceridemic, high atherogenic index, and low HDL states were also observed after adjusting for BMI, body fat mass, age, and diastolic blood pressure. These findings suggest that the hypoadiponectinemia observed in dyslipidemia may accelerate the atherosclerotic changes seen in the metabolic syndrome.

METABOLIC ROLES OF ADIPONECTIN

Adiponectin as a mediator of insulin action/resistance

A strong correlation between adiponectin and systemic insulin sensitivity has been well established both in vivo and in vitro in mice, other animals, and humans.^{11,17,27,36,37,38,39,40,41,42} In experiments conducted by researchers,²⁷

intraperitoneal injection of mammalian- expressed full-length adiponectin into fasting male wild-type mice and two models of type 1 diabetes insulinopenic nonobese diabetic and streptozotocin induced diabetic mice produced a significant transient reduction of glucose levels. Adiponectin did not appear to be acting primarily as an insulin secretagogue, since insulin levels were low at the beginning of the experiments in all animals and remained low even after adiponectin injection. Adiponectin injection into a type 2 diabetic model (ob/ob mice) also lowered glucose levels. Despite dramatically different insulin levels in the different mouse models, a common mechanism appeared to be responsible for the decreased plasma glucose sensitization of the liver to insulin-induced suppression of hepatic glucose output. Studies ¹¹ showed similar effects, namely improved insulin sensitivity and amelioration of hyperglycemia in mouse models of obesity, diabetes, and lipodystrophy, although following systemic infusion of physiological doses of the globular domain of adiponectin, not the full-length form. Euglycemic hyperinsulinemic clamp studies have shown that acutely increasing circulating adiponectin levels by infusion of recombinant full-length adiponectin improves insulin-induced suppression of hepatic glucose production in mice. This was associated with a reduced expression of the gluconeogenic enzymes PEPCCK and glucose- 6-phosphatase (G6Pase), indicating that transcriptional regulation of these two enzymes may contribute to the molecular mechanism of action of adiponectin.³⁶ Circulating adiponectin levels have been shown to decrease in parallel with progression of insulin resistance during development of type 2 diabetes in rhesus monkeys genetically predisposed to develop insulin resistance.³⁷ In this study, there was a negative correlation of adiponectin levels with body weight and fasting insulin levels and a positive

correlation with insulin-stimulated glucose uptake (a marker of insulin sensitivity). In these monkeys, the decline in adiponectin levels preceded overt hyperglycemia. Development of hyperinsulinemia is one possible mechanism for the suppression of adiponectin levels seen in these studies. However, hyperinsulinemia per se seems unlikely as a mediator of low adiponectin levels, since adiponectin levels remain low in the later stages of type 2 diabetes in association with decreased circulating insulin levels. Adipocyte insulin action or signal transduction rather than absolute levels of insulin may regulate adiponectin secretion. In support of this contention, Bogan and Lodish⁴³ have shown that secretion of adiponectin by 3T3-L1 adipocytes requires phosphatidylinositol 3-kinase (PI-3K), a major intermediate of insulin signaling activity. Insulin stimulated insulin receptor substrate 1 (IRS-1)-associated PI-3K activity has been shown to be decreased in adipocytes of type 2 diabetic subjects.⁴⁴ Thus it is possible that the decreased adipocyte PI-3K activity in type 2 diabetic patients may contribute to the decreased adiponectin levels. Additional investigations to test this hypothesis are warranted. Other investigators have presented data on the potential inverse relationship between adiponectin and insulin action. Euglycemic-hyperinsulinemic clamp studies in both humans and rats⁴⁵ have shown that insulin infusion leads to decreased circulating adiponectin levels, consistent with the interpretation that insulin exerts an acute effect on adipocytes to decrease production and/or secretion of this adipocytokine. There is published data³⁸ supporting a possible role of adiponectin in catecholamine-induced insulin resistance. They found that treating 3T3-L1 adipocytes with the adrenergic agonist isoproterenol reduced the level of adiponectin mRNA by 75% in vitro. This inhibitory effect of isoproterenol was almost completely reversed by

pretreatment of the cells with the adrenergic antagonist propranolol and the protein kinase A (PKA) inhibitor H-89. The authors concluded that catecholamines might induce insulin resistance at least partly by downregulation of adiponectin gene expression, and that this inhibitory effect was mediated via adrenergic receptors through a Gs protein (stimulatory guanine nucleotide binding) PKA-dependent pathway. Homozygous (adipo adiponectin-deficient mice have been shown to have significantly increased insulin resistance when compared with wild-type and heterozygous (adipo) adiponectin-deficient mice in certain studies.³⁹ This loss-of-function experiment provides further evidence that adiponectin is indeed required for normal regulation of insulin sensitivity and glucose homeostasis in vivo.

The connection between adiponectin levels and insulin resistance has been further confirmed by data obtained from treatment with TZDs. The peroxisome proliferator-activated receptor (PPAR) gamma is a ligand-activated transcription factor thought to be a master regulator of adipocyte differentiation and multiple adipocyte genes. TZDs are specific synthetic ligand activators of PPAR-gamma that improve glucose tolerance and insulin sensitivity in type 2 diabetic patients and in animal models of insulin resistance through mechanisms that are incompletely understood. The administration of TZDs has been shown to increase the plasma adiponectin concentrations in insulin resistant humans and rodents and in subjects with type 2 diabetes.^{14,15,16,17} The promoter activity of the adiponectin gene has been shown to be markedly enhanced by the TZDs,¹⁴ although the presence of a functional PPAR-gamma response element in the adiponectin gene remains controversial.^{46,47} The induction of adiponectin in fact

might be caused by secondary effects involving other PPAR inducible genes and not by specific activation of the PPAR response elements.⁴⁸ In support of an important role for PPAR-gamma in regulation of adiponectin synthesis, circulating adiponectin levels were found to be suppressed fivefold in patients with severe insulin resistance in association with dominant-negative PPAR-gamma mutations.¹⁷ Thus, induction of adipose tissue adiponectin expression and consequent increases in circulating adiponectin levels could potentially represent a novel potential mechanism for PPAR-mediated enhancement of whole body insulin sensitivity. Furthermore, adiponectin may be a biomarker of in vivo PPAR-gamma activation. Studies reported an increase in adiponectin levels in normal subjects after only 14 days of treatment with rosiglitazone. This finding was supported by a recent study in rats, which showed a similar increase in adiponectin levels after two weeks of TZD treatment.³⁷ In another study,¹⁶ an increase in the plasma adiponectin levels was observed along with weight gain after rosiglitazone treatment. These results may appear contradictory to the reported negative correlation between plasma adiponectin levels and body weight. The activation of PPAR-gamma by TZDs may promote weight gain by increasing adipocyte differentiation and the number of small adipocytes, as has been previously shown,⁴⁹ as well as enhance adiponectin gene transcription in existing mature adipocytes, thus increasing adiponectin levels. Adiponectin has also been proposed by some investigators as a reliable marker for insulin resistance in type 2 diabetes.

Researchers⁴⁰ used the hyperinsulinemic- euglycemic clamp to quantify glucose infusion rate (GIR) as an index for insulin sensitivity in 16 patients with

type 2 diabetes. GIR was most strongly correlated with circulating adiponectin levels and fasting plasma glucose. The role of adiponectin in mitigating insulin resistance has been further substantiated by studies in humans and mice with lipodystrophies.^{11,41,42} Lipodystrophies are characterized by selective but variable loss of body fat and insulin resistance. Serum adiponectin levels are extremely low in patients with generalized lipodystrophies and may be related to the general absence of adipose tissue and/or associated severe insulin resistance. Researchers¹¹ showed that treating lipoatrophic mice with physiological doses of adiponectin significantly but not completely ameliorated hyperglycemia and hyperinsulinemia. Adipose tissue expression and circulating adiponectin concentrations have also been found to be significantly decreased in HIV-positive patients with lipodystrophy treated with highly active antiretroviral therapy. Both serum and mRNA concentrations of adiponectin were found to closely correlate with features of insulin resistance, including hepatic fat content.⁵⁰ Thus, it may be reasonable to surmise that decreased production of adiponectin in lipoatrophic adipose tissue may contribute to the development of insulin resistance in these patients. Although a cause-and-effect association has not been definitely established, available evidence indicates that visceral fat is an important link between the many facets of the metabolic syndrome, including glucose intolerance, hypertension, dyslipidemia, and insulin resistance.⁵¹ Visceral adiposity is characterized by enhanced lipolysis¹ and augmented plasma FFA flux, especially into the portal circulation. Increased inflow of FFAs into the liver from the portal circulation is thought to retard insulin clearance and to enhance lipid synthesis, which may result in peripheral hyperinsulinemia and hyperlipidemia. FFAs have also been shown to induce hepatic insulin resistance

by inhibiting insulin suppression of glycogenolysis during euglycemic hyperinsulinemic clamp studies⁵² and to directly stimulate glycogenolysis and gluconeogenesis, thus contributing to mild fasting hyperglycemia in euglycemic subjects given lipid infusions.⁵³ Adiponectin mRNA and protein levels have been found to be reduced in omental fat compared with subcutaneous fat.⁵⁴ Visceral fat may also produce an as-yet-unidentified factor that destabilizes adiponectin mRNA.⁵⁵ The strong inverse correlation between serum adiponectin levels and intra-abdominal fat mass may in part underlie the link between visceral fat and insulin resistance. Although these epidemiological and experimental studies are suggestive of a role for adiponectin in insulin sensitivity and firmly establish an association between insulin resistance and low plasma adiponectin levels, it is not yet established whether decreased adiponectin levels are the cause or effect of this dysregulated metabolic state.

Adiponectin and atherosclerosis

Experimental studies have indicated that adiponectin has potential antiatherogenic and anti-inflammatory properties.^{19,20,21,56,57,58,59,60} Monocyte adhesion to the vascular endothelium and subsequent differentiation to macrophages and foam cells is considered crucial for the development of vascular disease. In certain studies¹⁹ it was found that adiponectin had effects on monocyte adhesion to endothelium, myeloid differentiation, and macrophage cytokine production and phagocytosis. Adiponectin has been shown to inhibit both the production and action of TNF-alpha, a cytokine that has direct effects on the adhesion molecules.^{3,19} Although its receptor has not been identified, adiponectin modulates signaling of nuclear factor beta (NF Beta) (a transcription factor

involved in the inflammatory response), at least partly through a cAMP-dependent pathway.⁵⁶ It has been shown that adiponectin suppressed macrophage to foam cell transformation in vitro.⁵⁷ Thus adiponectin probably serves as a modulator for macrophage foam cell formation and could provide an answer to the fundamental mechanism for the link between vascular inflammation and atherosclerosis. Furthermore, adiponectin mediated signaling has been shown to inhibit growth factor-induced human aortic smooth muscle cell proliferation and migration.²⁰ These in vitro studies demonstrate that adiponectin may act as an antiatherosclerotic factor through a direct effect on endothelial cells. Severe neointimal thickening and increased proliferation of vascular smooth muscle cells has been demonstrated in mechanically injured arteries of adiponectin knockout mice. Supplementation of adiponectin in this mouse model attenuated the neointimal proliferation.⁵⁸ This has been the first in vivo evidence that adiponectin might serve as a critical link bridging the adipose tissue-vascular axis. Amelioration of atherosclerosis associated with decreased expression of class A scavenger receptor and TNF-alpha has been demonstrated in globular adiponectin transgenic (gAd Tg) apo E-deficient mice.³⁰ This appears to be the first in vivo demonstration of a protective role of adiponectin against atherosclerosis. High-sensitive C-reactive protein (hs-CRP) is a well-known marker and risk factor for coronary artery disease. It was recently shown that CRP mRNA is expressed in human adipose tissue.⁵⁹ A significant inverse correlation has been observed between CRP and adiponectin mRNA levels in subcutaneous adipose tissue of human subjects with angiographically demonstrated coronary atherosclerosis.⁵⁹ The same negative correlation exists between plasma hs-CRP and adiponectin levels. This reciprocal association

between adiponectin and CRP levels in both human adipose tissue and plasma is supportive of a role for adiponectin against the development of atherosclerosis and vascular inflammation.

CLINICAL RELEVANCE OF ADIPONECTIN

Type 2 diabetes results from an interaction between genetic and environmental factors. Genome-wide scans have mapped a susceptibility locus for type 2 diabetes, metabolic syndrome, and coronary heart disease to chromosome 3q27, where the gene encoding adiponectin is located.^{60,61,62,63} In some studies⁶⁴ it was found that genetic variations resulting in reduced serum adiponectin levels are associated with increased risk for type 2 diabetes in the Japanese population. In another study, Japanese subjects carrying a missense mutation in the adiponectin gene associated with hypoadiponectinemia exhibited the phenotype of the metabolic syndrome, including insulin resistance and coronary artery disease.⁶⁵ Thus genetic polymorphisms of the adiponectin gene that result in lower production and secretion of adiponectin may be responsible, at least in part, for the pathogenesis of the insulin resistance syndrome and diabetes. Conversely, increased baseline concentrations of adiponectin may be associated with a reduced risk of developing type 2 diabetes.⁶⁶ Replenishment of adiponectin might represent a novel treatment strategy for insulin resistance and type 2 diabetes. Adiponectin might have several therapeutic advantages over antidiabetic drugs now used clinically. First, in addition to hypolipidemic and antidiabetic effects, adiponectin has potential anti-inflammatory properties that might prevent or retard atherogenesis. Second, adiponectin appears to exert these effects without increasing body weight.¹¹ Adiponectin might have therapeutic

implications as an anti-obesity drug as well, although there have been no studies in humans so far. In a study, administration of adiponectin slightly but not significantly reduced weight gain induced by a high-fat diet in mice.¹¹ In another study daily administration of a very low dose of gAd to mice consuming a high fat/sucrose diet caused profound and sustainable weight reduction without affecting food intake.²⁶ The effect of gAd on weight reduction may reflect its ability to stimulate lipid oxidation or some other yet-to-be-described mechanism. It remains to be determined whether adiponectin can be effectively and safely used as a pharmacologic means to treat obesity in humans. It is also important to note that although low concentrations of plasma adiponectin are observed in obese individuals, a prospective study done in Pima Indians found that circulating adiponectin levels did not predict future weight gain and thus did not appear to play an etiologic role in the development of obesity in these individuals.⁶⁷ Improvement in insulin sensitivity by weight reduction in obese subjects with gastric bypass surgery^{68,69} has been reported to increase adiponectin levels. However there has been conflicting data on whether improvement in insulin sensitivity with exercise training is associated with increased adiponectin levels. One study found a correlation between incremental increases in glucose infusion rates (a measure of insulin sensitivity) during euglycemic-hyperinsulinemic clamp studies and adiponectin levels with intensive aerobic exercise,⁷⁰ whereas another group of investigators found no increase in adiponectin levels even after six months of exercise training, although insulin action significantly improved.⁷¹ The differences in these observations may be in part due to the fact that there was improvement in body composition with the first study, whereas there was no loss

of body mass in the latter. In view of its potential beneficial effects, any measure that increases adiponectin levels would likely have some clinical significance.

Whether this improvement in insulin sensitivity by the above measures or by treatment with PPAR gamma agonists is mediated entirely or in part by adiponectin has yet to be determined.

Adiponectin and coronary artery disease

When the vascular endothelium is injured, adiponectin accumulates in the subintimal space of the arterial wall through its interaction with collagens in the vascular intima. Adiponectin attenuates TNF- α -induced expression of adhesion molecules in endothelial cells, which is an initial step of atherosclerosis. In a study¹⁸ it was shown that Plasma adiponectin concentrations in diabetic women without CAD were significantly lower than those in nondiabetic women (7.6 versus 11.7 μ gm/dl, $P = 0.001$). Diabetic women with CAD exhibited even lower plasma adiponectin concentrations (6.3 μ gm/dl). In men, diabetic subjects without CAD also showed lower plasma adiponectin levels compared with nondiabetic subjects (6.660.4 versus 7.960.5 μ gm/dl). Plasma adiponectin levels in diabetic men with CAD were even lower and statistically significant when compared with diabetic men without CAD (4.060.4 versus 6.660.4 μ gm/dl, $P=0.001$).

CLINICAL EVALUATION OF OBESITY

Body Mass Index

Quetelet was one of the leaders in developing “mathematical” methods to evaluate populations. He developed the concept of the “average man” and used the ratio of weight divided by the square of stature or height (kg/m^2) as a measure of an individual’s fatness. This unit, the body mass index, might be termed the Quetelet Index (QI) in honor of the man who developed what has become a widely used way of evaluating weight status.

Accurate measurement of height and weight which are used to calculate the BMI is the initial step in the clinical assessment of overweight. This index is calculated as the body weight (kg) divided by the stature (height [m]) squared (wt/ht^2). The BMI needs to be adjusted for ethnicity. Based on these differences and the observations that the risk for diabetes and hypertension had doubled when the BMI was 25 kg/m^2 , a task force from the Asia- Oceania section of the International Association for the Study of Obesity has proposed an alternative table where obesity is defined as a BMI more than 25 kg/m^2 and high-risk waist circumference at more than 90 cm for men and more than 80 cm for women.

Waist Circumference

The most accurate measurement of visceral or central fat is obtained from a computed tomogram or with MRI, but these are expensive and not generally available. For this purpose, waist circumference is the most practical clinical alternative. Waist circumference is measured with a flexible tape placed horizontally at the level of the natural waist line or narrowest part of the torso as

seen anteriorly. The ratio of waist circumference divided by hip circumference waist-hip ratio; (WHR) was used in the pioneering studies that brought scientific recognition in the 1980s to the relationship of centrally located fat to the risk of developing heart disease, diabetes, and other chronic problems associated with obesity. This concept was originally suggested by Vague in 1948 and is now widely accepted.

Some studies also showed that waist circumference was as good as or better than WHR or sagittal diameter in estimating visceral fat. For practical purposes, waist circumference alone and/or WHR are used as one criterion for evaluating the contribution of fat distribution to the health risk from obesity.

Studies have shown that anthropometric measures, BMI, WHR, and waist circumference, are not comparable measures across different racial populations. Age- and weight matched Asian Indian men had 21% more total body fat than Swedish men (multiscan CT).

The emerging typical Asian Indian urban/migrant has phenotype of higher percentage of body fat at a lower value of body mass index (BMI), high waist hip ratio (WHR) at a relatively low waist circumference and less lean body mass as compared to ethnic groups. Asian Indian migrants have higher values of BMI and WHR and thicker skin folds as compared to urban subjects in India, Asian Indian men had significantly thicker truncal skin folds as compared to Caucasians. High body fat, often at BMI values that are in non-obese range is another characteristic phenotypic feature of Asian Indians, reported by several groups, including that in Asian Indians in USA (mean BMI, 24.5 kg/ m², body fat ~33%) and in Asian

Indians in India (mean BMI 23.3 kg/m², body fat ~ 35%). This leads to abnormal lean body mass (muscle) to fat ratio; sarcopenia with higher body fat composition. The emerging Asian Indian Phenotype is high body fat with relatively less body BMI, less lean body mass (particularly in lower limbs), high BF/ BMI ratio (higher body fat per unit BMI), high waist-hip ratio (absolute value of waist circumference may not be excessive), variable subscapular/triceps ratio, high intramyocellular lipids. Thus Asian metabolic syndrome is the constellation of adverse metabolic and clinical effects of insulin resistance. Its high and increasing prevalence and its profound impact on the major diseases requires that clinicians consider its diagnosis and management on a routine basis. Recently published guidelines on its definition now make convenient and reliable diagnosis possible. ATP III/WHO guidelines spelt out criteria for metabolic syndrome. If three of five are present then a case qualifies for it. Auxological parameters and lipid levels are central to it. Researchers have also modified ATP III criteria in Urban Asian adults viz. with a modified waist circumference (WC) appropriate for Indians: raised WC (men more than or equal to 90 cm) and (women more than or equal to 85 cm) with rest of the values being the same as ATP III.

Table No. 1: Classification of Obesity as Recommended by the Asia-Pacific Task Force

Classification	BMI (Kg/m ²)	Waist circumference	
		<90 cm (men) <80 cm (women)	≥90 cm (men) 80cm (women)
Normal range	18.5-22.9		
Overweight	> 23	Average	Increase
At risk	23 to 24.9	Increase	Moderate
Obese I	25 to 29.9	Moderate	Severe
Obese II	≥30	Severe	Very severe

METHODOLOGY

The present study was conducted at KLES Dr. Prabhakar Kore Hospital and Medical Research centre during the period of Jan 2007 to May 2008.

Study Design

A case control study.

Source of Data

All male Type-2 diabetic patients attending the out patient department of KLES Dr. Prabhakar Kore Hospital and Medical Research Centre and those admitted in the wards.

Selection Criteria

Inclusion Criteria

- Male subjects of any age group diagnosed to have type 2 diabetes.
- HbA1c of participants should be <7.5% done within 2 months of screening.

Exclusion Criteria

- Patients having any abnormalities on ECG.
- Those patients who are on Thiazolidinedione group of drugs will be excluded.

Sample Size

Ninety obese and non obese male patients were selected for the present study.

Sampling Procedure

With the help of a statistical software, taking the likely difference in the mean value of adiponectin in the groups (Diabetic obese and non obese) to be 2.75 (on the basis of earlier studies), and taking standard deviation of four in each group, with α value at 0.05 and β value to be 0.20, the sample size required would be 33 in each group. Hence a total sample size of 70 patients was decided with 35 in each group. 20 control (Non diabetic) subjects were taken for comparison with the diabetic subjects. Finally data obtained was systematically tabulated and data analysis was done both quantitatively as well as qualitatively as mentioned in the analysis plan.

Procedure

All cases were evaluated and selected by detailed medical history and physical examination. The study was approved by the Ethical and Research Committee of J. N. Medical College, Belgaum.

All male Type-2 diabetic patients attending the out patient department of KLES Dr. Prabhakar Kore Hospital and Medical Research Centre and those admitted in the wards were screened for the eligibility into the study. After finding the suitability as per inclusion and exclusion criteria they were selected for the study and briefed about the nature of the study, the interventions used and written informed consent was obtained (Annexure-I). The consented patients were enrolled in the present study. Further, descriptive data of the participants like name, age, sex, detailed history, were obtained by interviewing the participants and recorded on predesigned and pretested proforma (Annexure-II).

Relevant diabetic history, clinical examination, Anthropometry and Serum Adiponectin levels were evaluated for each subject. Subjects were grouped as overweight (BMI more than or equal to 23) and non overweight (BMI less than 23) on the basis of the Asia pacific guidelines. Finally data obtained was systematically tabulated and data analysis was done both quantitatively as well as qualitatively.

Investigations done

- Assessment of Adiponectin
- Body Mass Index
- Waist Hip Ratio
- Other Investigations

Assessment of Adiponectin

Blood samples for measuring fasting plasma adiponectin were drawn with and transferred into prechilled EDTA tubes and immediately placed on ice. All tubes were cold centrifuged (4°C) within several minutes of collection and stored at 70°C until assay at the Department of Biochemistry.

Body Mass Index

Height was measured in meters in bare foot individuals. Body weight was measured by subjects wearing light summer clothing, less than 23 is considered is normal (Asia Pacific guidelines by WHO).

Waist Hip Ratio

To measure waist at the level in between the iliac crest and costal margin and hip circumference at maximum diameter at the buttocks, 0.90 or less was considered normal.

Other Investigations

Complete blood count including hemoglobin, total leucocyte count, differential leucocyte count, fasting blood sugar, post prandial sugar, glycated hemoglobin (HbA1c), blood urea, serum creatinine, urine routine, microalbuminuria (Whenever possible), electrocardiogram, echocardiography (Whenever possible), fundus examination were done wherever required.

Statistical Analysis

Mean and standard deviation (S.D.) was calculated for all the quantitative variables for both the overweight and non overweight subjects in both cases and controls. Students unpaired t test was used in comparison of two similar parameters in groups. Contingency tables were prepared for different levels of plasma adiponectin and other parameters. Chi-square test was used to find the association between level of these two variables in different tables. Karl pearson's correlation coefficient was calculated to correlate plasma adiponectin levels and different measures of obesity.

RESULTS

A total of 90 subjects were studied and were divided into four groups.

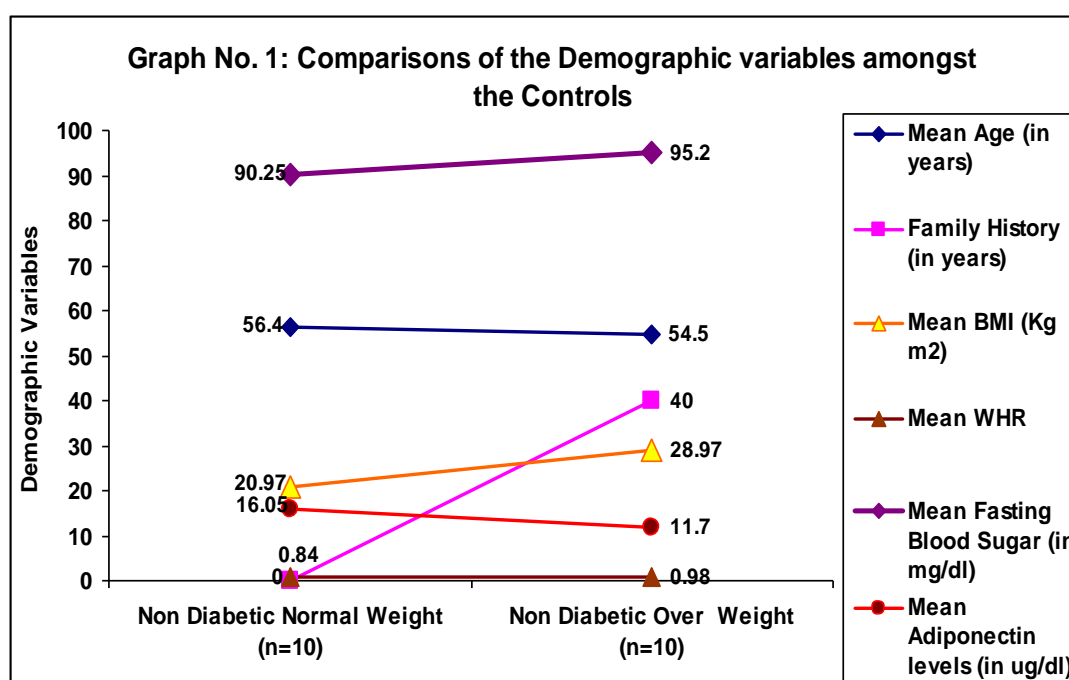
Table No. 2: Distribution of the study population

Groups	Normal Weight (BMI < 23)		Overweight (BMI ≥ 23)	
	Number	Percentage	Number	Percentage
Cases (Diabetic)	35	38.9%	35	38.9%
Controls (Non Diabetic)	10	11.1%	10	11.1%

The study subjects were stratified as cases and controls depending on the presence or absence of diabetes and further sub classified according to their obesity (as defined by their body mass index). We enrolled comparable subjects in both the Normal weight and the over weight category of both cases and controls.

Table No. 3: Comparison of the demographic variables among the controls

Demographic Variables	Non Diabetic Normal weight (n=10)	Non Diabetic Overweight (n=10)
Mean age (in years)	56.4 ± 10.32	54.50 ± 11.78
Family history of diabetes (in years)	NIL (0%)	4/10 (40%)
Mean Body Mass Index (BMI) (in Kg/m ²)	20.97 ± 1.28 (19.3 to 22.7)	28.97 ± 2.76 (23.1 to 31.4)
Mean Waist Hip Ratio (WHR)	0.84 ± 0.04	0.98 ± 0.07
Mean Fasting Blood sugar (in mg/dl)	90.25 ± 9.32	95.20 ± 11.63
Mean Adiponectin levels (in µg/dl)	16.05 ± 2.49	11.7 ± 4.25

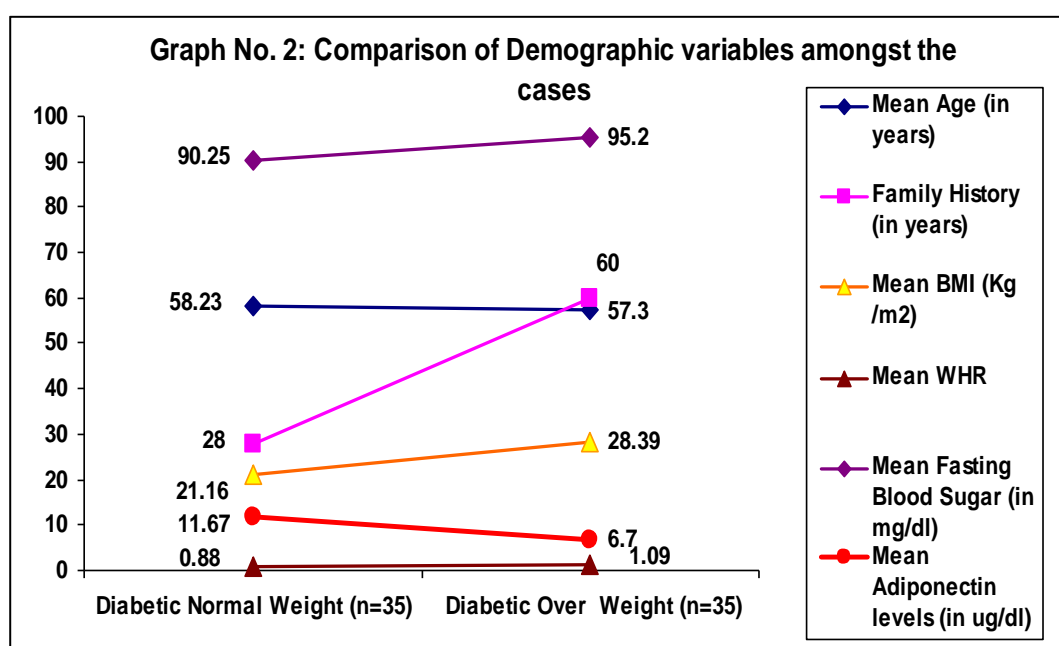


- The mean age and fasting blood sugar in the 2 groups are similar.
- Family history of Diabetes is more prevalent in the overweight group.

- The Abdominal obesity as measured by the WHR is more in the overweight group.
- The mean adiponectin levels are significantly lower in the overweight subjects when compared to normal weight subjects.

Table No. 4: Comparison of the demographic variables among the cases

Demographic Variables	Diabetic Normal weight (n=35)	Diabetic Overweight (n=35)
Mean age (in years)	58.23 ± 9.88	57.30 ± 11.41
Family history of diabetes (in years)	10/35 (28%)	21/35 (60%)
Mean Body Mass Index (BMI) (in Kg/m ²)	21.16 ± 1.49	28.39 ± 3.06
Mean Waist Hip Ratio (WHR)	0.88 ± 0.04	1.09 ± 0.06
Mean Adiponectin levels (in µg/dl)	11.67 ± 2.70	6.70 ± 2.74

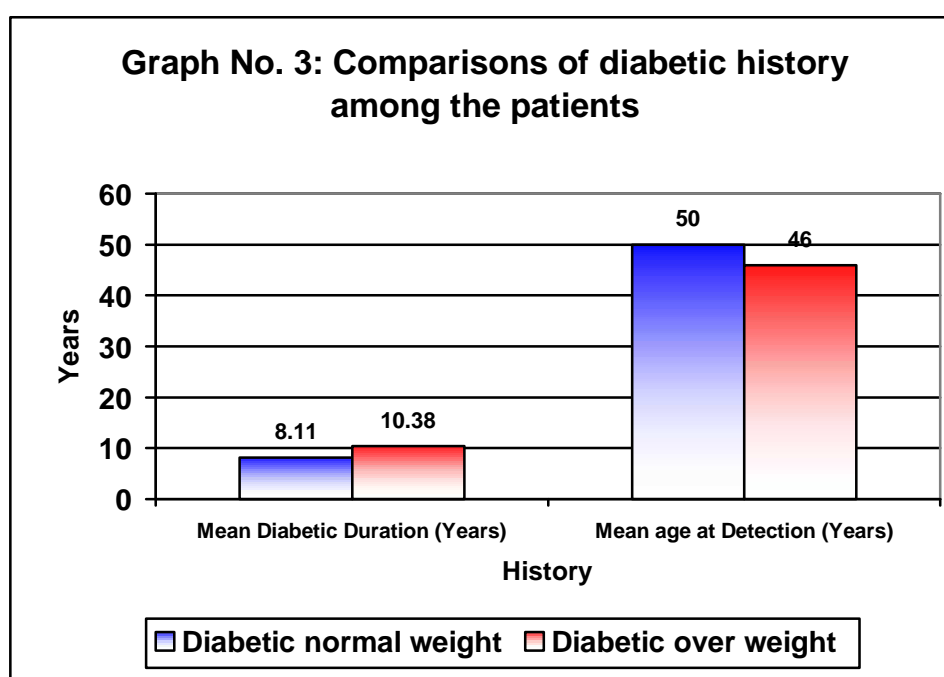


- The mean age in the 2 groups are comparable.
- Family history of Diabetes is more prevalent in the overweight diabetic group.
- The Abdominal obesity as measured by the WHR is more in the overweight diabetic group.
- The mean adiponectin levels are significantly lower in the overweight diabetic subjects when compared to non overweight diabetic subjects.

Table No. 5: Comparison of the diabetic history among the cases

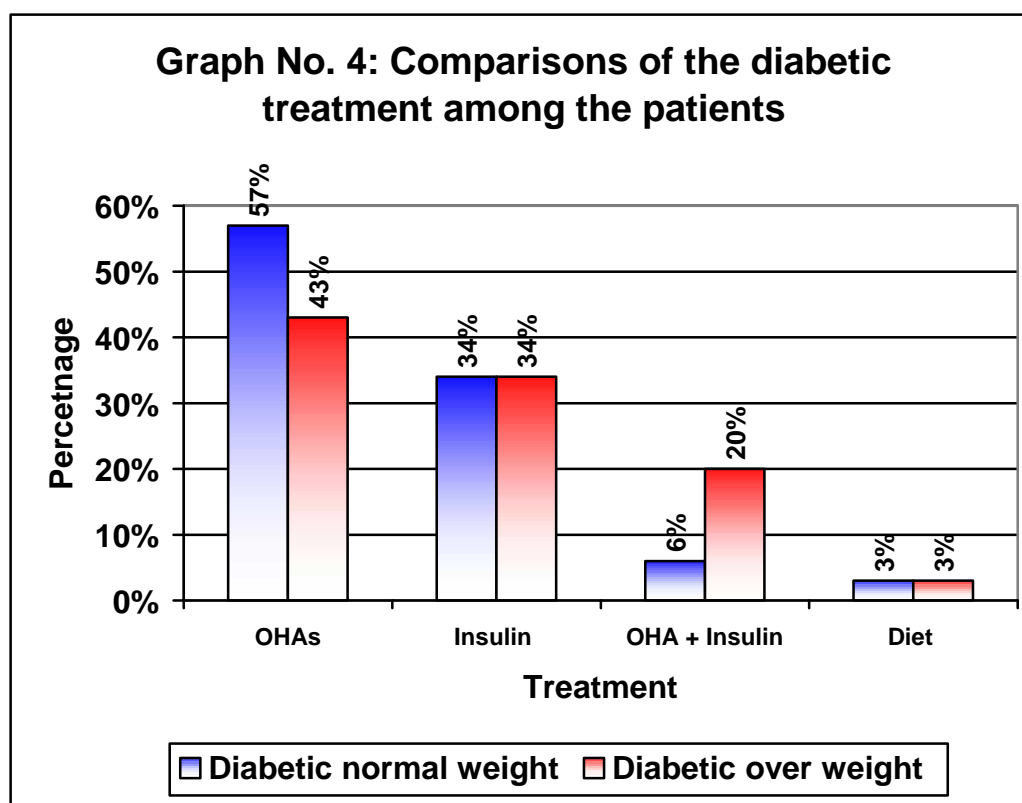
a. Diabetic History

Diabetic History	Diabetic Normal weight (n=35)	Diabetic Overweight (n=35)
Diabetic duration (in years)	8.11 ± 5.70	10.38 ± 7.86
Mean age at detection of diabetes (in years)	50.00 ± 10.9	46.00 ± 11.57



b. Diabetic treatment

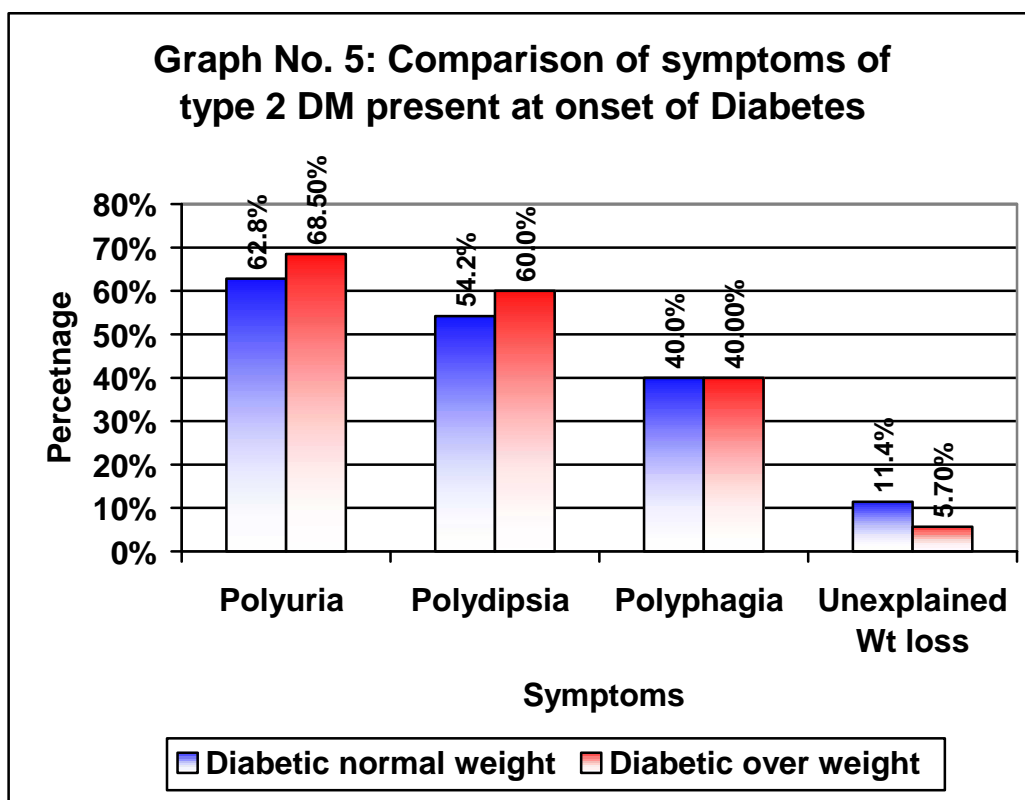
Diabetic Treatment	Diabetic Normal weight (n=35)		Diabetic Overweight (n=35)	
	No.	%	No.	%
Oral Hypoglycemic drugs (O.H.A's)	20	57%	15	43%
Insulin	12	34%	12	34%
Both (O.H.A'S + Insulin)	02	06%	07	20%
Diabetic diet	01	03%	01	03%



- The mean age at detection and duration of diabetes is comparable in the 2 groups.
- Insulin usage was more prevalent in the overweight diabetic subjects – 54% of the diabetic overweight subjects were insulin users as compared to only 40% in the diabetic non overweight group.

Table No. 6: Comparison of symptoms of type 2 DM present at onset of diabetes

Symptoms	Diabetic Normal weight (n=35)		Diabetic Overweight (n=35)	
	No.	%	No.	%
Polyuria	22	62.8%	24	68.5%
Polydipsia	19	54.2%	21	60%
Polyphagia	14	40%	14	40%
Unexplained weight loss	4	11.4%	2	5.7%

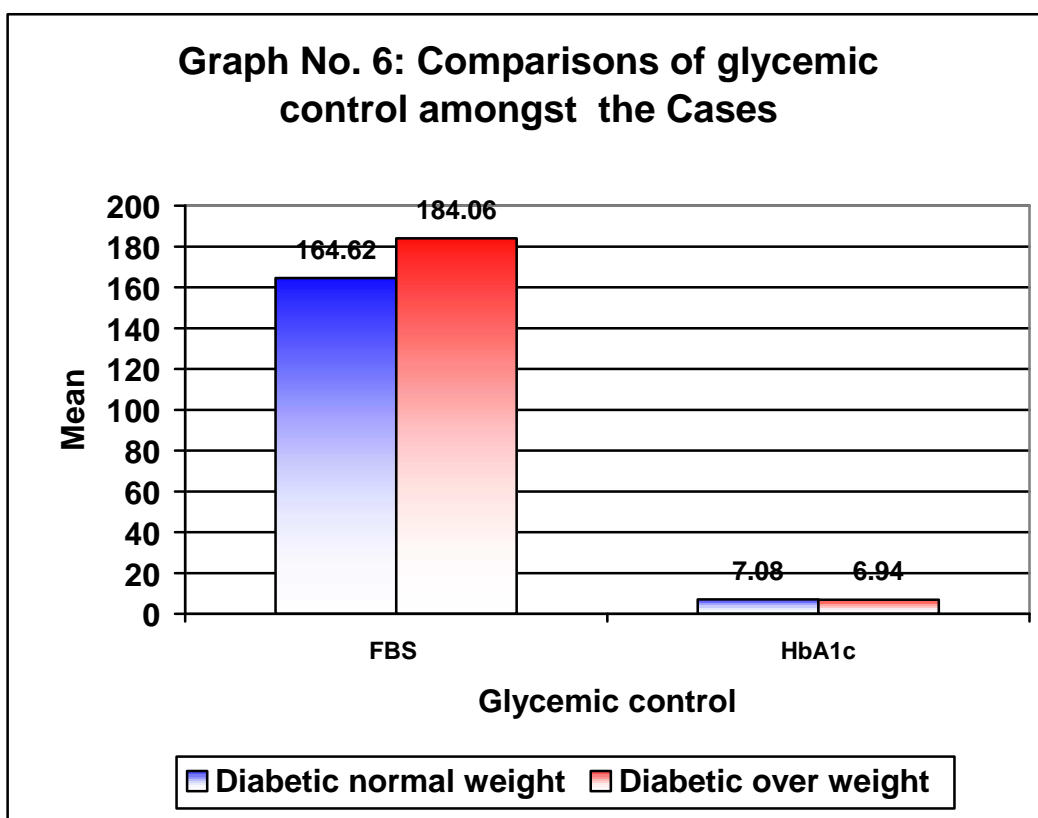


The hyperosmolar symptoms were similar in both the groups, of which polyuria was present in the majority (66%) of the subjects at the onset of diabetes.

Table No. 7: Comparison of glycemc control and complications among the cases

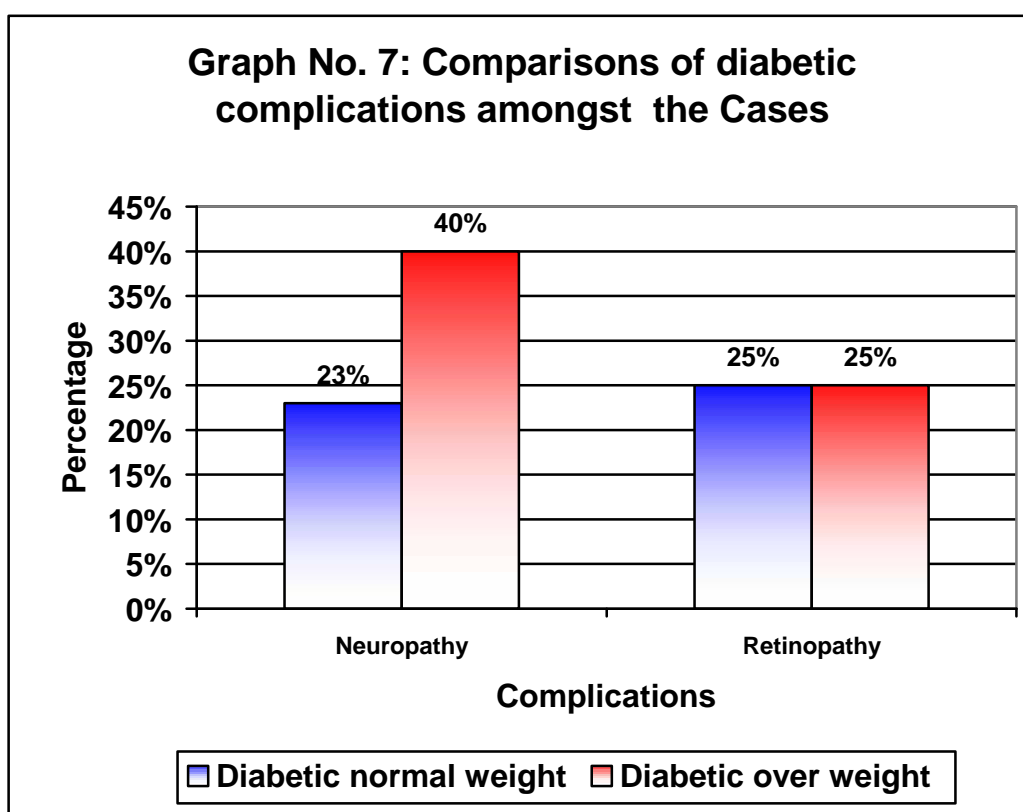
a. Glycemc control

Glycemc control	Diabetic Normal weight (n=35)	Diabetic Overweight (n=35)
Fasting Blood Sugar	164.62 ± 29.56	184.06 ± 30.12
HbA1c	7.08 ± 0.37	6.94 ± 0.28



b. Complications

Complications	Diabetic Normal weight (n=35)		Diabetic Overweight (n=35)	
	No.	%	No.	%
<u>Neuropathy</u>	08	23%	14	40%
<u>Retinopathy</u>	09	25%	09	25%



- The mean FBS was higher in the diabetic overweight group as compared to the normal weight group however the HbA1c was similar in both the groups.
- The prevalence of diabetic neuropathy was found to be more in the diabetic overweight group whereas diabetic retinopathy was similar in both the groups.

Table No. 8: Correlation of diabetic duration and adiponectin**a. Diabetic overweight**

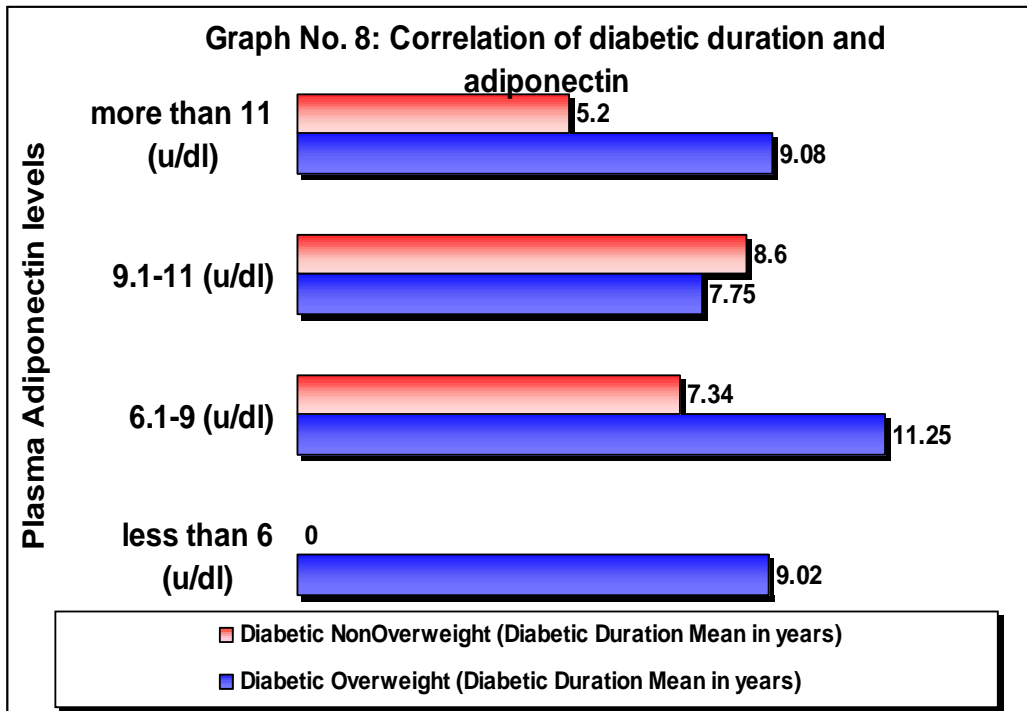
Diabetic Duration (Years)	Plasma adiponectin levels (μ /dl)			
	Less than or equal to 6	6.1 to 9	9.1 to 11	More than 11
Mean (in years)	9.02	11.25	7.75	9.08
Less than or equal to 5	6	3	2	1
6 to 10	3	4	1	0
11 to 15	4	2	2	0
More than 16	3	4	0	1

(p = 0.78)

b. Diabetic normal weight

Diabetic Duration (Years)	Plasma adiponectin levels (μ /dl)			
	Less than or equal to 6	6.1 to 9	9.1 to 11	More than 11
<u>Mean(in years)</u>	0	7.34	8.6	5.2
5 years	0	1	3	7
6-10 years	0	3	7	5
11-15 years	0	0	2	2
> 16 years	0	1	0	2

p = 0.049



There is no association between the duration of diabetes and adiponectin level in both the overweight (p value = 0.78) and the non overweight diabetic group (p value = 0.49). These results are similar to as found by Yamuchi; takayashi et al in their study.

Table No. 9: Correlation between glycemic control and adiponectin levels**a. HbA1c Vs Adiponectin****i. Glycated haemoglobin versus adiponectin levels in diabetic overweight**

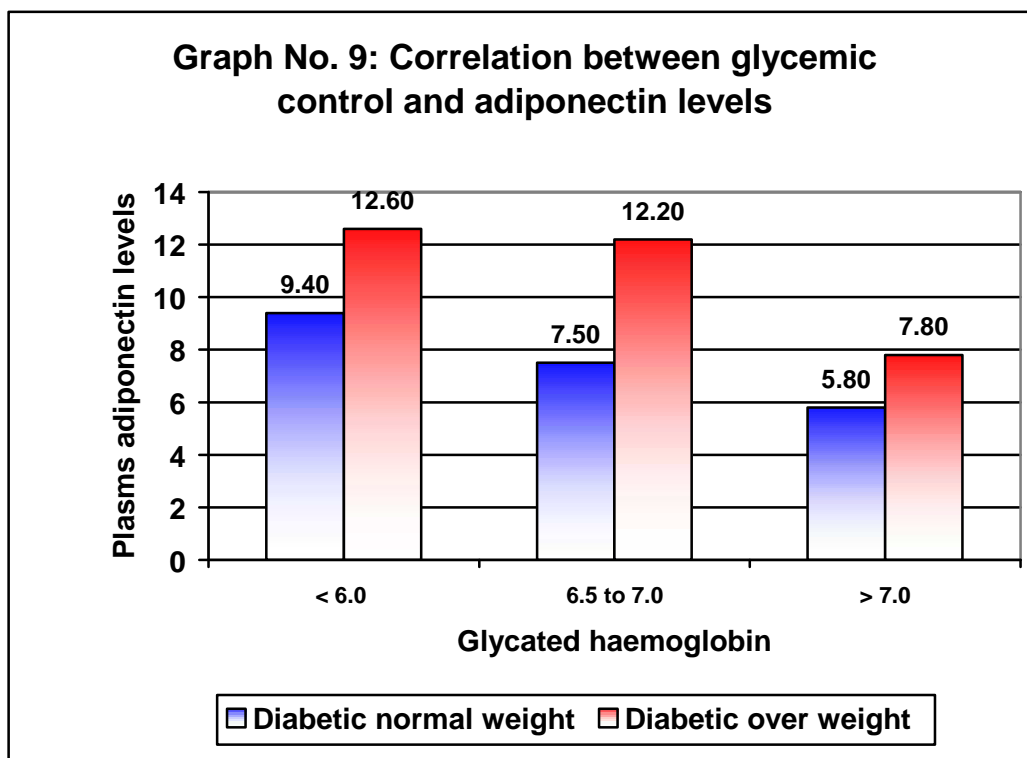
Plasma Adiponectin levels (μ /dl)	Glycated haemoglobin		
	Less than or equal to 6.5	6.5 to 7.0	More than 7.0
<u>Mean plasma adiponectin level</u>	9.4	7.5	5.8
Less than or equal to 6	1/15	3/15	11/15
6.1 to 9.0	0/13	5/13	8/13
9.1 to 11	0/5	2/5	3/5
More than 11	1/2	1/2	0/2

p = 0.006

ii. Glycated haemoglobin vs adiponectin levels in diabetic normal weight

Plasma Adiponectin levels (μ /dl)	Glycated haemoglobin		
	Less than or equal to 6.5	6.5 to 7.0	More than 7.0
<u>Mean plasma adiponectin level</u>	12.6	12.2	7.8
Less than or equal to 6	0/1	0/1	1/1
6.1 to 9.00	0/5	2/5	3/5
9.1 to 11	6/12	3/12	3/12
More than 11	9/16	6/16	1/16

p = 0.004



The Plasma adiponectin levels when compared with HbA1c shows poor glycaemic control in subjects with lower adiponectin levels in both the overweight ($p=0.0067$) as well as non overweight diabetics ($p=0.0046$), this is evident by a decreasing adiponectin levels as the HbA1c rises in both the groups.

b. Fasting Blood Sugar Vs Adiponectin

i. Correlation between glycaemic control and adiponectin levels in overweight

Plasma Adeponectin levels (µ/dl)	Fasting Blood Sugar (mg/dL)		
	Less than or equal to 100	101 to 125	More than 126
Less than or equal to 6	0	0	10/31(32%)
6.1 to 9.00	0	0	11/31(35%)
9.1 to 11	0	0	06/31(19%)
More than 11	0	0	04/31(13%)

$p = 0.003$

ii. Fasting blood sugar vs adiponectin levels in diabetic normal weight

Plasma Adiponectin levels (μ /dl)	Fasting Blood Sugar (mg/dL)		
	Less than or equal to 100	101 to 125	More than 126
Less than or equal to 6	0	0	08/28(30%)
6.1 to 9.00	0	1	06/28(22%)
9.1 to 11	0	0	08/28(30%)
More than 11	0	0	05/28(18%)

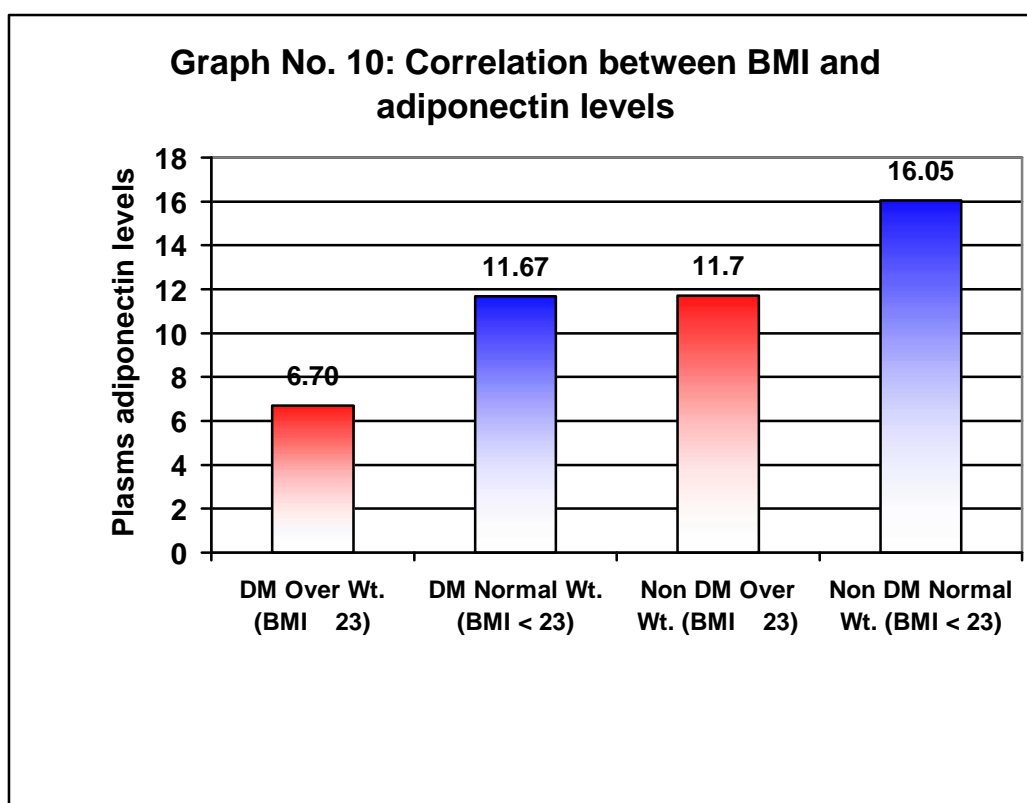
p = 0.039

67% of the overweight diabetic subjects and 52% of the normal weight diabetic subjects having uncontrolled fasting blood sugar (FBS >126%) had plasma adiponectin levels lower than normal (<9 μ /dl). This emphasizes that plasma adiponectin levels are inversely proportional to fasting blood sugar in both the overweight (p=0.003) and normal weight diabetic individuals (p=0.393).

Table No. 10: Correlation between body mass index & plasma adiponectin

Plasma adiponectin levels (μ /dl)	Diabetic		Non Diabetic	
	Over Weight. [BMI \geq 23]	Normal Weight [BMI < 23]	Over weight. [BMI \geq 23]	Normal weight [BMI < 23]
<u>MEAN</u>	6.70 \pm 2.74	11.67 \pm 2.70	11.7 \pm 4.25	16.05 \pm 2.49
Less than or equal to 6	16/35	00/35	1/10	0/10
6.1 to 9.00	13/35	05/35	3/10	0/10
9.1 to 11	05/35	13/35	1/10	1/10
More than 11	01/35	17/35	5/10	9/10

(p value <0.001)

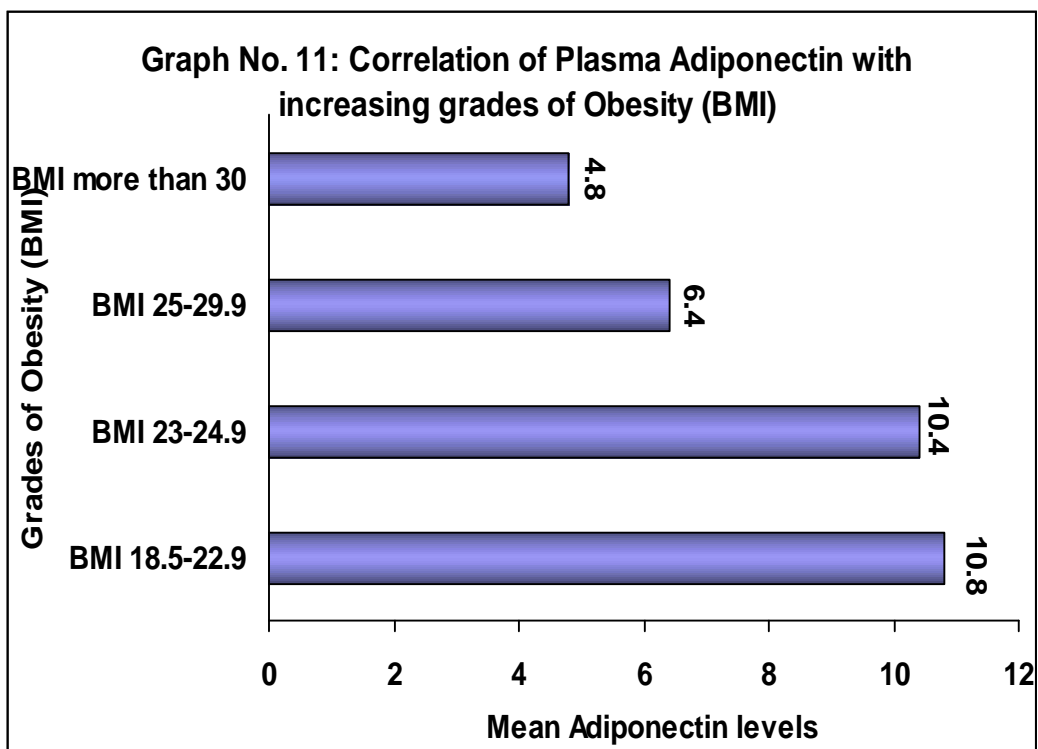


The mean adiponectin level is higher in the non diabetic subjects when compared to the diabetic subjects. Among the 2 groups it is higher among the non obese groups ($P < 0.001$). The correlation co-efficient between the 2 variables plasma adiponectin and BMI was +0.7494 in the diabetic group and +0.6924 in the non diabetic group, which means there is a strong positive correlation between these 2 variables.

Table No. 11: Correlation of plasma adiponectin with increasing grades of obesity (BMI)

Plasma adiponectin levels (μ /dl)	Grades of obesity			
	18.5 to 22.9	Overweight BMI (More than or Equal to 23)		
		23.0 to 24.9	25.0 to 29.9	30
<u>MEAN</u>	10.8	10.4	6.4	4.8
Less than or equal to 6	0/16	0/16	9/16	7/16
6.1 to 9.00	5/18	2/18	10/18	1/18
9.1 to 11	14/18	0/18	4/18	0/18
More than 11	15/18	2/18	0/18	1/18

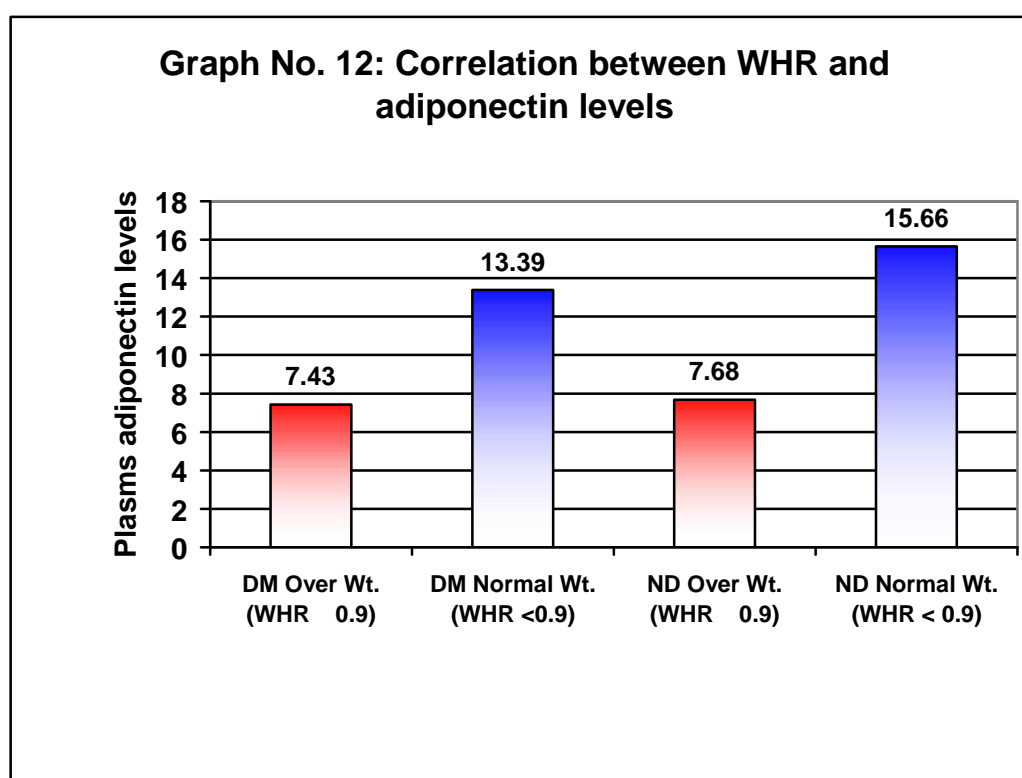
$p < 0.001$



This table reveals that there is a progressive decrease in the plasma adiponectin level with increasing grades of BMI ($P < 0.001$). 100% of subjects with low adiponectin levels ($< 6 \mu\text{dl}$) have their BMI more than 25 Kg/m^2 and 84% of subjects with high adiponectin levels have their BMI ≤ 23 .

Table No. 12: Correlation between waist hip ratio & plasma adiponectin

Plasma adiponectin levels (μ /dl)	Diabetic		Non Diabetic	
	Over Wt. (WHR \geq 0.9) (n=49)	Normal Wt. (WHR $<$ 0.9) (n=21)	Over Wt. (WHR \geq 0.9) (n=5)	Normal Wt. (WHR $<$ 0.9) (n=15)
MEAN	7.43 \pm 2.42	13.39 \pm 0.29	7.68 \pm 1.76	15.66 \pm 2.88
Less than or equal to 6	16/49	0/21	1/5	0/15
6.1 to 9.00	16/49	2/21	3/5	0/15
9.1 to 11	17/49	1/21	1/5	0/15
More than 11	00/49	18/21	0/5	15/15

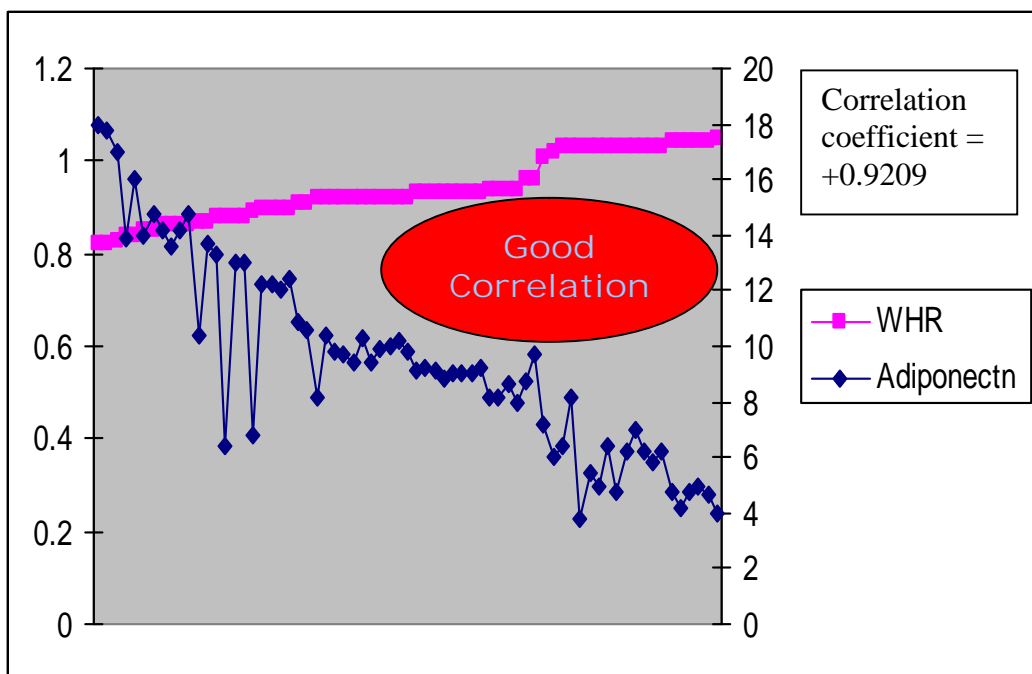
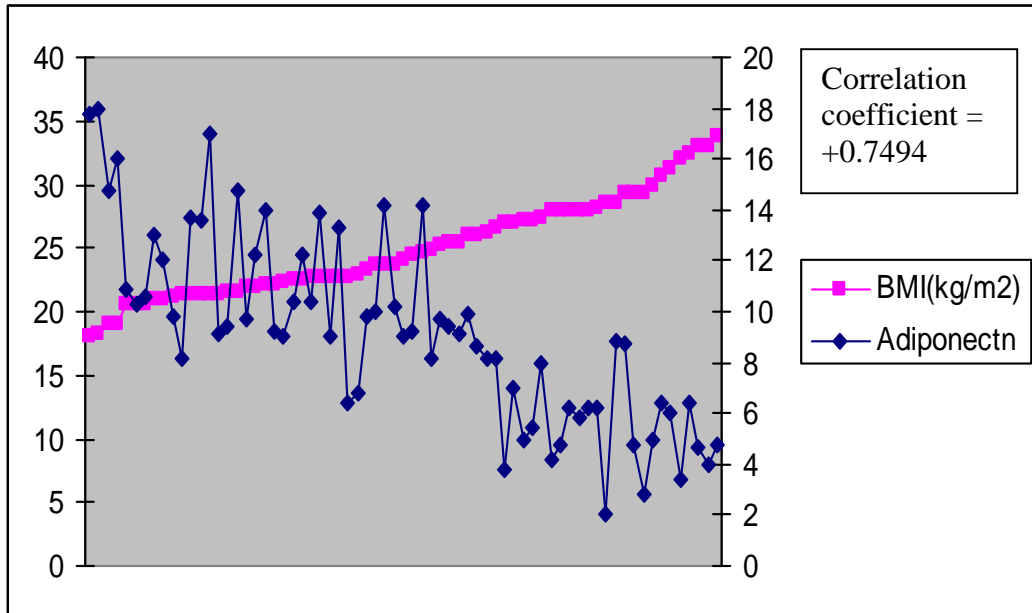


The mean adiponectin level is higher in the non diabetic subjects when compared to the diabetic subjects ($P < 0.001$). Among the 2 groups it is higher

among the non obese groups (as calculated by WHR). The correlation co-efficient between the 2 variables plasma adiponectin and WHR was +0.9209 in the diabetic group and +0.9690 in the non diabetic group, which means there is a strong positive correlation between these 2 variables.

Since the correlation coefficient is higher between the WHR and adiponectin than BMI (+0.7494 and +0.6924) and adiponectin, WHR (+0.9209 and +0.9690)is a better measure of obesity and better predicts insulin resistance and development of atherosclerosis with respect to plasma adiponectin levels.

Graph No. 13: Correlation between increasing grades of obesity (measured separately by body mass index and waist hip ratio) with plasma adiponectin levels



Plasma adiponectin and diabetic complications
Table No. 13: Correlation between plasma adiponectin & diabetic retinopathy
a. Diabetic over weight

Plasma adiponectin levels (μ /dl)	Diabetic Retinopathy	
	Present	Absent
<u>MEAN</u>	5.8	6.3
Less than or equal to 6	08	06
6.1 to 9.00	04	06
9.1 to 11	02	00
More than 11	00	01

p = 0.314

b. Diabetic normal weight

Plasma adiponectin levels (μ /dl)	Diabetic Retinopathy	
	Present	Absent
<u>MEAN</u>	11.4	10.6
Less than or equal to 6	00	00
6.1 to 9.00	00	05
9.1 to 11	04	07
More than 11	04	08

p=0.292

There is no significant correlation between plasma adiponectin levels and diabetic retinopathy in both the overweight and non overweight groups.

Table No. 14: Correlation between plasma adiponectin and diabetic neuropathy

a. Diabetic over weight

Plasma adiponectin levels (μ /dl)	Diabetic Neuropathy	
	Present	Absent
<u>MEAN</u>	<u>5.8</u>	<u>6.4</u>
Less than or equal to 6	06	10
6.1 to 9.00	02	11
9.1 to 11	01	04
More than 11	00	02

p = 0.439

b. Diabetic normal weight

Plasma adiponectin levels (μ /dl)	Diabetic Neuropathy	
	Present	Absent
<u>MEAN</u>	<u>10.8</u>	<u>11.3</u>
Less than or equal to 6	00	00
6.1 to 9.00	02	03
9.1 to 11	03	10
More than 11	04	12

p = 0.759

There is no significant correlation between plasma adiponectin levels and diabetic neuropathy in both the overweight and non overweight groups.

DISCUSSION

Recent research has shown that adipose tissue is not simply an inert storage depot for lipids but is also an important endocrine organ that plays a key role in the integration of endocrine, metabolic, and inflammatory signals for the control of energy homeostasis. The adipocyte has been shown to secrete a variety of bioactive proteins into the circulation, which have been collectively named adipocytokines. Adiponectin, is a novel and important member of the adipocytokine family and has been postulated to play an important role in the modulation of glucose and lipid metabolism in insulin- sensitive tissues in both humans and animals. Decreased circulating adiponectin levels have been demonstrated in genetic and diet-induced human models of obesity. In humans, plasma levels of adiponectin are significantly lower in insulin-resistant states including type 2 diabetes and can be increased upon administration of the insulin-sensitizing thiazolidinedione (TZD) class of compounds. Plasma adiponectin levels in diabetic subjects with coronary artery disease (CAD) are lower than in diabetic patients without CAD, suggesting that adiponectin may have anti-atherogenic properties. In studies done on human aortic endothelial cells, adiponectin has been shown to dose-dependently decrease the surface expression of vascular adhesion molecules known to modulate endothelial inflammatory responses. It also inhibits proliferation of vascular smooth muscle cells and concentrates within the vascular intima of catheter-injured vessels. In clinical studies, low adiponectin levels have been associated with an atherogenic lipid profile. The association of low adiponectin levels with obesity, insulin resistance,

CAD, and dyslipidemia indicates that this novel protein may be an important new marker of the metabolic syndrome.

This study has helped us to find the mean levels of adiponectin in our Asian, diabetic and non diabetic, population in relation to their obesity measured both by BMI and WHR, and thereby predicting its usefulness as a clinical marker of the vascular complications in diabetes and obesity. Asian Indians have a unique phenotype characterized by increased abdominal obesity and visceral fat despite low BMI. Though studies have indicated adiponectin to be associated with diabetes and obesity in the west, there are virtually no studies relating it with diabetes and obesity in the unique Asian-Indian phenotype.

In this study a total of 90 subjects both Diabetic (Cases =70) and Non-Diabetic (Controls=20) were enrolled, they were further classified according to their BMI as overweight (more than 23) and non overweight (less than equal to 23) individuals.

The demographic variables including their present age, sex, age at detection of diabetes, duration of diabetes, hyperosmolar symptoms and glycemic control) were statistically comparable among both the diabetic groups making them a similar cohort of subjects.

The Family history of diabetes was found to be more prevalent among the Overweight population in both the diabetic and non diabetic groups. This result is in concordance with the study conducted by Pascale et al.⁷² This result signifies the importance of the hypothesis of a common existing genetic locus designated

for all the components of metabolic syndrome. Thereby predisposing, offspring's of diabetic individuals, not only for diabetes but also for obesity.

The Abdominal obesity as measured by the increased waist hip ratio was found more in the overweight group. These results were as found by Booth et al⁷³ in their study. This was observed in both the diabetic and non diabetic groups and suggested the more prevalence of abdominal obesity in the overweight group.

The insulin usage was more prevalent in the overweight diabetic individuals than the non overweight group. These results were as published by Lazarus et al⁷⁴. This could be explained both as a cause and effect of each other. Obese individuals tend to have a higher insulin resistance and thereby require exogenous insulin in higher dosage and much earlier in the course of the disease. Those using insulin also tend to gain weight over a few years of treatment.

The diabetic duration when correlated with the plasma adiponectin levels had no association between them. These results are similar to as found by Helen et al.⁷⁵

The plasma adiponectin levels when compared with HbA1c showed poor glycemic control in subjects with lower adiponectin levels in both the overweight ($p = 0.0067$) as well as non overweight diabetics ($p = 0.0046$), this is evident by a decreasing adiponectin levels as the HbA1c rises in both the groups. These results are similar to those as found by Matthias et al.⁷⁶ In our study since all included subjects had a good glycemic control (HbA1c less than or equal to 7.5), even among them the plasma adiponectin levels have shown a strong association with

HbA1c. This implies the importance of adiponectin in controlling the sugar levels even among the well controlled diabetics.

The mean adiponectin level is higher in the non diabetic subjects when compared to the diabetic subjects. These results are similar to as found in by Weyer C et al¹³ in their study where adiponectin was inversely correlated with glycemic values. This suggests the role played by adiponectin in reducing Insulin resistance and thereby improving plasma sugar levels. Among the two groups (Diabetic and non diabetic) Plasma adiponectin was higher among the non obese groups ($p < 0.001$) as also found by Arita Y et al¹² in their study. The inverse relation between obesity and adiponectin levels is paradoxical as adiponectin is itself secreted by adipose tissue. However this paradox has been explained by the feedback inhibition caused by the other Adipocytokines that are secreted in large quantities (leptin, TNF- α) in Obese individuals which inhibit the Adiponectin secreting adiposities responsible in lower adiponectin secretion in obese individuals.

The correlation co-efficient between the two variables plasma adiponectin and BMI was + 0.7494 in the diabetic group and + 0.6924 in the non diabetic group, which means there is a strong positive correlation between these two variables.

When plasma levels of adiponectin levels were compared with increasing grades of obesity (as defined by BMI) there was a progressive decrease in the plasma adiponectin level. These results were as demonstrated by Arita Y et al¹² in their study.

When obesity was defined with the help of WHR then the following observations were made. The mean adiponectin level was higher in the non diabetic subjects when compared to the diabetic subjects ($p < 0.001$). Among the two groups it was higher among the non obese groups (as calculated by WHR). The correlation co-efficient between the two variables plasma adiponectin and WHR was + 0.9209 in the diabetic group and + 0.9690 in the non diabetic group, which means there is a strong positive correlation between these two variables. The inverse correlation between waist hip ratio and adiponectin was also demonstrated by Arita Y et al.¹²

Since the correlation coefficient was higher between the WHR and adiponectin than BMI (+ 0.7494 and + 0.6924) and adiponectin, WHR (+ 0.9209 and + 0.9690) is a better measure of obesity and better predicts insulin resistance and development of atherosclerosis with respect to plasma adiponectin levels.

When plasma adiponectin was compared to microvascular complications of diabetes, it was observed that in this study there was no correlation between plasma adiponectin levels and diabetic retinopathy or diabetic neuropathy, in both the overweight and non overweight groups. The existing literature about this matter is controversial, Kikuku et al in their study found no correlation between Diabetic microvascular complications and Plasma adiponectin levels. However S Hadjadj et al⁷⁷ did report a correlation between the severity of micro vascular complications of diabetes with Plasma Adiponectin levels. There is evidence that adiponectin increases the peripheral insulin sensitivity and thereby prevents the diabetic complications. However it is known that the microvascular complications are more dependent on the degree and duration of hyperglycemia

than insulin resistance which is a marker of macrovascular complications. Also in our study we have included only subjects with good glycemic control and therefore the existence of microvascular complications and its correlation with adiponectin may not be a true projection of the actual data.

CONCLUSION

The view of the adipocyte as simply a storage depot for fat is no longer tenable. Among the various “adipocytokines,” adiponectin, which is an abundant circulating protein synthesized solely in adipose tissue, appears to play a very important role in carbohydrate and lipid metabolism and vascular biology. Adiponectin appears to be a major modulator of insulin action and its levels are reduced in type 2 diabetes, which could contribute to peripheral insulin resistance. It has significant insulin-sensitizing as well as anti-inflammatory properties that include suppression of macrophage phagocytosis and TNF- alpha secretion and blockage of monocyte adhesion to endothelial cells in vitro.

- In this study it was found that the mean adiponectin levels were lower in diabetic subjects when compared to non diabetic subjects, this level was further reduced in the overweight subjects as compared to normal weight subjects in both the diabetic as well as non diabetic group.
- It was also found that lower plasma adiponectin levels were present in subjects with poor glycemic control, higher BMI and higher WHR. The best correlation was found with WHR, reflecting the importance of measuring visceral obesity as a marker of insulin resistance and atherosclerosis.
- It was also found that Plasma adiponectin level did not correlate to diabetic duration and the microvascular complications like diabetic retinopathy and neuropathy.

It is known that Genetic polymorphisms and environmental modifications resulting in lower production and secretion of adiponectin have exhibited a phenotype like metabolic syndrome and are associated with a higher risk of type two DM and cardiovascular mortality. Conversely, increasing the baseline concentrations of adiponectin by modifying the environmental factors it is possible to reduce the risk of developing insulin resistance / type 2 diabetes and coronary heart disease.

Adiponectin can simply be increased by administering insulin sensitizing drugs like Thiazolidinedione class of compounds and doing regular Exercise and by inducing weight loss. Also Adiponectin is grossly reduced in patients with coronary artery disease when compared to matched control subjects implicating its use as a marker of coronary artery disease. This study has explored, the association between excess body weight and plasma adiponectin in the Asian type - 2 diabetic individuals. It has correlated adiponectin levels in diabetic individuals who are not over weight by BMI but are metabolically obese as measured by waist hip ratio, thereby emphasizing on the body fat distribution as an important marker of obesity. This research also emphasizes on the importance of weight reduction in raising adiponectin levels in Indian population until adiponectin itself can be used as a therapeutic modality. This study will also act as a baseline study to conduct further prospective studies which could further prove the importance of Adiponectin as an important clinical marker of atherosclerosis and insulin resistance and also explore the therapeutic aspect of adiponectin.

Although further investigations are required, on adiponectin administration, as well as regulation of the pathways controlling its production, it represents a promising target for managing obesity, hyperlipidemia, insulin resistance, type 2 diabetes, and vascular inflammation. Numerous important questions about adiponectin await further study. The mechanisms by which adiponectin is synthesized and secreted need to be elucidated, as do the signals that reduce adiponectin expression in adipocytes with increasing adiposity. Similarly, the role and regulation of adiponectin oligomerization need to be defined. The molecular mechanisms by which adiponectin exerts its multiple functions and whether its actions are receptor mediated still remain a mystery. Is the primary activity of adiponectin antiatherosclerotic, or is it principally a modulator of lipid metabolism and regulator of insulin sensitivity or is it all of the above? The answers to these and other intriguing questions will undoubtedly provide additional insight into the metabolic roles of this new adipocyte hormone.

SUMMARY

Adiponectin has been labeled as the molecule of this decade due to its protective role in disease like diabetes and coronary artery disease. This study aimed at correlating plasma adiponectin with obesity in type 2 diabetes. 90 age matched male subjects, attending KLES Dr Prabhakar Kore Hospital and Medical Research Centre were enrolled and stratified into four groups based on BMI: Diabetic overweight DO (n=35), Diabetic normal weight DNW (n=35), Non Diabetic overweight NDO (n=10) and Non Diabetic Normal weight NDNW(n=10). Only well controlled (HbA1c < 7.5%) diabetics with normal ECG and not on Thiazolidinedione were included. All were studied with respect to anthropometry (BMI&WHR) and Plasma adiponectin (Measured by ELISA, both globular and full-length).

It was found that Mean adiponectin is higher in non-diabetics compared to diabetics. Among the 4 groups adiponectin levels were lower in the two overweight groups (DO, NDO) versus non-overweight groups (DNW, NDNW) ($p < 0.001$). There was a statistically significant ($P < 0.001$) inverse relation between the range of BMI values and Plasma Adiponectin amongst diabetics. Based on WHR, adiponectin levels were significantly lower ($p < 0.001$) in the centrally obese subjects compared to normal WHR subjects, whether diabetic or not. The correlation coefficient of reduced adiponectin is higher with WHR (+ 0.9209 and + 0.9690 for diabetics and non-diabetics respectively) than with BMI (+ 0.7494 and + 0.6924 for diabetics and non diabetics respectively). Although all diabetic subjects had good glycemic control (HbA1c < 7.5), there

was a statistically significant inverse relation between the range of HbA1c values and Plasma Adiponectin, whether in overweight ($p = 0.0067$) or normal weight ($p = 0.0046$). We concluded that both diabetes and obesity have an inverse correlation with adiponectin. Plasma adiponectin levels correlates better with WHR than BMI and probably predicts insulin resistance and development of atherosclerosis. Even within a normal range of HbA1c, lower values were associated with higher adiponectin.

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ANNEXURE I – CONSENT FORM

Objective and Purpose of the study

This study is a comparative study of plasma adiponectin levels between obese and non obese type 2 diabetic individuals. The principal investigator of the study is Dr. S.B. Kalagate and the co-investigator is Dr. Nitin Kapoor. This research is intended to study the comparison of plasma adiponectin levels in relation to obesity in type 2 diabetic individuals and my co-operation will be of great help to the patients of obesity and diabetes in future.

Procedure

If I agree to be a part of the study I will be asked the relevant history and will be subjected to relevant clinical examination like measuring of height, weight, waist circumference and body fat percentage. I will also have to give two cc of blood for assessment of plasma adiponectin level.

Risk and Benefit

The only risk and possible discomfort I might get is well taking blood from my arm for assessment of plasma adiponectin levels, it may cause swelling, pain, redness, bruising or infection (rarely happens) at the site where needle is inserted.

Alternatives

Taking part in this study is voluntary I may choose not to take part in this study, or if I decide to take part I can later change my mind and withdraw from

the study. My decision will not change the present or future health care or other services that I receive. The study doctor or sponsor may stop my participation in this study any time. If I choose not to take part in the study I will receive standard treatment for patients with my condition.

Privacy and Confidentiality

All information collected about me during the course of this study will be kept confidential to the extent permitted by law. The code numbers will identify me in this research record.

Institutional / Sponsors Policy

Does not apply to this research.

Financial Incentives for Participation

I will not be charged any amount for the investigations subjected to me. I will not receive compensation or reimbursement for taking part in this study.

Authorization to Publish Results

Information from this study may be published but my identity will be confidential in any publication.

Consent Statement

I voluntarily agree to take part in this study by signing below. I may withdraw at any time. I am not giving up any of any legal rights by signing this

form. My signature below indicates that I have read, or it has been read to me, this entire consent form, and have had all my questions answered.

Name of Study Participant or legally authorized representative:

Signature / Thumb Print:

In case of the queries during study or in future you may contact following person.

Principal investigator : Dr. S.B. Kalagate Phone: 0831-2473787

Co-investigator : Nitin Kapoor Phone: 9844169049

Name of Witness:

Signature:

Investigator Name:

Signature:

Date :

Place:

ANNEXURE II – PROFORMA

Name:

S. No. :

Age:

Case/Control:

Sex:

OPD / IPD Number:

Address:

Occupation:

Date of enrollment:

HISTORY

Presenting Complaints

History of presenting illness

Significant Past History

Diabetic history

- Polyuria
- Polyphagia
- Polydipsia
- Unexplained weight loss

Duration of Diabetes

Age at onset of Diabetes

Family history of diabetes

Treatment History of diabetes

Drug Name/Dosage/Frequency/Duration of treatment

Other Drugs (If any)

Significant personal history

Significant Family history

GENERAL PHYSICAL EXAMINATION

VITAL SIGNS

Pulse

Blood Pressure

- In Sitting position
- In Standing position

Respiratory rate

Temperature

Examination of peripheral pulses

ANTHROPOMETRY

Height

Weight

Body Mass Index

Waist circumference

Hip circumference

Waist hip ratio

Any significant findings (Pallor, Icterus Etc)

SYSTEMIC EXAMINATION

Respiratory system

Cardio-vascular system

Per-Abdominal examination

Central nervous examination

EVALUATION OF DIABETIC COMPLICATIONS

VASCULAR COMPLICATIONS

1. Microvascular complications

DIABETIC NEUROPATHY

Symptoms –

Tingling/Numbness/Burning

Neuropathic pain

Diabetic polyradicular pain

Mononeuropathy – Cranial/Peripheral

Examination –

Sensory loss

Loss of ankle reflex

Abnormal position sense

DIABETIC RETINOPATHY

Fundus examination

DIABETIC NEPHROPATHY

Microalbuminuria

(if available)

GFR Calculation – Cockcroft-Gault equation

$$\frac{(140 - \text{age}) * \text{body weight (kg)}}{72 * \text{plasma creatinine (mg/dl)}} =$$

2. Macrovascular complications

History suggestive of:

Coronary artery disease

Cerebro vascular disease

Peripheral vascular disease

NON VASCULAR COMPLICTIONS

Gastrointestinal Manifestations

Anorexia/Nausea/Vomiting/Bloating/ Nocturnal Diarrhea

Genitourinary Complications

History suggestive of Diabetic vesicopathy

History of Erectile dysfunction/retrograde ejaculation

Dermatological manifestations

Diabetic dermopathy

Scleroderma

Lipoatrophy/hypertrophy

Xerosis/Pruritis

INVESTIGATIONS

ROUTINE INVESTIGATIONS

COMPLETE BLOOD COUNT-

1. Haemoglobin
2. Total leucocyte count
3. Differential leucocyte count

BLOOD UREA

SERUM CREATININE

URINE ROUTINE

MICROALBUMINURIA

(Whenever possible)

SPECIFIC INVESTIGATIONS

FASTING BLOOD SUGAR/

POST PRANDIAL BLOOD SUGAR /

RANDOM BLOOD SUGAR –

GLYCATED HAEMOGLOBIN –

ADIPONECTIN LEVEL –

ECG –

ECHO (Whenever possible) -

FUNDUS EXAMINATION (Whenever possible) –

SIGNATURE OF THE GUIDE

ANNEXURE III – KEY TO MASTER CHART

AB	-	Absent
C	-	Case
CN	-	Control
DM	-	Diabetes Mellitus
DD	-	Diabetic diet
FBS	-	Fasting blood sugar
GFR	-	Glomerular filtration rate
HbA1c	-	Glycated haemoglobin
INS	-	Insulin
M	-	Male
MN	-	Month
NA	-	Not applicable
NC	-	Not Checked
OHA	-	Oral hypoglycemic agent
PR	-	Present
R	-	Random
RBS	-	Random blood sugar
Sr.	-	Serum
Sr. Creat	-	Serum creatinine
Sr. No.	-	Serial Number
WHR	-	Waist Hip Ratio
X	-	Not Applicable
YR	-	Year

MASTER CHART

Sr. No.	Group	Demographic Details					DM Status		Anthropometry					Diabetic Complications				Sr. Adiponectin
		Age (In Years)	Sex	Diab duration	Family history	Treatment	HBA1C	FBS/RBS	Height(cms)	Weight(kgs)	Waist circ(cm)	Hip circum(cm)	WHR	Gfr calculation	S.Creat	Neuropathy	Fundus-Diabetes Retinopathy	
1	C	70	M	8 MN	AB	OHA	6.5	183	159	78	111	107	1.03	47.00	1.6	AB	AB	6.4
2	C	54	M	2 YR	PR	OHA	7.3	217	169	84	112	108	1.04	125.00	0.8	AB	AB	4.8
3	C	40	M	3 YR	PR	OHA	6.8	190	168	64	84	96	0.87	126.00	0.7	AB	AB	10.4
4	C	48	M	1 YR	AB	OHA	7.3	105	188	76	93	101	0.92	194.00	0.5	AB	AB	8.2
5	C	62	M	7 YR	AB	INS	7.4	184	184	77	102	110	0.92	92.00	0.9	AB	PR	10.4
6	C	50	M	4 YR	PR	INS	7.3	148	178	75	100	116	0.86	78.12	1.2	AB	NC	14.2
7	C	50	M	10 YR	PR	INS	7.0	155	178	68	96	115	0.83	85.00	1.0	PR	NC	17.0
8	C	59	M	8 YR	AB	OHA	6.5	R126	180	81	101	107	0.94	151.87	0.6	AB	AB	8.2
9	C	70	M	20 YR	AB	OHA	7.2	159	187	75	95	110	0.86	60.70	1.2	AB	PR	13.6
10	C	72	M	30 YR	PR	INS	7.2	R210	167	82	98	102	0.96	36.80	2.1	AB	NC	8.7
11	C	52	M	15 YR	AB	INS	7.0	210	174	77	85	88	0.96	47.00	2.0	AB	NC	9.7
12	C	78	M	15 YR	AB	INS	7.3	210	166	63	80	95	0.84	27.12	2.0	AB	AB	13.9
13	C	52	M	5 YR	AB	OHA	7.2	141	165	70	86	92	0.93	171.11	0.5	AB	NC	9.1
14	C	78	M	4 YR	AB	OHA	7.0	164	167	62	80	94	0.85	26.69	2.0	AB	NC	14.0
15	C	58	M	5 YR	PR	OHA	6.8	138	167	64	83	94	0.88	91.11	0.8	PR	NC	13.3
16	C	55	M	5 YR	PR	OHA	7.3	196	169	77	95	92	1.03	53.47	1.7	AB	NC	8.2
17	C	46	M	4 YR	AB	OHA	7.0	176	187	75	85	97	0.87	89.01	1.1	PR	NC	13.7
18	C	52	M	20 YR	AB	OHA	7.4	220	188	88	108	125	0.86	46.76	2.3	AB	NC	14.2
19	C	56	M	6 YR	PR	INS	6.2	186	186	94	120	116	1.03	137.08	0.8	AB	NC	3.8
20	C	65	M	4 YR	AB	OHA	6.7	189	171	84	98	92	1.06	67.30	1.3	AB	AB	2.0
21	C	69	M	15 YR	AB	INS	6.8	180	178	87	100	97	1.03	57.19	1.5	AB	NC	5.4
22	C	65	M	20 YR	AB	OHA + INS	7.0	186	181	75	118	134	0.88	78.12	1.0	AB	AB	6.4
23	C	70	M	7 YR	AB	OHA	7.3	R92	176	71	108	121	0.89	98.61	0.7	PR	AB	6.8
24	C	58	M	8 YR	PR	INS	7.3	269	169	80	108	103	1.04	113.88	0.8	AB	NC	4.2
25	C	85	M	14 YR	PR	OHA + INS	7.2	130	167	76	112	108	1.03	52.77	1.1	AB	PR	5.0
26	C	58	M	12 YR	AB	INS	7.3	184	174	89	106	100	1.06	50.68	2.0	AB	PR	2.8
27	C	53	M	3 YR	PR	OHA	7.3	130	170	91	120	117	1.02	157.08	0.7	PR	AB	6.0
28	C	62	M	15 YR	AB	INS	6.9	R252	176	69	96	103	0.93	74.75	1.0	AB	NC	9.2
29	C	52	M	7 YR	AB	INS	7.0	R188	157	80	105	101	1.03	122.22	0.8	AB	NC	6.4

MASTER CHART

Sr. No.	Group	Demographic Details					DM Status		Anthropometry					Diabetic Complications				Sr. Adiponectin
		Age (In Years)	Sex	Diab duration	Family history	Treatment	HBA1C	FBS/RBS	Height (cms)	Weight (kgs)	Waist circ (cm)	Hip circum (cm)	WHR	GFR calculation	Sr. Creat	Neuropathy	Fundus-Diabetes Retinopathy	
30	C	55	M	12 YR	AB	OHA	6.8	R268	168	74	96	102	0.94	124.80	0.7	AB	AB	8.2
31	C	52	M	8 MN	AB	DD	6.1	164	161	86	120	114	1.05	150.15	0.7	AB	AB	4.0
32	C	62	M	8 YR	AB	INS	6.7	262	176	66	97	105	0.92	71.50	1.0	PR	NC	9.8
33	C	67	M	5 YR	AB	INS	6.8	172	177	69	78	84	0.92	46.60	1.5	AB	AB	9.7
34	C	65	M	12 YR	AB	OHA	7.3	R276	164	56	92	101	0.91	97.22	0.6	AB	AB	10.9
35	C	47	M	3 YR	PR	OHA	6.8	R184	176	67	95	102	0.93	86.54	1.0	PR	PR	9.1
36	C	60	M	12 YR	PR	OHA	6.5	164	169	75	98	104	0.94	166.66	0.5	AB	AB	8.6
37	C	62	M	6 MN	AB	OHA	7.4	146	159	73	89	95	0.93	60.83	1.3	AB	PR	8.8
38	C	60	M	6 YR	PR	INS	7.5	136	172	66	95	102	0.93	61.11	1.2	PR	AB	9.0
39	C	60	M	8 YR	PR	OHA+_INS	6.5	137	173	65	77	84	0.92	60.18	1.2	AB	AB	9.4
40	C	52	M	8 YR	PR	OHA	7.3	136	176	64	86	93	0.92	97.77	0.8	AB	PR	10.3
41	C	60	M	6 YR	PR	OHA+ INS	6.8	137	173	68	88	94	0.93	68.68	1.1	AB	AB	9.0
42	C	52	M	6 MN	AB	INS	6.9	R186	178	66	87	95	0.91	36.66	2.2	PR	AB	10.6
43	C	57	M	14 YR	PR	OHA	7.1	143	170	74	87	94	0.92	106.63	0.8	PR	NC	9.4
44	C	53	M	4 YR	AB	OHA	7.1	199	178	83	83	90	0.92	143.27	0.7	AB	PR	9.9
45	C	76	M	6.5 YR	AB	OHA	6.7	145	187	76	83	96	0.86	51.90	1.3	PR	PR	14.8
46	C	52	M	7 YR	PR	OHA	7.0	169	175	73	91	98	0.92	52.40	1.7	AB	PR	10.0
47	C	73	M	28 YR	PR	OHA	6.9	156	179	77	93	100	0.93	65.10	1.1	AB	PR	9.0
48	C	45	M	18 YR	PR	INS	7.4	179	169	80	102	99	1.03	117.20	0.9	PR	PR	4.8
49	C	63	M	2 YR	AB	OHA	6.6	156	156	78	108	122	0.88	104.20	0.8	AB	AB	13.0
50	C	80	M	25 YR	PR	OHA + INS	7.3	199	175	87	120	116	1.03	42.60	1.7	PR	PR	6.2
51	C	51	M	6 YR	AB	OHA	6.6	134	183	76	94	104	0.90	134.20	0.7	AB	PR	12.2
52	C	28	M	2 YR	AB	OHA	7.5	243	171	99	118	113	1.04	73.30	2.1	AB	PR	4.8
53	C	55	M	5 YR	AB	INS	6.4	145	188	65	79	96	0.82	85.20	0.9	AB	AB	18.0
54	C	56	M	6 YR	AB	INS	6.6	178	175	73	92	100	0.92	121.60	0.7	AB	AB	10.2
55	C	73	M	12 YR	PR	OHA	6.8	198	182	78	91	98	0.92	72.58	1.0	AB	AB	9.8
56	C	48	M	15 YR	AB	OHA + INS	6.7	176	177	69	94	104	0.90	110.20	0.8	AB	PR	12.2
57	C	61	M	9 YR	AB	OHA	6.7	167	186	66	84	98	0.85	103.45	0.7	AB	AB	14.8
58	C	55	M	5 YR	PR	INS	6.7	156	189	65	79	96	0.82	109.62	0.7	AB	AB	17.8

MASTER CHART

Sr. No.	Group	Demographic Details					DM Status		Anthropometry					Diabetic Complications				Sr. Adiponectin
		Age (In Years)	Sex	Diab duration	Family history	Treatment	HBA1C	FBS/RBS	Height(cms)	Weight(kgs)	Waist circ(cm)	Hip circum(cm)	WHR	Gfr calculation	S.Creat	Neuropathy	Fundus-Diabetes Retinopathy	
59	C	52	M	1 YR	AB	OHA	6.8	165	186	66	80	95	0.84	100.83	0.8	AB	AB	16.0
60	C	55	M	7 YR	PR	INS	6.9	167	185	72	98	111	0.88	94.44	0.9	AB	AB	13.0
61	C	60	M	7 YR	AB	INS	7.0	187	178	78	88	94	0.93	86.66	1.0	AB	PR	9.2
62	C	48	M	7 YR	AB	OHA	7.2	189	177	85	115	111	1.03	108.61	1.0	AB	AB	7.0
63	C	73	M	8 YR	AB	OHA	7.3	179	178	89	115	111	1.03	51.70	1.6	AB	AB	6.2
64	C	40	M	12 YR	PR	OHA + INS	7.5	196	172	89	118	113	1.04	72.70	1.7	PR	PR	5.0
65	C	42	M	10 YR	PR	INS	7.4	195	179	90	123	119	1.03	87.50	1.4	PR	PR	5.8
66	C	65	M	18 YR	PR	INS	7.4	193	173	84	130	126	1.03	46.05	1.9	PR	PR	6.2
67	C	48	M	2 YR	PR	OHA	7.3	194	179	88	108	114	0.94	102.20	1.1	AB	AB	8.0
68	C	44	M	25 YR	AB	INS	7.0	179	188	75	98	108	0.90	83.30	1.2	AB	AB	12.0
69	C	47	M	17 YR	PR	INS	7.5	185	161	99	124	118	1.05	37.60	3.4	PR	PR	2.6
70	C	50	M	20 YR	PR	INS	7.4	188	167	93	122	117	1.04	41.50	2.8	PR	PR	4.7
71	CN	46	M	X	PR	X	X	88	184	67	96	106	0.90	NA	NA	NA	NA	12.4
72	CN	44	M	X	AB	X	X	106	171	86	99	98	1.01	NA	NA	NA	NA	7.2
73	CN	50	M	X	AB	X	X	110	173	84	89	94	0.94	NA	NA	NA	NA	8.6
74	CN	54	M	X	AB	X	X	97	174	64	85	94	0.90	NA	NA	NA	NA	12.4
75	CN	48	M	X	AB	X	X	102	173	62	86	103	0.83	NA	NA	NA	NA	16.8
76	CN	52	M	X	AB	X	X	R99	180	73	96	114	0.84	NA	NA	NA	NA	15.0
77	CN	46	M	X	PR	X	X	97	180	74	93	112	0.83	NA	NA	NA	NA	15.0
78	CN	47	M	X	AB	X	X	106	181	81	91	103	0.88	NA	NA	NA	NA	13.8
79	CN	48	M	X	AB	X	X	R110	160	81	103	99	1.04	NA	NA	NA	NA	4.8
80	CN	44	M	X	AB	X	X	86	168	69	87	97	0.89	NA	NA	NA	NA	12.2
81	CN	48	M	X	AB	X	X	84	177	73	79	91	0.86	NA	NA	NA	NA	14.2
82	CN	54	M	X	AB	X	X	87	180	64	89	108	0.82	NA	NA	NA	NA	17.5
83	CN	46	M	X	AB	X	X	95	166	76	84	91	0.92	NA	NA	NA	NA	9.1
84	CN	60	M	X	AB	X	X	106	164	77	87	93	0.93	NA	NA	NA	NA	8.7
85	CN	70	M	X	AB	X	X	88	171	68	84	101	0.83	NA	NA	NA	NA	17.3
86	CN	74	M	X	AB	X	X	96	172	67	88	107	0.82	NA	NA	NA	NA	17.9
87	CN	78	M	X	AB	X	X	78	164	76	88	104	0.84	NA	NA	NA	NA	16.0

MASTER CHART

Sr. No.	Group	Demographic Details					DM Status		Anthropometry					Diabetic Complications				Sr. Adiponectin
		Age (In Years)	Sex	Diab duration	Family history	Treatment	HBA1C	FBS/RBS	Height(cms)	Weight(kgs)	Waist circ(cm)	Hip circum(cm)	WHR	Gfr calculation	S.Creat	Neuropathy	Fundus-Diabetes Retinopathy	
88	CN	67	M	X	PR	X	X	89	186	67	84	105	0.80	NA	NA	NA	NA	19.0
89	CN	67	M	X	PR	X	X	98	177	66	86	104	0.82	NA	NA	NA	NA	18.5
90	CN	65	M	X	AB	X	X	96	159	73	85	103	0.82	NA	NA	NA	NA	16.9