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To study the effect of high density lipoprotein and calcium on the levels of random blood sugar, body mass index, and age: A case control perspective

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Abstract
Type 2 Diabetes Mellitus (T2DM) is a disorder in which there is inefficient use of insulin hormone, leads to build up of glucose in the blood and eventually ends up with hyperglycemia. The aim of this study was to test the hypothesis of inter-relationship of age, body mass index (BMI), random blood sugar (RBS), Total Cholesterol (TC), Triacylglycerols (TAGs), High Density Lipoproteins cholesterol (HDLc), and Ca in patients with T2DM and also to compare these levels with that of healthy controls. Plasma glucose, serum TC, TAGs, HDLc, and Ca were estimated according to the instructions given in the methods. BMI was significantly higher and also the levels of RBS, TC and TAGs were significantly higher in T2DM patients compared with healthy controls. In addition, total serum Ca level was significantly higher in T2DM patients compared with healthy controls. No significant differences were observed in age and HDLc levels in T2DM patients compared with healthy controls. In the patient group positive correlation was observed between age and TAGs, age and HDLc, RBS and TC, RBS and TAGs, RBS and HDLc. In the control group, we found positive correlation between age and HDLc and a negative correlation between BMI and serum Ca. The authors conclude from the study that alterations in the study parameters in T2DM group are due to hyperglycemia, deficient scavenging action of HDLc and increase in serum calcium. Therefore, in patients with T2DM, any compensation mechanism may become insufficient.

Introduction

Type 2 Diabetes Mellitus (T2DM) is a disorder in which there is inefficient use of insulin hormone, which leads to build up of glucose in the blood and eventually ends up with hyperglycemia[1]. The risk of T2DM occurrence increases in an individual with genetic predisposition present in the family; if female gender; and also in ageing[2,3]. The contributing factors other than that of genetic factors include are diet, environmental pollutants, cigarette smoking, and obesity. Urbanization has urged the people to a diverted dietary intake[1-3], which accompanied with lack of exercise and improper sleep have alter the physiological responses of the cell to experience hyperglycemia[4]. Recent international diabetes

federation reports that there are around 1 billion adults are overweight worldwide, and 300 million of them are obese[5]. Most of the individuals included in the report have adopted the lifestyle of physical inactivity and western lifestyle, and most prominently India facing the greatest increase in the count of T2DM individuals[5,6]. The major disheartening problem is that overweight and obesity have a strong relationship towards atherosclerosis, cardio-vascular and cerebro-vascular accidents[7]. Thus, the research should be given high priority in a way to understand the factors that are solely responsible for the causation of obesity and also to know the preventive measures.

Recent reports have also suggests that the contributing factors have their affect on bio-molecules metabolism[8-10]. Normally insulin is released by the beta cells of pancreas in response to glucose present in the blood[11,12]. The released insulin sensitizes the insulin dependent tissues like skeletal muscle, adipose tissue, and liver to take up glucose for further metabolism[8-12]. But, chronic persistence of elevated glucose levels (hyperglycemia) impairs insulin stimulated glucose utilization in skeletal muscles and adipose tissues, leads to glucose toxicity[13]. Further, improper uptake of glucose, also activates Hydroxyl Methyl Glutaryl Coenzyme-A (HMG-Co-A) reductase, and decreases the oxidation of Triacylglycerols (TAG) at the extra-hepatic tissues resulting in increase of plasma glucose concentration and dyslipidemia in T2DM[14].

The important function of High Density Lipoprotein cholesterol (HDLc) is to remove fats and cholesterol from extra-hepatic cells and transport it back to the liver for excretion or re-utilization[15]. Lower levels of HDLc are consistently associated with increased risk of T2DM and also cardiovascular diseases and higher levels of HDLc are correlated with better cardiovascular health in reports[15-17]. Studies have also suggested that HDLc improves insulin secretion and also regulate the glucose levels in the animals[18-20]. There are also experimental evidences which suggest that levels of HDLc may contribute to the etio-pathogenesis of T2DM[17-19]. Calcium (Ca) is an element that plays an important role not only in cell signaling as second messenger but also in a wide range of biological functions[20]. In recent decades, it has been shown that Ca is related to insulin secretion, and insulin resistance[21]. Insulin secretion has been shown with respect to intracellular calcium which increases the expression of glucose transporters on insulin sensitive cells[22]. Studies have also shown association of elevated level of serum calcium with plasma glucose[21-24].

The above cited literature demonstrates that HDLc and Ca help in the secretion insulin, transport of glucose from outside to inside of the cell, and also in the utilization of glucose in the body respectively. More important function of HDLc is to scavenge the extra hepatic cholesterol to the liver.

Therefore in view of above-cited results, the aim of this study was to test the hypothesis of inter-relationship of age, body mass index (BMI), random blood sugar (RBS), TC, TAGs, HDLc and Ca in patients with T2DM and also to compare these levels with that of healthy controls.

Material & Methods

The study was conducted in the Department of Biochemistry, Maharani Laxmi Bai Medical College (MLBMC), Jhansi. Age & sex matched fifty human individuals having a normal glycaemic status were taken into healthy control group. Fifty T2DM subject, on treatment were included in T2DM group. The diagnosis of T2DM was made according to the norms laid by American Diabetes Association 2018. The diagnosis of T2DM group subjects was done by the consultants of General Medicine department of MLBMC. Exclusion criteria were type 1 diabetes individuals, less than five years of known duration of T2DM, and with complications. Inclusion criteria for healthy controls were non-diabetic, not taking supplementations, and having no other complications. Plasma glucose was

estimated by using the method Glucose Oxidase and Peroxidase (DPEC – GOD/POD) purchased from Arkray Healthcare Pvt. Ltd. Serum TC was estimated by using the method of Cholesterol Oxidase and Peroxidase (CHOD/POD) purchased from Arkray Healthcare Pvt. Ltd. Serum TAGs was estimated by using the method Glycerol Phosphate Oxidase and Peroxidase (Liquid stable) purchased from Arkray Healthcare Pvt. Ltd. Serum HDL was estimated by using the method polyethylene glycol (PEG) and phenol and 4-aminoantipyrine (PAP). Serum Calcium (Ca) was estimated by Arseno III method. Fasting venous blood (5ml) were drawn into EDTA and plane vials, after informed written consent from all the study group subjects with a disposable syringe & needle, under all aseptic conditions. Serum was separated by centrifuging the blood at 3000 rpm for 20 minutes. Samples were stored in aliquots at -20° C until assayed.

Statistical Analysis

IBM SPSS version 20 was used to perform statistical analysis. Unpaired 't' test was performed to compare the means of variables between two groups. Pearson correlation coefficient was considered to understand the association between two variables. Percentages were also calculated. $P < 0.05$ was considered significant.

Results

Age, BMI, RBS, TC, TAGs, HDLc and serum Ca levels are shown in Table 1. BMI was significantly higher and also the levels of RBS, TC (t=5.043, d=98) and TAGs (t=2.419, d=98) were significantly higher in T2DM patients compared with healthy controls. In addition, Total serum Ca (t=18.642, d=98) level was significantly higher in T2DM patients compared with healthy controls. No significant differences were observed in age and HDLc levels in T2DM patients compared with healthy controls.

In the patient group (Table 2) positive correlation was observed between age and TAGs (R=0.3458, P=0.01), age and HDLc (R=0.1401, P=0.03), RBS and TC (R=0.3770, P=0.03), RBS and TAGs (R=0.1241, P=0.01), RBS and HDLc (R=0.2060, P=0.02). In the control group (Table 3), we found positive correlation between age and HDLc (R=0.2655, P=0.01) and a negative correlation between BMI and serum Ca (R=-0.613, P=0.034).

Table 1: Findings in patients with T2DM and healthy control groups

| Variable	T2DM subjects (n=50)	Control subjects (n=50)	P- value
Age (years)	51.9±7.2	51±4.7	NS
BMI (kg/mt^2)	30.1±4.2	24.1±2.2	S
RBS (mg/dL)	195.7±61.8	125.9±11.4	S
TC (mg/dL)	168.5±27.7	146.6±12.9	S
TAGs (mg/dL)	159.9±55.4	139.6±21.4	S
HDLc (mg/dL)	41.2±4.6	45.8±3.4	NS
Total Ca (mg/dL)	10.2±0.8	9.1±0.2	S

Note: BMI- body mass index, Random Blood Sugar (RBS), TC-Total Cholesterol, TAGs-Triacylglycerols, HDLc-High Density Lipoprotein cholesterol, Calcium (Ca), S-Significant (<0.05), NS-Not Significant (>0.05)

Table 2: Pearson correlation in T2DM group

Variables	Age	Body Mass Index	Random Blood Sugar
Age (Years)	---	R=-0.0584	R=0.3088
Body Mass Index	R=-0.0584	----	R=0.1243
Total Cholesterol	R=-0.1142	R=-0.0960	R=0.3770
Triacylglycerols	R=0.3458	R=0.0391	R=0.1241
High Density Lipoprotein	R=0.1401	R=-0.1617	R=0.2060
Serum Total calcium	R=-0.124	R=-0.0826	R=-0.0677

Table 3: Pearson correlation in Control group

Variables	Age	Body Mass Index	Random Blood Sugar
Age (Years)	---	R=-0.1279	R=0.1494
Body Mass Index (BMI)	R=-0.1279	----	R=-0.3293
Total Cholesterol	R=-0.1427	R=0.1965	R=0.1027
Triacylglycerols	R=-0.1091	R=-0.0451	R=0.2049
High Density Lipoprotein	R=0.2655	R=0.0981	R=0.1233
Serum Total calcium	R= 0.1704	R=-0.0588	R=-0.1808

Figure 1: Pie chart depicting the gender distribution of subjects of T2DM group
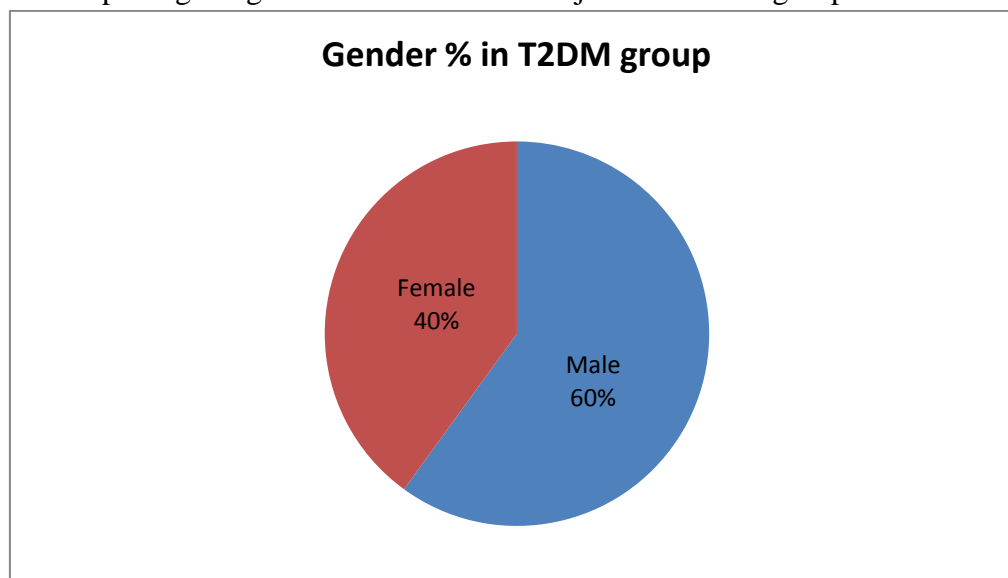
Figure 2: Pie chart depicting the gender distribution of Control subjects group
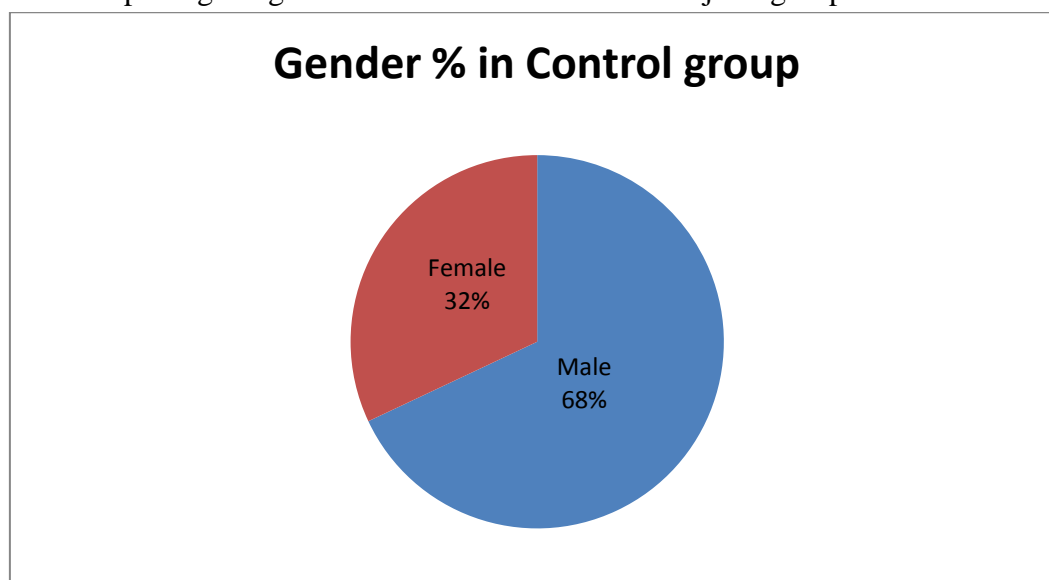
Discussion

Statistical significant differences were observed in BMI and in the levels of TC, TAGs, and Ca when compared between T2DM patients and healthy control. On the contrary, in the present study we did not observe statistical significant in the age group and also in the levels of HDLc when compared between T2DM patients and healthy control. In the patient group (Table 3 & 4) positive correlation was observed between age and TAGs, RBS and TC, RBS and TAGs, RBS and HDLc. T2DM is a common metabolic

disorder associated with profound alteration in lipid and lipoprotein profiles[25,26]. Hyperglycemia affects lipids by increasing the genes of key enzymes responsible for lipid metabolism[14,25-28]. The present study observed dyslipidemia in human. Previous studies have shown that in several models of T2DM including humans, lipoatrophic mice overexpressing SREBP-1c in adipose tissue, and insulin receptor substrate-2 (IRS-2) knockout mice in which insulin signaling is somehow impaired, hepatic SREBP-1c lipogenic transcription factors expression is instead activated[29,30]. A study demonstrated in insulin-depleted, STZ-administered mice that SREBP-1c induction does not require insulin[31]. Thus, continuous availability of glucose increased the formation of TC and TAG levels in the present study. HDLc is a lipoprotein, which carries cholesterol to the liver and transfer TAGs to other lipoproteins. Insulin is known to promote apolipoprotein A and HDL biosynthesis by liver [32,33]. Hyperglycemia inhibits this process and thus HDL secretion is impaired[34]. In addition, derangement in anabolic process i.e. more synthesis (cholesterol) and less utilization (TAGs) as observed in T2DM patients, is likely to cause decrease in the HDLc level[35]. Previous findings also demonstrated hyperglycemia and dyslipidemia in T2DM and different diabetic animal models[32-35]. Hyperglycemia induces alteration in weight in humans and as well as in rodents[35-37]. Studies on T2DM individuals and T2DM animal models have been shown to associate with body mass and hyperglycemia [38,39]. In the current study, we observed higher BMI in T2DM subjects. In the studies[35-39], it is not clearly stated whether T2DM comes first or change in the BMI. Nonetheless, the present study observed an increase in BMI compared to healthy controls. Studies revealed that increase weight can affect diabetes and diabetes can affect weight [40,41]. Overweight individuals particularly when body fat is distributed in the abdominal or visceral fat region, are associated with several metabolic abnormalities and diseases, including T2DM[42].

Even 10% increase in weight during their midlife enhances the risk of developing T2DM by more than 15 times when compared to a weight gain of after the young age[43]. Studies also demonstrate that weight loss through lifestyle intervention improved glycemic status and was effective in preventing the further development of diabetes [42,44,45]. Therefore, the increased scale between diabetes and BMI was due to hyperglycemia.

HDL particles export excessive lipid molecules present in the extra hepatic tissues to the liver for their further fate[15]. Such lipid molecules include cholesterol, phospholipid, and TAGs[15]. In the present study, we observed significant increase in TAGs in T2DM group as compared to control group TAGs. In addition, it is interesting that a correlation between age and HDLc was positive in the T2DM group but negative in the control group. At first the correlation between age and HDLc observed in the two groups seem contradictory, but possible explanation could be that the increase in HDLc and the TAG is compensatory to the increase in age. However, this increase is insufficient because T2DM have lower HDLc levels than their age-matched controls. There are many possible interpretations of this correlation in patients with T2DM because of the multifaceted pathogenesis of the disease. Lower level of HDLc was observed in the studies [15-20]. The studies also observed that individuals with T2DM are affected with vascular diseases in relation to lower HDLc levels[16-18]. In fact, the studies have referred lower level of HDLc to genetic factors, smoking, alcohol consumption and duration of the disease[18-20], but we attribute the lower HDLc level of the present study subjects to higher BMI as we observed increase in BMI in T2DM group subjects when compared to control group subjects. The fact that some variables affecting the status of HDLc were not controlled, e.g., age, the onset and duration of the disease, physical activity, and could be a limitation to our study, but the results were in accordance with previous research[18-20, 35-39]. However, we tried to overcome this handicap by taking blood samples

from all patients at the same time irrespective of physical activity of the subjects involved in the study.

In the present study we observed significant difference in the level of serum calcium (Ca) in T2DM group subjects when compared with control group subjects. Previous studies have reported that serum total calcium levels are higher in individuals with diabetes than in those without [21-24]. In addition, the current study observed negative correlation in BMI and serum Ca in control subjects. The secretion of insulin in response to an elevated concentration of plasma glucose is a Ca^{2+}-dependent process[22]. Alterations in insulin secretion have also been involved with disorders in blood glucose homeostasis[22,23], which was observed in the form of hyperglycemia in the present study. Thus, increase in serum Ca in T2DM subjects infers that the increase in BMI in order to compensate the metabolism of increased TC, TAGs in T2DM subjects. Subsequently, enhanced Ca levels can decrease the expression of glucose transporters and reduce glucose intake and, as a result, increase glucose plasma concentrations. Therefore, further studies are extensively needed on a multicountric, multicentric which should include all ethnic population to understand the mechanisms involved between serum calcium homeostasis, insulin, and glucose metabolism.

Conclusion

The authors conclude from the study that alterations in the study parameters in T2DM group are due to hyperglycemia, deficient scavenging action of HDLc and increase in serum Ca. Therefore, in patients with T2DM, any compensation mechanism may become insufficient. Studies have reported the beneficial effects of HDLc and Ca in humans but it has not been extensively studied in a multicountric, multicentric which should include all ethnic population.

Conflict of Interest: None declared.

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