DISCUSSION

Diabetes mellitus (DM) and obesity are the biggest public health challenges of the 21st century. Obesity is thought to be the primary cause of type 2 diabetes, especially in people who are genetically predisposed to disease. Rates of DM have increased markedly over the past 50 years in parallel with obesity. Indeed, this new unprecedented phenomenon has been referred to as “Diabesity”. Even in India, more than 65.1 million people are affected by diabetes and more than 5% of the country’s population has morbid obesity. This high incidence is attributed to a combination of genetic susceptibility plus adoption of high calorie, low activity life style by India’s growing middle class, population growth, aging and urbanisation. Both these disorders are associated with several comorbidities like hypertension, hyperlipidemia, cardiovascular diseases etc. Recently, ICMR-INDIAB study was conducted in four regions of India (Tamil Nadu, Maharashtra, Jharkhand and Chandigarh representing the South, West, East and North of India respectively) with a combined population of 213 million people. Physical activity was assessed using the Global Physical Activity Questionnaire (GPAQ) in 14227 individuals aged ≥20 years [urban-4173; rural-10054], selected from the above regions using the stratified multistaged design. Of the 14227 individuals studied, 54.4% (n=7737) were inactive (males:41.7%), while 31.9% (n=4537)[males:58.3%] were active and 13.7% (n=1953) [males:61.3%] were highly active. Subjects were more inactive in urban, compared to rural areas.
(65% versus 50%; p<0.001). Males were significantly more active than females (p<0.001). Subjects in all four regions spent more active minutes at work than in the commuting and recreation domains. Absence of recreational activity was reported by 88.4%, 94.8%, 91.3% and 93.1% of the subjects in Chandigarh, Jharkhand, Maharashtra and Tamil Nadu respectively. The percentage of individuals with no recreational activity increased with age (trend \( \chi^2:199.1, p<0.001 \)).

In addition, DM and obesity are closely related diseases in terms of onset and pathophysiology. Insulin resistance is believed to be a common etiologic mechanism in both T2DM and obesity. However, the clinical aspects of these diseases are somewhat different, even at similar levels of insulin resistance. Adipose tissue is no longer considered to be an energy storage depot having only a passive function. However, recent studies have revealed the endocrine functions of adipose tissue. Although it is now known to express and secrete a variety of metabolites, hormones and cytokines that have been implicated in the development of insulin resistance and CVDs, the molecular basis for the link between the obesity, DM and CVD remains poorly understood. Furthermore, clinical studies have shown that the plasma concentrations of several adipocytokines correlate with measures of adiposity, insulin resistance and endothelial function in humans.

In a view to gain better insight into the interplay of various hormones and cytokines in the pathophysiology of type 2 diabetes mellitus which can be
helpful in suitable drug development aimed at modulating serum leptin, adiponectin, insulin, C-peptide and lipid profile levels and might assist in better management of T2DM, the present study was undertaken in the Department of Biochemistry in collaboration with Department of Medicine, MM Institute of Medical Sciences and Research, Mullana, Ambala. Two hundred clinically diagnosed patients of T2DM with and without obesity in the age range of 30-70 years of either sex were selected to serve as subjects for the study. Equal numbers of non-diabetic subjects with and without obesity were included to serve as controls. Fasting plasma glucose, insulin, serum C-peptide, leptin, adiponectin, lipid profile (total cholesterol, triglycerides, HDL-cholesterol, LDL-cholesterol, and VLDL-cholesterol) levels and insulin resistance were estimated in all the subjects. The results thus obtained were statistically compared between diabetic and non-diabetic subjects.

The age of diabetic subjects, both with and without obesity, was almost comparable with difference between two groups being statistically insignificant (Table 2). A notable difference was found between obese and non-obese diabetics in terms of their body mass index (BMI) (Figure 19). The fasting plasma glucose levels were higher in non-obese than obese diabetics and the difference between the two groups was found to be statistically significant (p<0.05) as shown in Figure 20. Paradoxically, obese, type 2 diabetics had significantly higher fasting plasma insulin levels than non-obese diabetics clearly indicating the presence of severe hyperinsulinemia and
subsequent insulin resistance in obese than non-obese (Figure 21). The probable reason for the higher plasma glucose levels in non-obese diabetics compared to obese diabetics could be – i) non-obese diabetics might be taking oral hypoglycaemic agents irregularly ii) non-obese diabetics might not be adhering to dietary and/or lifestyle modifications iii) better response of obese diabetics to oral hypoglycaemic agents. This finding pertaining to low insulin levels in non-obese diabetics is in accordance with the findings of Barma et al who also reported lower insulin levels in lean type 2 diabetics than in obese diabetics. Snehlata et al conducted a study which analyzed the insulin secretion in Asian Indians and observed lesser insulin levels in non-obese compared to obese thereby suggesting that obese patients are hyperinsulinemic. Obese diabetics had elevated C-peptide levels than non-obese but there was no significant difference in C-peptide levels between obese and non-obese diabetics (p>0.05) as revealed in Figure 22. This again confirmed that obese patients are hyperinsulinemic. Similar results were reported by Abdullah et al and Barma et al. Even Jones et al also observed that plasma insulin and C-peptide concentrations are higher in obese than non-obese subjects. Insignificant difference in C-peptide levels despite higher insulin levels observed in obese than non-obese diabetic patients may be due to excess extraction of insulin by the liver.

Leptin is a multifunctional adipocytokine involved in the regulation of food intake, energy storage, and carbohydrate and lipid metabolism. Impaired
regulation of food intake is seen in case of obesity and insulin resistance but its role in the development of these diseases is still not well elucidated. There is also a growing evidence that leptin is an independent risk factor for cardiovascular disease and is likely to be an important link in the development of cardiovascular risk and obesity too. Markedly increased plasma leptin levels were found in obese individuals in a study conducted by Wannamethee et al. Serum leptin levels were reportedly higher in diabetics as compared to non-diabetics in a number of studies. Similarly, the present study also found higher serum leptin levels in obese, diabetics in comparison to non-obese type 2 diabetics (Figure 23). Being the product of the \( ob \) gene secreted from adipose tissue, leptin signals the amount of energy stores to the brain and is implicated in the regulation of food intake and energy balance. Hyperleptinemia seen in obesity may be due to leptin resistance, which may arise from impaired leptin transport across blood brain barrier (BBB), defects in leptin receptor signalling, and blockades in downstream neuronal circuitries. In addition, insulin resistance has been reported to contribute to hyperleptinemia indirectly. Hyperleptinemia that frequently accompanies obesity, as suggested by Abdella et al, results in increased \( ob \) gene expression and thus higher leptin levels.

Adiponectin, an adipocytokine secreted by fat cells, has regulatory functions on energy metabolism. In contrast to all other adipokines known till date, plasma adiponectin concentrations were found to be decreased in obesity,
T2DM and CVD, conditions commonly associated with insulin resistance and hyperinsulinemia rather to the degree of adiposity and glucose intolerance. The mechanism underlying this association between plasma adiponectin concentration and hyperinsulinemia/insulin resistance is not well known but hyperinsulinemia appears to down regulate apM1 gene expression in adipose tissue.\textsuperscript{221} The results of the present study also found significantly lower levels of adiponectin in obese diabetics than non obese diabetic subjects (Figure 24). This is in accordance with study done by Bu et al which observed significantly decreased adiponectin levels in obese diabetics.\textsuperscript{222} However, unlike the above findings, no difference in adiponectin levels was observed in a study done by Annuzzi et al between type 2 diabetic patients and healthy subjects.\textsuperscript{223}

DM has been associated with dyslipidemias whose prevalence is variable, depending on the type and severity of diabetes, glycemic control, nutritional status, age and other factors. This may be related to insulin resistance which has been closely associated with diabetic dyslipidemia and hypertension. In the present study, it was observed that the lipid profile was not significantly deranged which may be due to rural setting of the study area selected where subjects were relatively more physically active and less exposed to fast/junk food (Tables 3-7). This is in accordance with study done by Barma et al\textsuperscript{212} and Mukhyaprana et al.\textsuperscript{191} The most characteristic lipid abnormality in diabetics was hypertriglyceridemia which was similar to the findings of Dixit et al\textsuperscript{224} and Zargar et al.\textsuperscript{190} Sinharoy et al also reported elevated triglycerides in lean
type 2 diabetics compared to normal weight and obese type 2 diabetics.\textsuperscript{192} Similarly total cholesterol, HDL-cholesterol, LDL-cholesterol and VLDL-cholesterol did not differ significantly amongst obese and non obese type 2 diabetics. Most of the diabetic patients are found to have variable combination of triglyceride over production or under utilisation. In severe insulin deficiency, lipoprotein lipase (LPL) activity is markedly impaired. However, in mild to moderate type 2 diabetics, LPL activity is relatively intact which enhances endogenous triglyceride synthesis particularly in presence of obesity and adequate amount of insulin.\textsuperscript{190} Overall, there was increase in VLDL-cholesterol levels observed amongst diabetics, both obese as well as non obese. The reason may be increase in fatty acid transport to liver owing to increased lipolysis in adipose tissue ensuing from relative deficiency of insulin. Insulin directly degrades the apo B (which is major protein of VLDL particles) and thus it may increase secretion of apo B and then VLDL.\textsuperscript{225}

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 (Figure 25). 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. 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.\textsuperscript{226} Significant degree of insulin resistance have been reported in diabetics as well as obese subjects by Bu et al.\textsuperscript{222} Mohammadzadeh et al has also shown in their study that type 2 diabetics had significant levels of insulin resistance, as calculated by HOMA-IR.\textsuperscript{30}

The mean age of obese, non-diabetics was comparable to that of non obese, non-diabetics as shown in table 8. In addition, there was statistically highly significant difference in body mass index (BMI) amongst obese and non obese, non-diabetics; BMI being higher in obese subjects (Figure 26). 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 (Figure 27). Furthermore, differences in fasting plasma insulin levels while comparing obese and non obese, non-
diabetics were found to be statistically highly significant in our study (p<0.001) (Figure 28). These findings were similar to those observed by Carnethon et al in their study on Black and White adults and Urbanavicius et al while working on pre-diabetes and early type 2 diabetes.\textsuperscript{227, 228} Interestingly, it is also known that significantly high insulin levels (hyperinsulinemia) with normal FPG, as seen in obese individuals are features of insulin resistance which may further be implicated in the development of CVD.\textsuperscript{158}

It is well documented that high insulin levels are associated with elevated C-peptide levels as both are produced in equimolar amounts but the significance of C-peptide estimation in obese individuals lies in its ability to assess endogenous insulin reserve which can help in early screening of subjects with positive family history and thus, creating awareness about lifestyle modifications to prevent obesity-related disorders in the future. Keeping in view the above fact, the serum C-peptide levels were estimated and compared between obese and non obese, non diabetic subjects. The serum C-peptide levels were found to be significantly higher in obese than non obese, indicating insulin resistance. These findings were similar to those obtained in study done by Abdullah et al and Abdullah BB et al\textsuperscript{12, 174} (Figure 29).

It has been reported that the insulin resistance in obese individuals may also be associated with higher levels of serum leptin and low levels of adiponectin. Both are adipocyte-derived hormone involved in the pathogenesis of atherosclerosis which may place obese subjects at greater risk for CVD.\textsuperscript{229} The
results of the present study also confirmed that leptin levels were significantly higher in obese non-diabetics compared to non-obese subjects (Figure 30). The levels of adiponectin were significantly lower in obese than non-obese non-diabetics (Figure 31). Studies conducted by Jaleel et al\textsuperscript{230}, Kolahi et al\textsuperscript{231} and Weyer et al\textsuperscript{221} have shown high leptin levels and low adiponectin levels in overweight and obese subjects. Serum leptin levels are significantly elevated in obese subjects in proportion to the degree of adiposity, suggesting that hyperleptinemia may play a role in the pathogenesis of obesity related complication.\textsuperscript{232}

Leptin receptors are present most abundantly in brain besides various peripheral tissues. Mutation in the gene encoding the receptor results either in the impairment in transport of leptin across blood brain barrier or impaired signal transduction. This prevents leptin from acting on neuropeptides Y and therefore, causes more food intake and less energy expenditure leading to obesity. The net result is leptin being unable to perform its functions despite being produced in excess. This phenomenon is known as leptin resistance which leads to increased concentration of leptin in obese subjects.\textsuperscript{233}

Adiponectin production is negatively regulated in obesity leading to its decrease concentration. It has been suggested that hypoadiponectinemia in obesity is in large part attributable to insulin resistance and/or hyperinsulinemia.\textsuperscript{221} 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) as shown in tables 9-13. This may be due to rural setting of the study area selected where subjects were relatively more physically active and less exposed to fast/junk food.

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 (Figure 32). Similarly, studies done by Abdullah A et al\textsuperscript{12} and Urbanavicius et al\textsuperscript{228} have reported higher levels of insulin resistance amongst obese subjects, as calculated using HOMA method of insulin resistance. 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.\textsuperscript{234}

On comparing the mean age of subjects included in the four groups under study, it was found that the non diabetic subjects were younger in age than the diabetes patients, both with and without obesity (Table 14). Overall, the mean age of subjects studied was 49.76 ± 10.65 years. There were fifty males and fifty females in each group of subjects. Most of the parameters were not gender dependent. However, the pertinent variables i.e. leptin, adiponectin and
HDL-Cholesterol are dependent on gender of the subjects. The mean values of these parameters were compared amongst males and females under study and the difference between the two genders was found to be statistically insignificant (Tables 15-17) which restricted further analysis on the basis of gender. The fasting plasma glucose levels were found to be significantly higher amongst diabetic patients both with and without obesity than non diabetic subjects, indirectly indicating the presence of insulin resistance (Table 18). The fasting plasma insulin levels were higher amongst obese diabetics and obese non-diabetics. Non obese subjects with and without diabetes had significantly lower insulin concentrations than obese subjects (Table 19). This suggests that in obese subjects, insulin resistance associated with hyperinsulinemia, can predispose them towards the development of DM and CVD. Along with plasma insulin levels, serum C-peptide concentrations was also found to be elevated in obesity associated with and without DM clearly depicting that obese patients are more insulin resistant than non obese (Table 20).

Higher levels of serum leptin were found in obese subjects both with and without T2DM in the present study (Table 21). The serum leptin levels were lower in non obese diabetics and non-diabetics. Similarly, fasting leptin concentrations in Caucasian, Afro-Caribbean and Asian diabetic subjects have been shown to be higher in obese than in normal weight subjects with non-insulin dependent DM and non diabetic subjects as reported by Widiaja and
colleagues.\textsuperscript{235} Few studies done by Buyukvese et al\textsuperscript{236} and Zimmet et al\textsuperscript{237} found reduced levels of leptin in type 2 diabetics which has been related to insulin deficiency and difference in distribution of fat tissue throughout the body. Markedly increased serum leptin levels seen in obese individuals may be due to presence of leptin resistance following its excessive production.\textsuperscript{238}

Adiponectin is a hormone of adipocyte origin which is involved in the homeostatic control of glucose and lipid levels, so the levels of adiponectin are significantly reduced in obesity and T2DM in association with insulin resistance.\textsuperscript{239} The present study demonstrated significantly lower concentrations of adiponectin in obese subjects both with and without diabetes as compared to non obese subjects (Table 22). Lower concentration of adiponectin in diabetic obese than non diabetic obese in the present study is related to more deteriorated glucose metabolism owing to cumulative effects of T2DM and obesity. Surprisingly, although adipose tissue is the main source of adiponectin, research findings suggest that the blood level of adiponectin is reduced in obese or type 2 diabetics who have large reserves of fat tissue. This may in part be related to hyperinsulinemia and chronic insulin resistance seen in T2DM due to overproduction of TNF-\(\alpha\) by adipose tissue and interference in its signalling in endothelial cells by adiponectin.\textsuperscript{240} Reduction in metabolic function of adipocyte with aging leading to decreased adiponectin mRNA expression in adipose tissue has also been postulated for low serum adiponectin levels in obesity. Previous studies performed by Al-Kayatt et al\textsuperscript{241},
Mohammadzadeh et al\textsuperscript{30} and Abdelgadir et al\textsuperscript{242} also confirmed hypoadiponectinemia in obesity and T2DM.

Both lipid profile and DM have been shown to be the important predictors for metabolic disturbances including dyslipidemia, hypertension, CVDs, hyperinsulinemia etc.\textsuperscript{243} The present study has shown that diabetic obese subjects had higher serum total cholesterol, LDL-C, HDL-C and VLDL-C and triglycerides than non diabetic obese subjects (Tables 23-27). This is in agreement with the findings of the study done by Kim and co-authors.\textsuperscript{211} Zargar et al showed that all lipid fractions (except HDL-C) are abnormally elevated in obese diabetics when compared with obese non-diabetics.\textsuperscript{190} In the present study, significant elevation of total serum cholesterol, triglycerides and VLDL-C were observed in diabetes obese group as compared to non diabetic non obese group. Serum HDL-C and LDL-C levels did not show any significant variations. Similar findings have been reported by Al-Kayatt et al.\textsuperscript{241}

DM and obesity are closely related in terms of their onset and pathophysiology with insulin resistance being a common defect underlying both diseases. In an attempt to measure the extent of insulin resistance using HOMA-IR amongst the subjects included, it was observed that the subjects with both diabetes and obesity were significantly insulin resistant than those suffering from either of two diseases which may be due to cumulative effects of both DM and obesity (Table 28). Accumulation of adipose tissue in organs such as liver and/or
skeletal muscle underlies insulin resistance in obesity. Overproduction of counter regulatory hormones like TNF-α, IL-6, resistin has also been suggested in the development of insulin resistance seen in obesity and T2DM.\textsuperscript{234}

In order to study the correlation between various parameters under study, it was found that there were significant positive correlations of serum total cholesterol with HDL-C, LDL-C and VLDL-C (Figures 33-35). Triglyceride showed significant positive correlation with VLDL-C (Figure 36). These findings are in accordance with those depicted by Dixit and colleagues.\textsuperscript{224} In addition the correlation between plasma insulin and insulin resistance (HOMA-IR) also came out to be significantly positive (Figure 37).

Overall, the results of the present study demonstrated that the plasma levels of insulin, C-peptide, leptin and adiponectin are altered in subjects with T2DM and obesity, clearly suggesting the presence of pro-inflammatory, atherogenic and pro-diabetogenic adipocytokine profile which may be related to obesity, hypertension and CVD (Figure 38). However, the interplay of various hormones and adipocytokines in the pathophysiology of T2DM is rather complex and needs further elucidation. The adipocytokines may serve as an important link between increased fat mass, insulin resistance, deranged lipid and glucose metabolism and endothelial dysfunction especially in diabetic patients. Future studies with larger sample size and newly identified adipocytokines like resistin, vaspin, TNF-α, IL-6 and soluble leptin receptor
isoforms and their signalling pathway may shed new light on their function, molecular targets and potential clinical relevance in the prevention and treatment of obesity and obesity related diseases like T2DM, CVD etc. Novel adipocytokine-related treatment strategy may offer exciting new opportunities in a spectrum of metabolic diseases with several unmet clinical needs.