ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Effect of Pioglitazone on Abdominal Fat Distribution in Type 2 Diabetic Patients

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ABSTRACT

Diabetes mellitus is a significant, growing threat to global health. Indians have got high central adiposity and high percentage body fat in comparison with many other populations, which is responsible for insulin resistance. Pioglitazone by acting through the PPAR gamma receptor, effectively mobilises the visceral fat to subcutaneous areas, which highly correlates with insulin resistance. The aim and objective of this study was to evaluate the effect of Pioglitazone on abdominal fat distribution in type 2 diabetic patients. Type 2 diabetic patients with fasting plasma glucose 140 – 240 mg / dl and BMI < 35 kg / m^2 were selected for the study.T.Pioglitazone 15-30 mg was administered depending upon the individual blood glucose levels. Plasma glucose – fasting, postprandial, lipid profile, visceral and subcutaneous fat levels, were measured both prior and after drug administration, by using CT scan. Visceral fat was found to be decreased and subcutaneous fat was increased, which was found to be statistically significant($P < 0.001$).Also plasma glucose levels were found to be reduced significantly($p<0.001$). Thus from this study it was observed, that pioglitazone reduced visceral fat level & improves insulin sensitivity, which was reflected through improvement in plasma glucose homeostasis and a favourable lipid profile, which is essential in the prevention of macrovascular(coronary, cerebral) complications in patients with type 2 diabetes mellitus.

Keywords: Diabetes mellitus, visceral fat, subcutaneous fat.

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ISSN: 0975-8585

INTRODUCTION

Diabetes mellitus is defined as a state of chronic hyperglycemia, which is due to either inappropriate secretion of insulin and / or non responsiveness of the target tissues to the actions of insulin. It is a significant, growing threat to global health, probably affecting 250 million people worldwide [1].

India is currently experiencing an epidemic of diabetes mellitus. According to the World Health Organisation (WHO), India has the unique distinction of being the country, with the greatest proportion of diabetic patients in the world [2].

Indians have got an ethnic susceptibility to type 2 diabetes mellitus. This susceptibility is contributed mainly by insulin resistance, which may be due to environmental and / or genetic factors. Despite having a low body mass index (BMI), insulin resistance is a characteristic feature seen in Asian Indians [3]. This is explained by the fact, that higher level of plasma insulin is required by Asian Indians to maintain normoglycemia. They have got high central adiposity and high percentage body fat in comparison with many other populations, which is responsible for insulin resistance. This gets worsened with even small increments in weight and with lack of physical activity [2].

Insulin resistance is a poorly defined term, which refers to the decreased biological activity of insulin, and is usually equated with impaired glucose lowering[1]. Central (intra-abdominal) fat depots are much more strongly linked to insulin resistance, type-2 diabetes and cardiovascular disease than the peripheral (sub-cutaneous) fat depots[4]. Reduction of visceral fat either by surgical removal or by restricting the intake of calorie has been shown to reverse the hepatic insulin resistance [5].

Long term tissue damage due to chronic hyperglycemia, includes both micro and macrovascular complications, which are the major burden and the most expensive item in the diabetes health care budget. These complications are responsible for coronary artery disease, stroke and peripheral arterial diseases. So the effective management of diabetes mellitus would prevent all these complications resulting in a better quality of life index for the diabetic patients.

In 1955, the first generation sulfonylureas were introduced in the treatment of hyperglycemia[6]. Life – style modification, dietary modification and drugs like metformin and thiazolidinediones are shown to be beneficial in restoring insulin sensitivity[2]. Numerous oral hypoglycemic agents are now used clinically.

Thiazolidinediones are a newer class of insulin-sensitising agents. The glucose lowering effect of this group of drugs, needs insulin for their action[6].So they are effective only in patients with type 2 diabetes mellitus. Numerous studies have shown that this group of drugs, by acting through the nuclear receptor PPAR-r, decrease the visceral fat content[7,8]. Thus they also ameliorate dyslipidemia in patients with type-2 diabetes [9].

Since thiazolidinediones improve insulin sensitivity by reducing the visceral fat content, in this study, an attempt was made to evaluate the effect of pioglitazone on

abdominal fat distribution and glucose homeostasis in patients with type-2 diabetes mellitus.

MATERIALS AND METHODS

This study was a prospective, open labeled, single centered study, carried out in the out-patient department of Diabetology, Govt Rajaji Hospital, Madurai,over a period of twelve months, after obtaining Instituitional ethical committee clearance.Subjects who were willing to participate in the study was obtained written informed consent, after being explained personally about the purpose, potential risks and other critical issues regarding the study.

Both males and females of age between 30-60 years, with type – 2 diabetic patients who were on sulfonylureas, for a period of atleast 3 months prior to the study with BMI - < 35 kg / m^2 and fasting glucose levels - 140 – 240 mg / dl were included.The following patients were excluded: Patients with IDDM, Women who were pregnant or breast feeding, Patients with type 2 diabetes, who were on insulin, metformin or thiazolidinediones for the past 3 months, patients with hypersensitivity to pioglitazone, patients with other concurrent diseases like hypertension, congestive cardiac failure, coronary artery disease, renal or hepatic impairment, diabetic neuropathy/nephropathy/retinopathy, diabetic ketoacidosis, patients who were on other medications which induces or inhibits the Cyp 3A4 enzymes and patients who were enrolled for any study other than this study were excluded.

The study groups were selected over a period of 6 months. Written informed consent was obtained from all patients personally, dated and signed by the patients.The details of the investigator (Name, Phone Number and contact address) were given to each and every patient to enable them to contact for any ailments, at anytime during the study period.Three weeks prior to the study all the patients underwent counseling by a dietician. They were explained about the effects of food on blood glucose, and were instructed to follow a standard diet which will contain - carbohydrate 50%, fat 30% and protein 20%. A diet chart was given to all the patients.Patients were reviewed after one week of screening. The subjects who still continued to meet the inclusion criteria underwent the following; A written informed consent was obtained.Socio demographic data like age, sex, residential address, occupation, contact number were recorded. Physical examination was performed.Urine pregnancy test was done for female patients. Blood samples were obtained for clinical laboratory tests (Blood sugar fasting / post prandial, HbA1C, urea, creatinine, Lipid profile). Skin fold thickness was measured at the level of triceps on the right arm at a fixed point below the shoulder joint [10]. Skin fold measurement was made to the nearest one mm using skin fold calliper(fig.1).Body fat ratio was measured by using body fat analyzer(fig.2). Bio-electric impedance measurements were made using a hand to hand impedance analyzer (Omron body fat monitor, model HBF 302, Japan). The patient details like age, sex, height and weight were fed into the instrument. This device was held in both hands, while stretching both the arms horizontally in front of the body. The patients should be on empty stomach, for at least 6 hours and should be refrained from exercise for atleast 12 hours. They were advised to empty their urinary bladder prior to the measurement session [11]. .Measured subcutaneous and visceral fat ratio using computed tomography scan at the level of umbilicus. The visceral and subcutaneous fat mass was estimated with the help of computed tomography (CT) image [12]. All CT scans were performed in the

supine position and most of the photographs were made using a window close to 500 and centre around -40 Hounsefield unit. The subcutaneous fat layer was clearly defined between the skin and muscle. The intra-peritoneal part having the same density as the subcutaneous fat layer, was considered as the visceral fat area. The study drug was then dispensed, the dose depending upon the individual baseline glucose values. Instructed the patients regarding the study medication- dose and time of intake. The date of first administration of the study medication was documented. Instructions were given to the patients, to take the drug in the morning, along with their routine dose of sulfonylureas, except on the days of clinical visits. On these occasions the assigned drug was taken after withdrawing blood, for measuring fasting blood glucose and lipid profile. The patients were advised not to take any other medications without the knowledge of the investigator. The required number of tablets for 28 days was given to the patients and they were asked to return the empty strips during their next visit to ensure their compliance. Patients were reviewed at an interval of 4 weeks gap and were enquired about their well being. Vital parameters were measured. Blood sample was collected for clinical laboratory investigations as mentioned earlier. Skin fold thickness at the level of triceps – Right arm. Body fat ratio was estimated. The patients were assessed for any adverse events. The empty strips of study medication dispensed at previous visits were collected for checking compliance. Study drug was dispensed to the patients for the next 28 days. At the end of 16 weeks of treatment with pioglitazone, all the parameters were repeated as mentioned earlier .The data were analysed with SPSS statistical software package (version 13.0 SPSS Inc., Chicago, USA). The results obtained before and after pioglitazone therapy were analysed by using student's paired 't' test. P value < 0.05 was considered to be statistically significant.

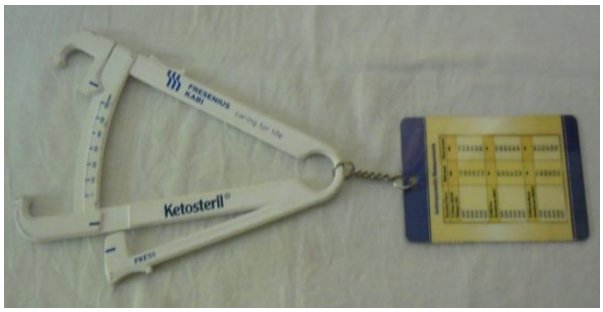

FIG:1 CALIPERS

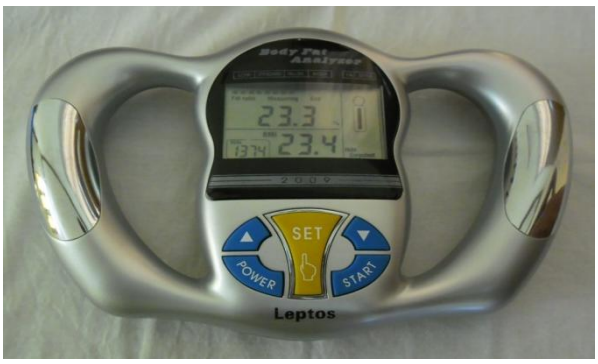

FIG: 2 BODY FAT ANALYSER

ISSN: 0975-8585

RESULTS

30 Patients were enrolled in this study, of which 15 were males (50%) and 15 were females (50%).The age of these patients range between 30 – 60 years (mean49.7 ± 7.5). Table 1 provides the detail regarding the clinical profile of these patients.After 16 weeks of therapy with pioglitazone, the body weight was found to be increased from an average of 61 ± 9.7 to 63 ± 9.9. This increase in body weight was found to be statistically significant (P <0.001). There was an increase in BMI from 24.9 ± 3.6 to 25.8 ± 3.6, which was statistically significant (P<0.001).There was a reduction in fasting blood glucose from 206.5 ± 48.5 to 132.8 ± 10.5, which was found to be statistically significant (P<0.001). The post – prandial glucose levels have reduced from 292.7 ± 71.4 to 186 ± 11.3, which was found to be statistically significant (p < 0.01). The level of HbAIC prior to therapy with pioglitazone was 10.3 ± 2, which after 16 week of pioglitazone therapy reduced to 6.8 ± 0.3. The reduction was found to be statistically significant (P<0.001). The blood urea levels reduced from 24.4 ± 5.9 to 23.9 ± 5 after 16 weeks of treatment with pioglitazone; but this reduction was not statistically significant (P>0.05). The levels of serum creatinine were reduced from 0.88 ± 0.16 to 0.87 ± 0.1, which was not significant statistically (P>0.05). The levels of total cholesterol was found to be decreased from 204.7 ± 53.2 to 183.9 ± 48.5. This reduction was found to be statisfically significant. (P<0.001).The levels of Triglycerides decreased from 216.2 ± 79.5 to 172.4 ± 62.6, which was found to be highly significant statistically (P <0.001).The levels of HDL were found to be increased from 37.9 ± 4.2 to 39 ± 4, which was statistically significant (P<0.001). The levels of VLDL has decreased from 43.1 ± 15.6 to 35.1 ± 12.2, which was found to be statistically significant (P<0.001). The levels of LDL had decreased from 124.1 ± 46.6 to 110.3 ± 41.7, which was statistically significant (P<0.001). The skin fold thickness was found to be increased from 14.7 ± 5.8 to 16.4 ± 5.9, which was found to statistically significant (P<0.001). The total body – fat ratio increased from 31.9 ± 7.7 to 33.4 ± 7.5, which was significant statistically (P<0.001). The visceral fat mass was found to be decreased from 140.9 ± 39.2 to127.6 ± 36.4(fig.3,4), after treatment with pioglitazone which was found to be statistically significant. The subcutaneous fat mass was found to be increased from 204.6 ± 65.9 to 231.4±78.6(fig.3,4), after treatment with pioglitazone which was found to be statistically significant.The visceral: subcutaneous fat mass was found to be decreased from 0.71 ±0.2 to 0.57±0.1, after treatment with Pioglitazone which was found to be statistically significant.

TABLE: 1: CLINICAL PROFILE OF PATIENTS BEFORE STARTING PIOGLITAZONE

N	30
Age (Year)	49.7 ± 7.5
Sex (M/F)	15/15
BMI (kg/m^2)	24.9 ± 3.6.
Average duration of diabetes	5.5 ± 2.1
Hb A1C	10.3 ± 2

ISSN: 0975-8585

Fig.3: CT IMAGES OF SUBCUTANEOUS AND VISCERAL FAT BEFORE THERAPY 41 YEARS/ FEMALE

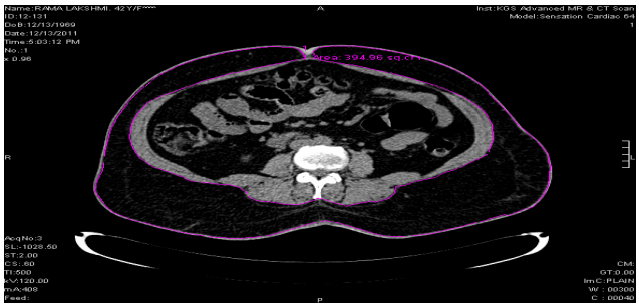

FIG: 3 SUBCUTANEOUS FAT

Subcutaneous fat area : 394.96 cm^2

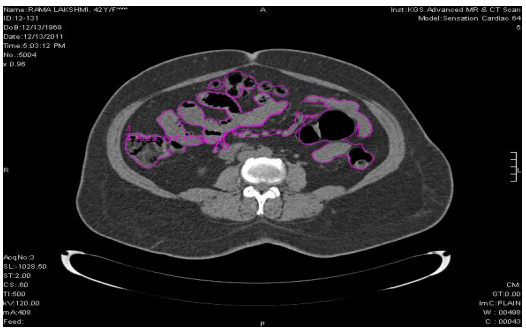

FIG: 4 VISCERAL AREA

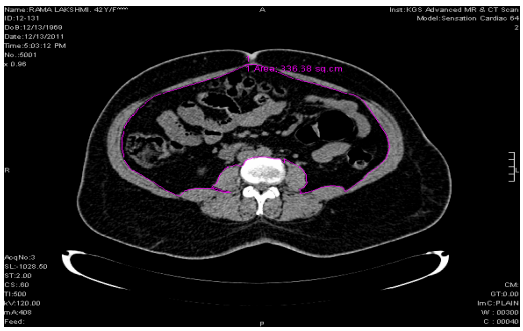

FIG: 5 INTRA PERITONEAL AREA

VISCERAL FAT AREA = INTRA PERITONEAL AREA - VISCERAL AREA
Visceral fat area : 199.21 cm^2

Fig.4: CT IMAGES OF SUBCUTANEOUS AND VISCERAL FAT AFTER 16 WEEKS OF THERAPY
41 YEARS/ FEMALE

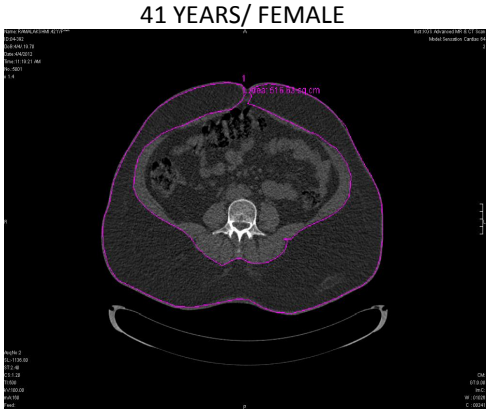

FIG: 6 SUBCUTANEOUS FAT

Subcutaneous fat area : 516.63 cm^2

ISSN: 0975-8585

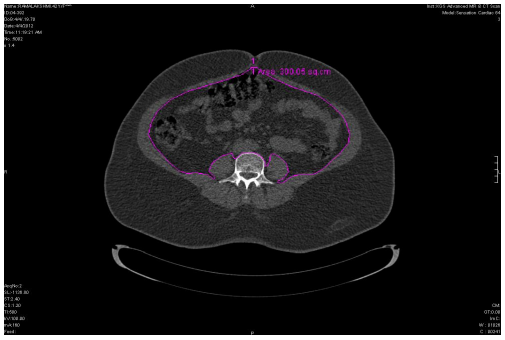

FIG: 7 INTRAPERITONEAL AREA

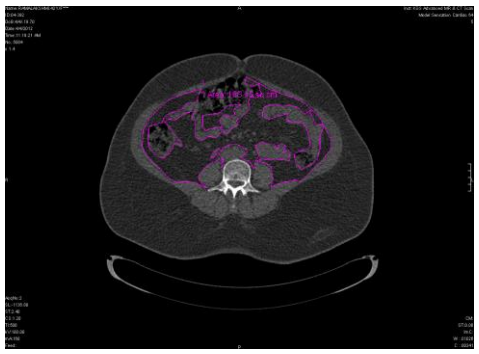

FIG: 8 VISCERAL AREA

VISCERAL FAT = INTRAPERITONEAL AREA - VISCERAL AREA.

Visceral fat area : 188.45 cm^2.

TABLE: 2: RESULTS BEFORE AND AFTER PIOGLITAZONE THERAPY

SL. No.	PARAMETER	BEFORE	AFTER	P-Value
1.	Body Weight (kg)	61 ± 9.7	63 ± 9.9	<0.001
2.	Body mass index (kg/m^2)	24.9 ± 3.6	25.8 ± 3.6	<0.001
3.	Plasma glucose (mg/dl) • Fasting • Post – prandial	 206.5 ± 48.5 292.7 ± 71.4	 132.8 ± 10.5 186.0 ± 11.3	 <0.001 <0.001
4.	HbAIC (%)	10.3 ± 2	6.8 ± 0.3	<0.001
5.	Blood Urea (Mg/dl)	24.5 ± 6	23.9 ± 5.1	>0.05
6.	(S) creatinine (mg/dl)	0.9 ± 0.2	0.9± 0.1	>0.5
7.	Serum Lipid • Total cholesterol (mg/dl) • Triglycerides (mg/dl) • HDL-c (mg/dl) • VLDL -c(mg/dl) • LDL –c (mg/dl)	 204.7 ± 53.2 216.2 ± 79.6 37.9 ± 4.2 43.1 ± 15.6 124.1 ± 46.6	 183 .9± 48.5 172.4 ± 62.6 39 ± 4 35 .1±12.2 110 .3± 41.7	 <0.001 <0.001 <0.01 <0.001 <0.001
8.	Body – fat ratio (%)	31.9 ± 7.7	33.4 ± 7.5	<0.001
9.	Visceral fat mass	140.9 ± 39.2	127.6 ± 36.4	<0.001
10.	Subcutaneous fat mass	204.6 ± 65.9	231.2 ± 78.6	<0.001
11.	Visceral : Subcutaneous fat mass	0.71 ±0.2	0.57±0.1	<0.001

DISCUSSION

This study was undertaken with the aim of evaluating the efficacy of pioglitazone in mobilizing the visceral fat and its effect on glucose homeostasis and lipid profile.30 patients were included in this study, of which 15 were males and 15 were females. After 16 weeks of treatment with pioglitazone, the body weight and subsequently the body mass index (BMI) of almost all the patients had increased, which was found to be statistically significant. This increase in body weight may be due to increase in subcutaneous adipose tissue mass,[7] or may be due to fluid retention [13]. In spite of this weight gain, the glycemic control of all the patients was found to be improved. This improvement in glucose homeostasis was reflected

by a decreased levels of fasting / postprandial blood glucose and HbA1C,after 16 weeks of treatment with pioglitazone. This reduction was found to be statistically significant. This improvement in hyperglycemia could be multifactorial. a) Increased insulin mediated glucose uptake in the skeletal muscle and in the liver, due to enhanced expression of the glucose transporters (GLUT-1 & 4) in these organs. b) Decreased hepatic glucose output. Pioglitazone mediates these effects by binding to the nuclear receptor PPAR-gamma.

From this study it was observed that pioglitazone alters the lipid profile in a favourable manner. The levels of total cholesterol, LDL-c, VLDL-c & triglycerides were found to be decreased and HDL-c was slightly increased. This change in lipid profile was found to be statistically significant. Type-2 diabetic patients exert a destined feature of diabetic dyslipidemia. This is a characteristic feature of insulin resistance, which renders the diabetic patients, more prone for macrovascular diseases. Previous studies have shown that visceral adipocytes are lipolytically more active than subcutaneous fat cells resulting in dyslipidemia .Pioglitazone, by decreasing the amount of visceral fat, could have altered the lipid profile. The body fat distribution was compared in this study, both before and 16 weeks after treatment with pioglitazone. Total body fat was found to be increased, which may be due to increase in the subcutaneous fat mass, and the skin fold thickness, measured at the level of triceps had also increased significantly. This may be attributed to the redistribution of visceral fat to the subcutaneous areas by pioglitazone, through the activation of PPAR-gamma receptor. This evidence is supported by previous studies, in which pioglitazone was shown to reduce the hepatic lipid content by mobilizing the hepatic fat to peripheral subcutaneous tissues[14]. Insulin resistance and type-2 diabetes are most strongly associated with the presence of increased abdominal or visceral fat [12]. Visceral fat can be estimated by performing abdominal CT or MRI Scan [15,16]. Computed tomography(CT) is an optimal technique for the accurate assessment of intra-abdominal fat, because magnetic resonance imaging(MRI) equipment is more expensive and less commonly available than is CT equipment.[17] It was known from several studies, that visceral fat areas obtained from a single scan at the level of umbilicus, (approximately at the level of L4 & L5) highly correlated with the total visceral fat volume [18]. Based on this, the visceral and subcutaneous fat ratio was estimated in this study, by performing computerized tomography (CT) scan at the level of umbilicus. It was observed that after16 weeks of treatment with pioglitazone, this ratio was reduced, which was statistically significant.

Thus from this study it was observed, that pioglitazone mediates various actions through transactivation of PPAR-gamma receptors, thereby reducing visceral fat & improving insulin sensitivity which was reflected through improvement in glucose homeostasis and a favourable lipid profile, which is essential in the prevention of macrovascular(coronary, cerebral) complications in patients with type 2 diabetes mellitus.

CONCLUSION AND SUMMARY

Diabetes is a significant and growing threat to global population. Type-2 Diabetes mellitus which is a heterogeneous condition is most commonly diagnosed in those >40 year of age and it accounts for 85 to 90% of diabetes worldwide, with striking geographical variation. An important specific risk factor for type-2 diabetes, which aggravates insulin resistance, is obesity. Visceral obesity, due to the accumulation of fat in the omental and

mesenteric regions, correlates with insulin resistance. These individuals have increased risk for elevated plasma triglycerides, lower high density lipoproteins (HDLs), elevated low – density lipoproteins (LDL), hyperuricemia, prothrombotic state with increased levels of plasminogen activator inhibitor type-1 (PA1-1) and pro – inflammatory state. These clusters of abnormalities significantly increase the risk of atherosclerotic disease.So, the therapeutic goals in the treatment type-2 diabetes are not only to correct hyperglycemia but also to manage the elevated blood pressure and dyslipidemias that result in increased cerebro-vascular and cardiac morbidity and mortality in these patients. Studies have shown that pioglitazone is effective in reducing the visceral fat, which highly correlates with insulin resistance and its related cerebro-vascular and cardiovascular morbidities and mortalities, by acting through the nuclear receptor PPAR-gamma.In this study, the effects of pioglitazone, on various parameters like, plasma glucose levels,HbA1c, plasma lipid profile, reduction of visceral fat, the changes in the subcutaneous and total body fat ,both before and after 16 weeks of treatment, was carried out in type-2 diabetic patients. Treatment with pioglitazone had a favourable outcome on glycemic control. There was an overall improvement in the fasting plasma glucose levels and HbA1C. Pioglitazone was also observed to have a positive effect on almost all lipid indices. Total cholesterol, LDL-cholesterol and triglyceride levels were decreased. HDL- cholesterol levels had increased after treatment with pioglitazone. Visceral fat was found to be decreased in these patients, after treatment with pioglitazone and there was a reduction in the visceral: subcutaneous fat ratio. There was a significant increase in the skin fold thickness and total body fat mass, which may be due to the increased subcutaneous fat mass. From this study, we can conclude that treatment with pioglitazone, not only reduces the blood glucose levels and the fraction of glycated hemoglobin (HbA1c), but also by mobilizing the visceral fat to the subcutaneous areas, it improves the insulin sensitivity and exerts a positive effect on lipid profile in type 2 diabetic patients thereby preventing the macrovascular complications and reducing the cerebro-vascular and cardio-vascular risk in these patients.

ACKNOWLEDGEMENT

The authors are very thankful to the of Institute of Pharmacology, Madurai Medical College, Madurai, Department of Diabetology, Department of Radiology , and Department of Biochemistry, Govt. Rajaji Hospital, Madurai.

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