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ORIGINAL RESEARCH

STUDY OF LIPID PROFILE AND MACRO MINERALS - CALCIUM, MAGNESIUM ANDPHOSPHOROUS IN SERUM IN CASES OF TYPE II DIABETES MILLETUS

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ABSTRACT

Background:Diabetes Mellitus is the most common metabolic disease and is posing as a major public health problem in the world, developing countries like India in particular. India has the second highest number of diabetics worldwide after China. Diabetes Mellitus is characterized by chronic hyperglycemia due to defective insulin secretion and / or insulin resistance. Non-insulin dependent diabetes mellitus (NIDDM) or type 2 diabetes mellitus accounts for more than 85% of the all the diabetics. It can occur at any age but is most common between 40 to 80 years of age. Macro minerals (calcium, magnesium, chloride, sulphur and phosphorus) play an important role in intermediary metabolism and cellular function, including enzyme activities and electrical gradients. The present study of these parameters is essential in clinically diagnosed patients of type 2 diabetes mellitus to show their role in the pathogenesis and to ascertain their role as possible biochemical markers of the disease progress.

Materials and Methods: Observational cross-sectional study was done. STUDY SUBJECT AND SIZEA total of one hundred patients aged between 30 years to 60 years, both male and female patients were selected from King George Hospital attached to Andhra Medical College, Visakhapatnam. Fifty patients diagnosed with type 2 diabetes mellitus attending OP at Department of Endocrinology and fifty healthy non-diabetic subjects were selected. The subjects are divided into two groups, fulfilling the inclusion and exclusion criteria. Group A (Cases): consisting of 50, type 2 diabetes mellitus diagnosed patients Group B (Controls): consisting of 50 healthy non diabetic subjects. Study Period: This study was conducted between December 2020 to November 2021 in the Department of Biochemistry, King George Hospital, Visakhapatnam. Sample Collection: After 12 hours of fasting, 5ml of venous blood is collected from the antecubital vein from each subject under aseptic conditions. Blood was collected in a

clot activator vacutainer (red cap tubes) and allowed to clot spontaneously in the tube, and then centrifuged for about 10 minutes at 3000rpm. In case of delay, the sample was stored at -20 degree Celsius for further analysis on the next working day. Carewas taken to prevent hemolysis.The findings were recorded and then tabulated in excel sheets, statistically analyzed using SPSS software. Unpaired t-test was done and expressed in terms of mean and standard deviation. A p-value of < 0.05 is considered as statistically significant.

Results: The (mean±SD) serum triglyceride levels in T2DM cases were 228±116.03 mg/dl and in healthy controls 103±18.23 mg/dl. The (Mean±SD) serum total cholesterol levels in T2DM cases were 208.26±48.14 mg/dl and in controls 164.18±12.71 mg/dl. The (mean±SD) serum HDL-C levels in T2DM cases were 33.82±7.89 mg/dl and in controls 45.16±3.35 mg/dl. The (mean±SD) serumLDL-C levels in T2DM cases were 128.36±44.80 mg/dl and in controls 99.74±9.23 mg/dl. The (mean±SD) serum VLDL-C levels in T2DM cases were 45.68±23.20 mg/dl and in controls 20.47±3.31 mg/dl. The (mean±SD) serum calcium levels in T2DM cases were 9.12±0.79 mg/dl and in controls 9.83±0.53 mg/dl. The (mean±SD) serum magnesium levels in T2DM cases were 1.87±0.35 mg/dl and in controls 2.30±0.36 mg/dl. The (mean±SD) serum phosphoruslevels in T2DM cases were 2.92±0.64 mg/dl and in controls 3.06±0.70 mg/dl. There is no statistical significance in the serum phosphorus levels in between the two groups.

Conclusion: in this present study. The serum triglyceride (TG) levels, serum total cholesterol levels, serum LDL-C Levels,VLDL-C Levels were significantly increased in the T2DM cases compared to the controls. The serum HDL-C levels were significantly decreased in the T2DM cases when compared to the controls. The serum calcium levels, Serum magnesium levels and serum phosphorous levels were significantly decreased in the T2DM cases when compared to the controls.

Keywords: Diabetes, Lipid Profile, Calcium, Magnesium,Phosphorous.

INTRODUCTION

Diabetes mellitus is one of the leading causes of blindness and kidney failure, heart attacks, stroke and lower limb amputation. Insulin deficiency or insulin resistance causes over metabolization of free fatty acids. This may lead to disorders in lipid metabolism resulting in dyslipidemia. Insulin resistance and type 2 diabetes mellitus are associated with clustering of interrelated lipid and lipoprotein abnormalities.[1,2] Early detection and management of dyslipidemia in diabetics can help prevent its progression and thereby decrease the risk of developing atherogenic cardiovascular complications. Diabetes mellitus leads to many complications, one of them is electrolyte imbalance. The study of the disturbances in the levels of serum electrolytes in type 2 diabetes mellitus has been a slightly ignored subject. Macro minerals (calcium, magnesium, chloride, sulphur and phosphorus) play an important role in intermediary metabolism and cellular function, including enzyme activities and electrical gradients.[3] Divalent macro metals (calcium and magnesium) play a significant role in the metabolism of carbohydrates, especially diverting assimilable glucose for utilization. Phosphate ions are essential during phosphorylation, a key enzyme reaction which diverts

glucose to metabolic pathway.[4] Serum concentrations of these macro minerals have shown to change with changes in plasma glucose levels.[5] Alterations in levels of calcium, magnesium and phosphorus were found to be associated with diabetes mellitus.[6,7] The disturbances in the levels of the macro minerals appear to have a negative effect on the glucose homeostasis and insulin sensitivity in type 2 diabetes mellitus and evolution of its complications like retinopathy, nephropathy and thrombosis. Dyslipidemia or lipid abnormalities like increase in the serum levels of triglycerides and LDL-C and decrease in HDL-C are expected in patients with type 2 diabetes mellitus.

Aim:

To study the levels of Lipid profile (total cholesterol, triglycerides, low density lipid LDL, high density lipids HDL and very low density lipids VLDL) and macro minerals – serum calcium, serum magnesium and serum phosphorus in cases of Type 2 Diabetes Mellitus.

Objectives:

1. To estimate the levels of Lipid profile – total cholesterol (TC), triglycerides (TG), low density lipids (LDL), high density lipids (HDL) and very low-density lipids (VLDL) in serum in cases of type 2 diabetes mellitus and to compare with that of healthy controls.
2. To estimate the levels of serum calcium in cases of type 2 diabetes mellitus and to compare with that of healthy controls.
3. To estimate the levels of serum magnesium in cases of type 2 diabetes mellitus and to compare with that of healthy controls.
4. To estimate the levels of serum phosphorus in cases of type 2 diabetes mellitus and to compare with that of healthy controls.
5. Serum calcium, serum magnesium and serum phosphorus levels are expected to decrease in patients with type 2 diabetes mellitus.

MATERIALS & METHODS

Observational cross-sectional study was done with a total of one hundred patients aged between 30 years to 60 years, both male and female patients were selected from King George Hospital attached to Andhra Medical College, Visakhapatnam.

Group A (Cases): consisting of 50 type 2 diabetes mellitus diagnosed patients.

Group B (Controls): consisting of 50 healthy non diabetic subjects Period- December 2020 to November 2021

Inclusion Criteria

- Patients diagnosed with type 2 diabetes mellitus
- Age between 30 – 60 years
- The healthy non diabetic subjects are taken as controls
- Those who have provided informed consent

Exclusion Criteria

- Age below 30 years and above 60 years
- Patients who are on statins or other lipid lowering therapy
- Patients with hypertension
- Patients with familial hypercholesteremia
- Patients with kidney disease
- Those who have declined to give informed consent

The following steps were taken during the course of this study:

- Approval of the study protocol from the ETHICS COMMITTEE, Andhra Medical College was taken.
- Informed consent was taken from each and every participant before their recruitment into the study in their local language.
- Detailed history was taken from every patient tor the purpose of fulfilling the selection criteria.
- A thorough clinical examination of each patient was carried out.
- Risk factors of type 2 diabetes mellitus like obesity, family history, impaired glucose tolerance or prediabetes, physical inactivity were assessed in all the patients and history was taken.
- An examination of dermatological manifestations of dyslipidemia also, like extensor digitoriumxanthoma, xanthelasma, Achilles tendon xanthomas was done.
- Drug history of lipid lowering drugs like statins, mineral supplements and other medications if any was taken.

Sample collection:

After 12 hours of fasting, 5ml of venous blood is collected from the antecubital vein from each subject under aseptic conditions. Blood was collected in a clot activator vacutainer (red cap tubes) and allowed to clot spontaneously in the tube, and then centrifuged for about 10 minutes at 3000rpm. After centrifuge, whenever possible, the analysis was done immediately. In case of delay, the sample was stored at -20 degree Celsius for further analysis on the next working day. Care was taken to prevent hemolysis. The findings were recorded and then tabulated in excel sheets, statistically analyzed using SPSS software. Unpaired t-test was done and expressed in terms of mean and standard deviation. A p-value of < 0.05 is considered as statistically significant.

Parameters

The following parameters were estimated from the collected sample in the present study:

1. Serum Glucose by Hexokinase G6P-DH Enzymatic UV method
2. Serum lipid profile:

- Serum Total cholesterol by CHOD – PAP Enzymatic photometric method.
- Serum HDL – Cholesterol by Direct Enzymatic Cholesterol – HDL estimation in the presence of detergent.
- Serum Triglycerides by GPO – POD method
- Serum LDL – Cholesterol estimated by Friedewald formula (FF)

- SerumVLDL–Cholesterol isestimated automaticallyby dividing Triglycerides by 5 when thevalue is in mg/dL.

3. Serum Calcium estimated by Arsenazo method
4. Serum Magnesium estimated by Xylidyl blue method
5. Serum Phosphorus estimated by Phosphomolybdate method

A. Glucose:

Reference range: (serum) Fasting – Adults70 to 106 mg/dLChildren60 to 100 md/dL

B. Total Cholesterol: Reference range:Total Cholesterol:< 200 mg/Dl – Desirable200 - 240 mg/dL – Borderline high>240 mg/dL – High.

C. HDL -C: Reference Range: 23 – 92 mg/dL.1) < 40 mg/dL is a major risk factor for CAD 2) > 60 mg/dL is a negative risk factor for CAD.

D. Triglycerides: Reference levels: Triglycerides level< 150mg/dL

- Normal,150 to 199 mg/dL
- Borderline high,200 to 499 mg/dL
- High>500 mg/dL

Very high Levels of 1700mg/dL or more show negative interference with this methodology.

E. LDL – C:(41) and 6. VLDL – C:Reference range for LDL – C:

<100mg/dL-good control,130 to159 mg/dL-Moderately elevated>160 mg/d-High

F. Reference range for VLDL – C: 2mg/dL to 30 mg/dL

G. Calcium: Reference Range:8.6 to 10.3 mg/dL in Serum.

H. Magnesium: Reference Range: 1.9 – 2.7 mg/dL in Serum

I. Phosphorus: Reference Range: 2.5 – 5.0 mg/dL in Serum

RESULTS

Table 1: Mean age of T2DM cases and non-diabetic controls

Parameter	Cases (T2DM)Mean±SD	Controls (Healthy) Mean±SD	't' value	'p' value	Significance
Age	47.80±6.12	42.28±8.20	-3.81	<0.0001	Statistically Significant

Table 2: Mean FBS (Fasting Blood Sugar) of T2DM cases and controls

Parameter	Cases (T2DM)Mean±SD	Controls (Healthy)Mean±SD	't' value	'p' Value	Significance
FBS	182.76±50.3	99.74±6.74	-11.56	<0.0001	HighSignificance

Serum Triglycerides (TG):

Serum Triglyceride levels in type 2 DM cases and healthy controls are shown in [Table 3].

Table 3: Serum Triglycerides in T2DM cases and controls

Parameter	Cases (T2DM)Mean±SD	Controls (Healthy) Mean±SD	't' Value	'p' Value	Significance
Triglycerides (TG) in mg/dl	228.3±116.03	103±18.23	-7.51	<0.001	High Significance

The (mean±SD) serum triglyceride levels in T2DM cases were 228±116.03 mg/dl and in healthy controls 103±18.23 mg/dl. A high statistical significance is present in the serum triglyceride levels in between the two groups.

Serum Total Cholesterol (TC):

The serum total cholesterol (TC) levels of T2DM cases and healthy controls.

Table 4: Serum Total Cholesterol (TC) levels in T2DM cases and controls

Parameter	Cases (T2DM)Mean±SD	Controls (Healthy) Mean±SD	't' value	'p' value	Significance
Total Cholesterol (TC) in mg/dl	208.26±48.14	164.18±12.71	-6.26	<0.001	High Significance

The (Mean±SD) serum total cholesterol levels in T2DM cases were 208.26±48.14 mg/dl and in controls 164.18±12.71 mg/dl. There is high statistical significance in the serum total cholesterol levels in between the two groups.

HDL-Cholesterol:

The serum HDL -Cholesterol levels of T2DM Cases and Controls are shown in [Table 5].

Table 5: Serum HDL-c levels in T2DM cases and controls

Parameter	Cases (T2DM)Mean±SD	Controls (Healthy) Mean±SD	't' value	'p' value	Significance
HDL-C(mg/dl)	33.82±7.89	45.16±3.35	9.36	<0.001	High significance

The (mean±SD) serum HDL-C levels in T2DM cases were 33.82±7.89 mg/dl and in controls 45.16±3.35 mg/dl. There is high statistical significance in the serum HDL-C levels in between the two groups.

LDL-Cholesterol:

The serum LDL -Cholesterol levels of T2DM Cases and Controls are shown in [Table 6].

Table 6: Serum LDL-C levels in T2DM cases and controls

Parameter	Cases (T2DM) Mean±SD	Controls (Healthy) Mean±SD	't' value	'p' value	Significance
LDL-C (mg/dl)	128.36±44.80	99.74±9.23	-4.42	<0.001	High significance

The (mean±SD) serum LDL-C levels in T2DM cases were 128.36±44.80 mg/dl and in controls 99.74±9.23 mg/dl. There is high statistical significance in the serum LDL-C levels in between the two groups.

Serum Calcium:

The serum Calcium levels of T2DM Cases and Controls are shown in [Table 7].

Table 7: serum calcium levels in T2DM cases and controls

Parameter	Cases (T2DM) Mean±SD	Controls (Healthy) Mean±SD	't' value	'p' value	Significance
Calcium (mg/dl)	9.12±0.79	9.83±0.53	5.33	<0.001	High significance

The (mean±SD) serum calcium levels in T2DM cases were 9.12±0.79 mg/dl and in controls 9.83±0.53 mg/dl. There is high statistical significance in the serum calcium levels in between the two groups.

Serum Magnesium:

The serum magnesium levels of T2DM Cases and Controls are shown in [Table 8].

Table 8: Serum magnesium levels in T2DM cases and controls

Parameter	Cases (T2DM) Mean±SD	Controls (Healthy) Mean±SD	't' value	'p' value	Significance
Magnesium (mg/dl)	1.87±0.35	2.30±0.36	6.14	<0.001	High significance

The (mean±SD) serum magnesium levels in T2DM cases were 1.87±0.35 mg/dl and in controls 2.30±0.36 mg/dl. There is high statistical significance in the serum magnesium levels in between the two groups.

Serum phosphorus:

The serum phosphorus levels of T2DM Cases and Controls are shown in [Table 9].

Table 9: Serum Phosphorus Levels in T2DM Cases and Controls

Parameter	Cases (T2DM) Mean±SD	Controls (Healthy) Mean±SD	't' value	'p' value	Significance
Phosphorus (mg/dl)	2.92±0.64	3.06±0.70	1.07	0.29	Not significant

The (mean±SD) serum phosphorus levels in T2DM cases were 2.92±0.64 mg/dl and in controls 3.06±0.70 mg/dl. There is no statistical significance in the serum phosphorus levels in between the two groups.

DISCUSSION

Age and Sex: The mean±SD age in the present study was 47.80±6.12 years in T2DM cases and 42.28±8.20 years in the non-diabetic controls. This is in accordance with some other studies in developing countries like India. The mean age of the study population of the present study was similar to that of other Indian studies like MamathaV. et al.[9]

Fasting Blood Sugar (FBS): The (mean±SD) of fasting blood sugar levels in T2DM cases is 182.76±50.30 mg/dl, and the (mean±SD) of FBS levels in controls is 99.74±6.78 mg/dl. There is high statistical significance in between the two groups. The results obtained in the present study are in accordance with the studies of FKN Arthur, et al,[8] MamathaV. et al,[9] and many other studies on type 2 diabetes mellitus as T2DM is essentially characterized by hyperglycemia.

Serum Triglycerides (TG): The (mean±SD) of serum triglyceride levels in T2DM cases is 228.30±116.03 mg/dl, and the (mean±SD) of triglyceride levels in controls is 103.54±18.23 mg/dl. There is high statistical significance in between the two groups. The results obtained in the present study are in accordance with the studies of SaurabhSultania et al.[10]

Serum Total Cholesterol (TC): The (mean±SD) of serum triglyceride levels in T2DM cases is 208.26±48.14 mg/dl, and the (mean±SD) of triglyceride levels in controls is 164.18±12.71 mg/dl. There is high statistical significance in between the two groups. The results obtained in the present study are in accordance with the studies of Marwan Khalid Sabahelkheir et al.[11]

SERUM HDL- The (mean±SD) of serum HDL- 33.82±7.89 mg/dl, and the (mean±SD) of HDL-C levels in controls is 45.16±3.35 mg/dl. There is high statistical significance in between the two groups. The results obtained in the present study are in accordance with the studies of SaurabhSultania, et al,[10] Dr.Bhagyashree.[12]

SERUM LDL- The (mean±SD) of serum LDL- 128.36±44.80 mg/dl, and the (mean±SD) of LDL-C levels in controls is 99.74±9.23 mg/dl. There is high statistical significance in between the two groups. The results obtained in the present study are in accordance with the studies of Dr. ZA Manzar, Dr.Bhagyashree.[12]

SERUM VLDL- The (mean±SD) of serum VLDL- 45.68±23.20 mg/dl, and the (mean±SD) of VLDL-C levels in controls is 20.47±3.30 mg/dl. There is high statistical significance in between the two groups. The results obtained in the present study are in accordance with the studies of SohanLalNigah, et al.[15]

Serum Calcium: The (mean±SD) of serum calcium levels in T2DM cases is 9.12±0.79 mg/dl, and the (mean±SD) of serum calcium levels in controls is 9.84±0.53 mg/dl. There is high statistical significance in between the two groups. The results obtained in the present study are in accordance with the studies of QaziNajeeb, et al,[13] Dr.Romy.W.Marshnilet al,[14] and SohanLalNigah, et al.[15]

Serum Magnesium: The (mean±SD) of serum magnesium levels in T2DM cases is 1.87±0.35 mg/dl, and the (mean±SD) of serum magnesium levels in controls is2.30±0.35 mg/dl. There is high statistical significance in between the two groups. The results obtained in the present study are in accordance with the studies of Revathi.R, et al,[16] QaziNajeeb et al,[13] and SiddanagoudaBiradar, et al.[17] The results of the present study are contradictory to the studies of Linyan Fang, et al.[18]

Serum Phosphorus: The (mean±SD) of serum phosphorus levels in T2DM cases is 2.92±0.64 mg/dl, and the (mean±SD) of serum phosphorus levels in controls is 3.06±0.70 mg/dl. There is no statistical significance in between the two groups. The results obtained in the present study are in accordance with the studies of Lena Haglin, et al.[19]

CONCLUSION

There is a very high prevalence of type 2 diabetes mellitus, in developing countries like India, in particular.

1. The etiology is multifactorial, mainly involving genetic factors, lifestyle factors and family history.
2. This study estimated the serum lipid profile (TG, TC, HDL-C, LDL-C, VLDL-C) levels in 50 patients with T2DM and comparing of these results with those of 50 healthy non diabetic controls.
3. The following lipid abnormalities were found in the T2DM cases in this present study when compared to the healthy controls.
4. The serum triglyceride (TG) levels were significantly increased in the T2DM cases compared to the controls.
5. The serum total cholesterol (TC) levels were significantly increased in the T2DM cases when compared to the controls.
6. The serum HDL-C levels were significantly decreased in the T2DM cases when compared to the controls.
7. The serum LDL-C levels were significantly increased in the T2DM cases when compared to the controls.
8. The serum VLDL-C levels were significantly increased in the T2DM cases when compared to the controls.
9. The serum calcium levels were significantly decreased in the T2DM cases when compared to the controls.
10. The serum magnesium levels were significantly decreased in the T2DM cases when compared to the controls.
11. The serum phosphorus levels were decreased in the T2DM cases when compared to the controls, but it is statistically insignificant.

Further in-depth studies in this area will help to establish better and rational interventional strategies to manage the dyslipidemia and macro mineral abnormalities in T2DM cases,

thereby preventing further complications and ultimately improving the patients' outcome and quality of life.

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