*Original Research Article*

# 'Diabesity' and Insulin Resistance: Results from a Study in a Rural Setting of Haryana, India

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## ABSTRACT

**Introduction:** Diabetes mellitus and obesity are closely related in terms of both pathogenesis and pathophysiology. Both metabolic disorders in turn result in vascular complications such as cardiovascular diseases through unknown mechanisms which may be linked to a cluster of risk factors including insulin resistance, hyperglycemia, dyslipidemia, hyperinsulinemia and systemic inflammation. Dyslipidemia, an important component of insulin resistance syndrome, type 2 diabetes mellitus (T2DM) and obesity, is characterized by increased triglycerides, low density lipoprotein-cholesterol and decreased high density lipoprotein-cholesterol.

**Aims and Objectives:** The present study was undertaken to estimate and compare the levels of fasting plasma glucose, serum lipid profile and insulin resistance in type 2 diabetes mellitus patients and non-diabetic subjects with and without obesity.

**Materials and Methods:** A total of 400 subjects comprising 200 T2DM patients (with and without obesity) attending the OPDs and wards of Department of Medicine, M.M Institute of Medical Sciences and Research, Mullana, Ambala and 200 non-diabetic subjects (with obesity and without obesity) from amongst the attendants of the patients and volunteers in the age range of 30-70 years of either sex were selected for the study. Fasting plasma glucose (FPG) and serum lipid profile levels including total cholesterol, triglycerides, LDL-C, HDL-C and VLDL-C were measured while insulin resistance was calculated using homeostasis model assessment method (HOMA).

**Results:** It was observed that fasting plasma glucose levels were significantly higher in non-obese than obese diabetics (p<0.05) but lipid profile was not significantly deranged amongst diabetic subjects. In addition, diabetics who were obese were found to be more insulin resistant than non-obese ones. Similarly, non-obese non-diabetic subjects had slightly higher FPG levels than their obese counterparts. Further, on comparing the levels of blood lipids between obese and non-obese non-diabetic subjects, statistically insignificant difference was observed (p>0.05), although obese individuals were more insulin resistant than non-obese subjects.

**Conclusion:** The subjects with both DM and obesity were significantly more insulin resistant than those suffering from either of two diseases. It is imperative that persons with co-existent DM and obesity should be regularly monitored for their blood lipid levels in order to reduce the associated cardiovascular disease risk.

*Keywords:* Diabetes mellitus, obesity, insulin resistance, dyslipidemia, cardiovascular disease.

## INTRODUCTION

Obesity, a growing health concern, is associated with hyperinsulinemia, insulin resistance, atherogenic lipid profile and the development of type 2 diabetes mellitus (T2DM) and cardiovascular disease. [1]

Indeed, insulin resistance is a common pathophysiologic mechanism underlying both obesity and T2DM which could result in vascular endothelial dysfunction, an abnormal lipid profile, hypertension and vascular inflammation. [2] DM has been associated with dyslipidemia which is usually presented as increased serum triglycerides, low density lipoprotein-cholesterol (LDL-C), very low density lipoprotein-cholesterol (VLDL-C) and decreased high density lipoprotein-cholesterol (HDL-C). [3] The prevalence of dyslipidemia in diabetic patients is quite variable which depends on the type and severity of diabetes, glycemic control, nutritional status, age and other factors, all of which may be related again to insulin resistance. [4] However, the higher prevalence of dyslipidemia could be attributed to certain metabolic derangements seen in DM including impaired apoprotein production, regulation of lipoprotein lipase (LPL), cholesteryl ester transfer protein (CETP) action, and impaired hepatic and peripheral actions of insulin. [5-6] In addition, the associated hyperglycemia, obesity and insulin changes seen in diabetes greatly accelerate the progression to atherosclerosis and other cardiovascular diseases. [4] So, the present study was conducted to estimate and compare the levels of fasting plasma glucose, serum lipid profile and insulin resistance in type 2 diabetes mellitus patients and non-diabetic subjects with and without obesity.

## MATERIALS AND METHODS

The present study was conducted in the Department of Biochemistry, Maharishi Markandeshwar Institute of Medical Sciences and Research, Mullana, Ambala (Haryana). A total of 400 subjects in the age range of 30-70 years of either sex were selected for the study, out of which 200 patients were suffering from type 2 diabetes mellitus and 200 were normal subjects (included as controls) from amongst the attendants of the patients and volunteers. Patients were diagnosed as diabetic according to the diagnostic criteria for diabetes mellitus issued by American Diabetes Association (ADA). [7] All the subjects include in the study were divided into 4 groups in the following manner:

Group I: 100 subjects having T2DM and obesity.

Group II: 100 subjects having T2DM and no obesity.

Group III: 100 subjects having only obesity and no diabetes.

Group IV: 100 subjects having no obesity and no diabetes.

Anthropometric indices including height, weight, hip and waist circumference were measured while subjects were in the standing position and wearing light clothing without shoes. Body Mass Index (BMI) was calculated by dividing body weight (in kilograms) by squared height (in square metres) according to WHO procedure. [8] Waist to Hip Ratio (WHR) was calculated as waist circumference divided by hip circumference. Informed consent, both in English as well as vernacular language, was taken from all the participants included in the study.

## EXCLUSION CRITERIA

1. The patients suffering from type 1 diabetes mellitus, rheumatoid arthritis and coronary artery disease.
2. The patients taking antioxidant drugs, corticosteroids, insulin.
3. Pregnancy
4. Hyper or Hypothyroidism, Cushing syndrome or any other endocrinopathy besides diabetes mellitus.

The study was approved by Institutional Ethics Committee of M.M Institute of Medical Sciences and Research, Mullana, Ambala.

Seven milliliters (7 ml) of venous blood sample was collected in dry disposable syringe under aseptic conditions from ante-cubital vein of the subjects after an overnight fasting of 10-12 hours. Four milliliters (4 ml) of blood was transferred to a sterile, dry and acid washed vial, allowed to stand for half an hour and after the clot formation, the supernatant fluid was

centrifuged to perform analysis of various parameters of lipid profile. The remaining 3 ml of blood was transferred to sodium fluoride and potassium oxalate containing vial, and centrifuged to separate plasma which was then used for estimation of fasting plasma glucose and insulin resistance. Plasma glucose levels were measured using glucose oxidase – peroxidase (GOD-POD) method using AutoZyme STAT glucose kit of Accurex Biomedical Private Ltd. Serum total cholesterol was measured by enzymatic CHOD – PAP method with lipid clearing agent using cholesterol esterase, cholesterol oxidase and peroxidase. [9] High density lipoprotein-cholesterol (HDL-C) was measured using precipitation method for direct measurement of HDL-C using ERBA diagnostic kits. [10] Serum triglyceride levels were estimated with an enzymatic glycerol phosphate oxidase (GPO)–Trinder method involving glycerol kinase, lipoprotein lipase,

glycerol-3-phosphate oxidase and finally peroxidase enzyme. Very low density lipoprotein- cholesterol (VLDL-C) and low density lipoprotein-cholesterol (LDL-C) levels were calculated using Friedewald's formula [11] which is based on assumption that VLDL is present in serum at a concentration equal to one-fifth of triglyceride concentration.

Statistical analyses were carried out using SPSS version 20.0 software. Descriptive statistics were calculated for different characteristics of the subjects. Student t-test and one-way ANOVA (Analysis of Variance) were used to compare the statistical differences between continuous variables.

## RESULTS

The physical parameters of diabetic subjects with and without obesity i.e. age and body mass index (BMI) are summarized in Table 1.

**TABLE 1: AGE AND BMI IN DIABETIC SUBJECTS**

| PHYSICAL PARAMETERS | DIABETIC, OBESE SUBJECTS N=100 | DIABETIC, NON-OBESE SUBJECTS N=100 | p-value |
|---|---|---|---|
| Age (years) | 50.68±9.41 | 51.69±9.41 | p > 0.05 |
| BMI (kg/m$^2$) | 32.4±2.84 | 23.55±1.77 | p < 0.001 |

The BMI in diabetic, obese subjects was higher than in non-obese subjects with difference amongst two groups being statistically highly significant (p<0.001).

On comparing the fasting plasma glucose (FPG) levels amongst diabetic subjects, it was observed that non-obese diabetic subjects had higher mean FPG levels than obese diabetics. There was

statistically highly significant difference in mean FPG levels amongst two groups as shown in Table 2. Furthermore, diabetic subjects who were obese had lower serum total cholesterol, triglyceride, HDL-C, LDL-C and VLDL-C levels than those without obesity (Table 2). There was statistically insignificant difference in lipid profile parameters amongst two groups.

**TABLE 2: FPG AND LIPID PROFILE IN DIABETIC SUBJECTS**

| BIOCHEMICAL PARAMETERS | DIABETIC, OBESE SUBJECTS N=100 | DIABETIC, NON-OBESE SUBJECTS N=100 | p-value |
|---|---|---|---|
| FPG (mg/dl) | 146.54±56.15 | 167.37±69.99 | p <0.05 |
| Total cholesterol (mg/dl) | 170.62±38.95 | 183.63±55.73 | p >0.05 |
| Triglycerides (mg/dl) | 184.23±104.52 | 200.53±112.41 | p >0.05 |
| HDL-C (mg/dl) | 40.88±11.32 | 42.74±10.49 | p >0.05 |
| LDL-C (mg/dl) | 93.74±25.71 | 100.78±45.19 | p >0.05 |
| VLDL-C (mg/dl) | 39.18±22.22 | 40.10±22.48 | p >0.05 |

In addition, the extent of insulin resistance was calculated using Homeostasis Model Assessment Method (HOMA) amongst obese and non-obese type 2

diabetic subjects. It was found that obese diabetics were more insulin resistant than non-obese diabetic subjects. Further,

statistically significant difference was observed amongst two groups (p<0.05).

The mean age of obese, non-diabetics was comparable to that of non-obese, non-diabetics as shown in Table 3. In addition, there was statistically highly significant difference in BMI amongst obese and non-obese, non-diabetics; BMI being higher in obese subjects (Table 3). The fasting plasma glucose levels were higher in non-obese, non-diabetics as compared to obese counterparts, with difference amongst two groups being statistically significant (Table 4). All the parameters of lipid profile namely total cholesterol, triglycerides, HDL-C, LDL-C and VLDL-C showed statistically insignificant difference amongst obese and non-obese, non-diabetic subjects. The mean insulin resistance in obese, non-diabetics (calculated using HOMA method) was higher than that observed in non-obese, non-diabetics with difference between two groups being statistically highly significant (p<0.001).

**TABLE 3: AGE AND BMI IN NON-DIABETIC SUBJECTS**

| PHYSICAL PARAMETERS | NON-DIABETIC, OBESE SUBJECTS N=100 | NON-DIABETIC, NON-OBESE SUBJECTS N=100 | p-value |
|---|---|---|---|
| Age (years) | 48.17±11.71 | 48.49±11.26 | p > 0.05 |
| BMI (kg/m$^2$) | 33.39±5.12 | 23.6±0.96 | p < 0.001 |

**TABLE 4: FPG AND LIPID PROFILE IN NON-DIABETIC SUBJECTS**

| BIOCHEMICAL PARAMETERS | NON-DIABETIC, OBESE SUBJECTS N=100 | NON-DIABETIC, NON-OBESE SUBJECTS N=100 | p-value |
|---|---|---|---|
| FPG (mg/dl) | 83.53±9.84 | 86.75±11.76 | p <0.05 |
| Total cholesterol (mg/dl) | 158.32±39.04 | 165.34±45.32 | p >0.05 |
| Triglycerides (mg/dl) | 160.68±83.43 | 161.02±75.66 | p >0.05 |
| HDL-C (mg/dl) | 39.29±9.43 | 41.19±10.74 | p >0.05 |
| LDL-C (mg/dl) | 88.53±32.30 | 91.94±27.70 | p >0.05 |
| VLDL-C (mg/dl) | 34.07±16.89 | 32.19±15.13 | p >0.05 |

## DISCUSSION

Obesity is a major risk factor for DM with as many as 80% of patients with type 2 diabetes mellitus being obese. [12,13] Obesity is associated with altered body fat distribution which in turn is linked with changes in body lipids and lipoproteins and hence, increased coronary heart disease risk. Diabetics usually present with several forms of dyslipidemia. In particular, diabetic dyslipidemia is characteristic of type 2 DM patients with obesity as seen in insulin resistance syndrome. The major features of this type of dyslipidemia include high serum triglycerides, low HDL-C and presence of small dense form of LDL particles. [14] Both lipid profile and DM have been shown to be important predictors for metabolic disturbances including dyslipidemia, hypertension, CVDs, hyperinsulinemia etc. [15] So, determination of serum lipid levels in patients with diabetes mellitus is now considered standard of care along with exercise, dietary modifications and oral hypoglycemic agents (OHAs).

In our study, the fasting plasma glucose levels were significantly higher in non-obese than obese diabetics (p<0.05). The probable reasons for this finding could be – i) non-obese diabetics might be taking OHAs irregularly; ii) non-obese diabetics might not be adhering to dietary and/or lifestyle modifications; and iii) better response of obese diabetics to OHAs. In the present study, it was observed that the lipid profile was not significantly deranged which may be due to the rural setting of the study area selected where subjects were relatively more physically active and less exposed to fast/junk food. This is in accordance with study done by Barma et al and Mukhyaprana et al. [16,17] However, the most characteristic lipid abnormality in diabetics was hypertriglyceridemia which was similar to the findings of Dixit et al and Zargar et al. [4,18] Sinharoy et al also reported elevated triglycerides in lean type 2 diabetics as compared to normal weight and obese type 2 diabetics. [19] Diabetic patients usually tend to have variable combination of

triglyceride over-production or under-utilization. Lipoprotein lipase (LPL) activity is markedly impaired in severe insulin deficiency. However, this LPL activity is relatively intact in case of mild to moderate insulin deficiency states which increases endogenous synthesis of triglycerides especially in presence of obesity and sufficient amount of insulin. [4] Overall, there was increase in VLDL-C levels observed amongst diabetics, both obese as well as non-obese which may be associated with increase in fatty acid transport to liver owing to increased lipolysis in adipose tissue resulting from relative insulin deficiency. In addition, insulin directly degrades apoprotein B (which is major protein of VLDL particles) and thus, there may be increased secretion of apo B and then VLDL in relative insulin deficient condition. [20]

Insulin resistance is a fundamental defect that precedes the development of full insulin resistance syndrome as well as beta cell failure and type 2 diabetes. So, the insulin resistance was calculated by homeostasis model assessment method (HOMA-IR) and then compared amongst obese and non-obese type 2 diabetics. The results clearly showed that diabetics, who were obese, were more insulin resistant than those without obesity. The mechanisms via which obesity causes insulin resistance of glucose or free fatty acid metabolism are still incompletely understood. It has been suggested that in obese subjects, the excess release of free fatty acids from adipose tissue inhibits glucose uptake in peripheral tissues and stimulates hepatic glucose production. Another possibility is that accumulation of adipose tissue in organs such as the liver and/or skeletal muscle underlies insulin resistance in obese subjects. Counter regulatory hormones, e.g. tumour necrosis factor (TNF-α) and resistin secreted from adipose tissue could also hypothetically cause insulin resistance via direct action on insulin sensitive tissues such as skeletal muscle. As far as the molecular mechanisms underlying insulin resistance in obesity are concerned, obese subjects have decreased insulin-stimulated tyrosine kinase activity of insulin receptor in skeletal muscle. [21] Significant degree of insulin resistance has been reported in diabetics as well as obese subjects by Bu et al. [22] Mohammadzadeh et al has also shown in their study that type 2 diabetics had significant levels of insulin resistance, as calculated by HOMA-IR. [23]

The mean age of obese, non-diabetics was comparable to that of non-obese, non-diabetics. In addition, there was statistically highly significant difference in BMI amongst obese and non-obese, non-diabetics; BMI being higher in obese subjects. The fasting plasma glucose levels were well within the normal range for both obese as well as non-obese subjects without diabetes. But non-obese subjects had slightly higher FPG levels than their obese counterparts which may be due to the nature of the participants included. Furthermore, on comparing the levels of blood lipids namely serum total cholesterol, triglycerides, HDL-C, LDL-C and VLDL-C between obese and non-obese non-diabetic subjects, statistically insignificant difference was observed (p>0.05).

Studies have demonstrated that obesity is strongly associated with insulin resistance and obesity is considered the most important risk factor for T2DM apart from well-documented genetic predisposition. The present study also indicated the similar trends with the extent of insulin resistance being higher in obese individuals. Similarly, Abdullah et al and Urbanavicius et al have reported higher levels of insulin resistance amongst obese subjects, as calculated using HOMA method of insulin resistance. [24,25] It has been suggested that TNF-α, produced by adipocytes, plays a key role in insulin resistance of obesity which may contribute to the development of T2DM. Several studies have documented increased adipose expression of TNF-α mRNA in non-diabetic subjects with obesity dependent insulin resistance. [26]

## CONCLUSION

The results of the present study showed that the subjects with both diabetes and obesity were significantly more insulin resistant than those suffering from either of two diseases. Although lipid profile was not significantly deranged amongst the subjects studied, realizing the fact that diabetics are at high risk for development of cardiovascular disease, it is quite imperative that an individual with both obesity and diabetes having two strong risk factors for cardiovascular diseases should undergo routine lipid profile estimation in order to reduce the morbidity and mortality associated with DM and its complications.

### *Conflict Of Interest*

The authors declare no conflict of interest.

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