

Cardiometabolic Profile Status in Diabetic Children and Adolescents

Manoj Kumar Nandkeoliar¹, Rajeev Goyal², Rahul Saxena³

¹Assistant Professor, Department of Biochemistry, School of Medical Sciences and Research; Sharda University; Greater Noida, U.P., India

²Associate Professor, Department of Biochemistry, VMMC & Safdarjung Hospital, New Delhi, India

³Assistant Professor, Department of Biochemistry, School of Allied Health Sciences, Sharda University, Greater Noida, U.P., India

Original Research Article

*Corresponding author

Rahul Saxena

Article History

Received: 27.09.2017

Accepted: 06.10.2017

Published: 30.10.2017



Abstract: Assessment of cardiometabolic profile in children and adolescents with type 2 diabetes mellitus (T2DM) is a serious concern and a matter of interest for researchers. However, limited studies have been documented on abnormality in glycemic and lipid profile in young diabetic patients. The objective of present study was to determine the glycemic and lipid profile in children and adolescents with T2DM and to determine their role in disease complexity. Fasting blood glucose levels, glycosylated hemoglobin along with serum total cholesterol, triglycerides, HDL, LDL and VLDL levels were estimated by using standard methods in 26 children of either sex (08-18 years age group) suffering from T2DM (Group II) and in 26 normal healthy individuals, served as healthy controls (Group I). The observed values were expressed as Mean \pm SD and data from patients and controls were compared using students't' test. Fasting blood glucose, serum total cholesterol, triglycerides, LDL and VLDL levels were significantly high ($p < 0.05$) and serum HDL levels were significantly low ($p < 0.05$) in T2DM patients as compared to healthy controls. Similarly, glycosylated hemoglobin (HbA1c) was also found towards the higher end of the normal range in Group II subjects. These finding suggest that diabetic children and adolescents are at enhanced risk to develop cardiovascular complication due to their altered glycemic and lipid profile with respect to non-diabetic controls. Therefore, control on blood sugar level and maintenance of normal lipid profile along with regular monitoring of cardiac markers in Indian children can prove to be protective against cardiovascular complications in early stage of life.

Keywords: Hyperglycemia, Dyslipidemia, Cardiovascular disease, Triglycerides.

INTRODUCTION

Among the top five causes of mortality, Type 2 diabetes mellitus (T2DM) is one of the most common chronic diseases globally and now emerging in epidemic proportions among pediatric group [1]. The global prevalence of diabetes among adults is estimated to be 6.4%, affecting 285 million people, in 2010, and is expected to increase to 7.7%, affecting 439 million people by 2030[2]. The prevalence of type 2 diabetes mellitus is rising much more rapidly because of increasing obesity and reduced body activity levels, as countries become more industrialized. Environmental and genetic factor including family history have been reported to predispose the children and adolescents to develop T2DM. In addition, a plethora of studies have evidenced an array of complex intertwining biochemical, genetic, and abnormal metabolic profile mirroring this pathophysiological association [3,4].

With aging, there is an increase in cardiovascular disease (CVD) risk which is mostly attributable to abnormal metabolic profile. However, there is a paucity of evidences related to CVD risk in early stage of life. Moreover, the increased cardiovascular risk in different age group subjects has been found to be related to the deranged lipid profile, endothelial dysfunction, metabolic, hormonal, and hemodynamic changes and coagulation disturbances [5]. By virtue of this type of association of altered metabolic profile with future cardiac complications, diabetic patients are considered to be at high risk of cardiovascular diseases. Interestingly, it is conceivable that children with T2DM may be hyperlipidemic, as seen in adults [6]. Therefore, the objectives of present study were to estimate glycemic and lipid profile in diabetic children and adolescent patients and to determine the relationship of these profiles with disease complexity which may confer us more rational

approach to the treatments of such complicated conditions.

MATERIAL AND METHODS

In the present study, 26 children and adolescents of either sex with Type 2 diabetes mellitus (11 males and 15 females) belonging to age group 08 to 18 years, were recruited as patient group, after taking their informed consent and approval of protocol by ethics committee of college. 26 age and sex matched healthy subjects with fasting blood glucose less than 100 mg/dl were recruited as controls. A general information or pre-experimental questionnaire regarding demographic information, detailed clinical and family history; and limited physical examination including waist-hip measurement was completed in all the subjects. All patients had T2DM, defined as per revised American Diabetic Association criteria (ADA 2013) [7].

Inclusion criteria

Children and adolescents belonged to 08 to 18 years of age; who gave informed consent for study, newly diagnosed, not under any medical treatment (anti-inflammatory drug) or taking antioxidant supplement for at least one month prior to blood collection were included.

Exclusion criteria

Children and adolescents above 18 and below 08 years of age; those with maturity onset diabetes of the young (MODY); adolescents girls with sexual activity; and with other connective tissue disease like systemic sclerosis; those with acute and chronic infections, fever, malignancy, renal disease, hepatic disease, hypertension, those taking antioxidant vitamin supplements or non-steroidal anti-inflammatory drugs were excluded.

Fasting blood sample (5 ml) was collected from the antecubital vein of the study group subjects and divided into three parts. First part was collected in fluoride vial for glucose estimation, second part in EDTA vial for HbA1c estimation and third part was kept in a syringe for half an hour for proper coagulation followed by serum separation at 2000 rpm to estimate serum lipid profile.

Fasting blood glucose levels were measured by using enzymatic kit based on glucose oxidase method. Glucose, in presence of glucose oxidase, converted into gluconic acid along with production of Hydrogen peroxide, which later oxidatively coupled with 4-aminoantipyrine /phenol (in presence of peroxidase) and red quinoneimine dye was produced. The intensity of the color complex was directly proportional to the glucose in specimen and showed absorption maxima at 505 nm [8].

Glycosylated hemoglobin (HbA1c) was estimated by using the VITROS chemistry products HbA1c reagent kit in conjunction with the VITROS chemistry products Calibrator Kit 18 and VITROS chemistry products FS Calibrator 1 on the VITROS 5.1 FS. Whole blood samples were hemolyzed on the VITROS 5.1FS and the concentration of HbA1c was measured in the hemolyzed samples, controls and calibrators. After performing a calibration for each reagent lot, HbA1c concentration in the unknown samples was determined using the stored calibration curve and absorbance was measured at 340 nm after a fixed incubation time.

Serum total cholesterol was estimated by enzymatic kit method which involves the conversion of cholesterol ester into free cholesterol and fatty acid by cholesterol esterase. In the second reaction, cholesterol oxidase acts on cholesterol and produce cholest-4-ene3-one and hydrogen peroxide. H₂O₂ oxidatively couples with 4-aminoantipyrine and phenol to produce red quinoneimine dye. This dye had absorbance maximum at 510 nm [9].

Enzymatic kit method was also used in the estimation of serum triglyceride. Triglyceride was hydrolysed by lipoprotein lipase to release glycerol which was converted into glycerol 3 phosphate by glycerol kinase. In addition, glycerol phosphate oxidase converts glycerol 3 phosphate into dihydroxy acetone phosphate and hydrogen peroxide. In presence of peroxidase, hydrogen peroxide oxidizes phenol chromogen to red color compound. The intensity of color was directly proportional to concentration of triglyceride and measured at 510 nm [10].

Serum high density lipoprotein (HDL) was estimated by using phosphotungstic acid/Mg²⁺ which precipitates chylomicrons, VLDL and LDL fraction whereas HDL fraction remains unaffected in supernatant. Cholesterol content of HDL fraction was assayed using Autozyme cholesterol [11].

Serum LDL-cholesterol and VLDL-cholesterol levels were calculated by Friedwald's formula [12].

$$\text{LDL-C} = \text{TC} - [(\text{TG}/5) + \text{HDL-C}]$$

$$\text{VLDL cholesterol} = \text{Total cholesterol} - (\text{HDL} + \text{LDL})$$

STATISTICAL ANALYSIS

The data collected from patients and control were entered separately in Microsoft Excel sheet of windows 2007 and values were expressed as Mean \pm SD. The significance of mean difference between groups was compared by using Student's t-test and distribution of probability (P).

RESULT

The clinical and demographic profile of the study group children and adolescents with T2DM and healthy controls are represented in Table 1. In the present study, the children without and with diabetes belonged to age group 08- 18 years i.e. 13.4 ± 3.5 and 14.6 ± 3.2 years in Group I and Group II respectively, as represented in Table 1.

Out of the selected 26 subjects of T2DM, 11 patients were male and 15 were female. The recruited children and adolescents with diabetes have positive family history of T2DM i.e. in 78%. In addition, height and weight measurement followed by BMI calculation revealed that Group II subjects had significantly high ($p < 0.05$) BMI as compared to healthy non diabetic control group subjects. The observation made reveal insignificant increase ($p < 0.1$) of waist hip ratio in the patients group subjects as compared to healthy controls.

As compared to normal healthy controls, abnormalities in glycemc and lipid profile were observed in study group subjects with diabetes, as represented in Table 2. In the Group II subjects, fasting blood glucose level was increased significantly ($P < 0.001$; 142.35 ± 14.46 mg%) along with increased glycosylated hemoglobin (5.10 ± 0.58) i.e. towards the higher end of the normal range, with respect to Group I subjects or healthy controls.

It was observed that dyslipidemia is highly prevalent and significantly associated with T2DM in children and adolescents. In children and adolescents with T2DM, significantly increased level of total cholesterol ($P < 0.05$; 19.09 % high), triglycerides ($P < 0.05$; 17.15% high), LDL-cholesterol ($P < 0.001$; 30.85% high) and VLDL ($P < 0.05$; 23.95% high) were observed whereas HDL-cholesterol levels were significantly reduced in Group II ($P < 0.05$; 11.22% low) subjects as compared to non-diabetic children and adolescents.

Table-1: Clinical characteristics of the study group subjects (Mean \pm SD).

S.No.	Parameters	Group I (n=26)	Group II (n=26)
1	Age (years)	13.4 ± 3.5	14.6 ± 3.2
2	Males/Females	13/13	11/15
3	Family history	-	78%
4	Height (meter)	150.6 ± 10.4	148.85 ± 11.0
5	Weight (Kg)	41.5 ± 3.8	52.8 ± 4.5
6	BMI (Kg/m ²)	17.90 ± 1.25	$24.32 \pm 1.40^{**}$
7	Waist: Hip ratio	0.79 ± 0.03	$0.96 \pm 0.03^*$

Where,

* $p < 0.1$: Non-significant

** $p < 0.05$: Significant;

*** $p < 0.001$: Highly Significant;

Table-2: Glycemic and lipid profile of the study group subjects (Mean \pm SD).

S.No.	Parameters	Group I (n=26)	Group II (n=26)
1	HbA1c (%)	5.10 ± 0.58	$6.28 \pm 0.57^*$
2	Fasting Blood Glucose (mg/dl)	78.47 ± 11.2	$142.35 \pm 14.46^{***}$
3	Total cholesterol (mg/dl)	148.71 ± 9.48	$167.80 \pm 12.50^{**}$
4	Triglyceride (mg/dl)	98.5 ± 10.52	$115.40 \pm 9.48^{**}$
5	HDL-cholesterol (mg/dl)	40.24 ± 2.85	$36.18 \pm 2.45^{**}$
6	LDL-cholesterol (mg/dl)	78.52 ± 12.28	$102.75 \pm 13.35^{***}$
7	VLDL-cholesterol (mg/dl)	28.35 ± 2.65	$35.14 \pm 3.22^{**}$

Where,

* $P < 0.1$: Non significant

** $P < 0.05$: Significant

*** $P < 0.001$: Highly significant

DISCUSSION

T2DM is frequently asymptomatic and may go undiagnosed in children. Despite intense research efforts to uncover the etiology of diabetes in children and adolescents, it remains inscrutable. However, a growing body of evidence supports the understanding that the disease begins with reduced glucose absorption from gastrointestinal tract accompanied by prolonged peripheral glucose accumulation, gluconeogenesis, reduced disposal of glucose and diminished hepatic glucose output, which are considered as hallmarks of T2DM development [13].

T2DM owes its pathological origin to inappropriate secretion of insulin, due to defective islet cell function or beta cell mass. Continuous consumption of calories-rich meals, junk food and sedentary lifestyle have culminated into an epidemic of diabetes projected to afflict around 300 million people across the globe by 2020 [14]. Arslanian *et al.* also reported in white youth that family history and excessive body weight are associated with increased risk of T2DM in children and adolescents of ethnic background [15]. Certain clinical features characteristics of T2DM found in adults have also been observed in children. In the present study, family history of T2DM and increased body weight were prevalent along increased fasting blood glucose and glycosylated hemoglobin in children with diabetes, as compared to non diabetic children. Our results are in concordance with the findings of Ramchandran *et al.* who studied on Asian- Indian urban children with T2DM [16]. The most probable mechanism underlying the etiology of diabetes in children was suggested to be the increased body weight, perturbed genetic expression of insulin along with impaired glucose utilization in muscles, overproduction of hepatic glucose, and enhanced absorption of splanchnic glucose [14, 17-19]. In addition, the present study also showed significant increase in the serum total cholesterol LDL-c, VLDL and triglyceride levels along with reduced HDL levels in the children with diabetes as compared to healthy controls. Our findings were in congruence with the findings of previous investigators where association of T2DM with altered lipid profile are well documented and the main pathophysiological basis underlying dyslipidemia along with glucose intolerance, and increased body weight has been attributed to IR [20]. Interestingly, our findings also reflect that due to elevated levels of serum total cholesterol along with LDL and depleted levels of HDL, children and adolescents with T2DM are at enhanced risk of future cardiovascular complications.

CONCLUSION

Thus, it is apparent that the incidence of T2DM is independent of age and lays his icy hands even on the children and adolescents. Hence, screening of T2DM in children and adolescents is an essential

step to reduce the morbidity and mortality caused by T2DM and its associated complications. The lifestyle change and diet therapy must be emphasized with appropriate levels in terms of patient education. Apart from general monitoring of weight and dietary pattern in children with positive family history of T2DM, regular monitoring of metabolic profile including glycosylated hemoglobin estimation and proper physical exercise are necessary steps to prevent the development of childhood diabetic population. Furthermore, in a country of scarce resources and unaffordability of tools for cardiac complication detection in children like India, with having high burden of diabetes; these simple clinical diagnostic metabolic profiles are useful for screening of adolescent diabetic population for cardiovascular complications. Therefore, regular monitoring of metabolic profile such as glycemic and lipid profile along with body mass index and waist hip ratio may not only predict T2DM development in early age but also prevent and regulate the development of diabetes associated complications such as CVD in children and adolescents.

REFERENCES

1. Hannon TS, Rao G, Arslanian SA. Childhood obesity and type 2 diabetes mellitus. *Pediatrics*. 2005; 116: 473-480.
2. Shaw JE, Sicree RA, Zimmet PZ. Global estimates of the prevalence of diabetes for 2010 and 2030. *Diabetes Res Clin Pract* 2010;87:4-14.
3. Brenta G, Danzi S, Klein I. Potential therapeutic applications of thyroid hormone analogs. *Nature Clinical Practice Endocrinology and Metabolism*. 2007, 3(9): 632-640.
4. Goglia F, Moreno M, Lanni A. Action of thyroid hormones at the cellular level: the mitochondrial target. *FEBS Letters*. 1999, 452(3): 115-120.
5. Mithal A, Majhi D, Shunmugavelu M, Talwarkar PG, Vasnawala H, Raza AS. Prevalence of dyslipidemia in adult Indian diabetic patients: A cross sectional study (SOLID). *Indian J Endocr Metab*. 2014;18:642-7.
6. Bloomgarden ZT. Type 2 diabetes in the young. The evolving epidemic. *Diabetes care*. 2004; 27:998-1010.
7. Association AD. 2. Classification and Diagnosis of Diabetes. *Diabetes care*. 2016; 39(1):S13-S22.
8. Trinder P. Determination of glucose in blood using glucose oxidase with an alternative oxygen receptor. *Ann Clin Biochem*. 1969; 6:24-27.
9. Richmond W. Preparation and properties of a cholesterol oxidase from *Nocardia* sp. and its application to the enzymatic assay of total cholesterol in serum. *Clin Chem*. 1973; 19(12): 1350-6.
10. Fossati P, Prencipe L. Serum triglycerides determined colorimetrically with an enzyme that

- produces hydrogen peroxide. Clin Chem. 1982;28 (10): 2077-80.
11. Castelli WP. HDL in assessing risk of coronary heart disease. Metabolic therapy. 1977; 6:1-5.
 12. Friedewald WT, Levy RI, Friedrickson DS. Estimation of the concentration of LDL – cholesterol in plasma, without use of the preparative ultracentrifugation. Clin. Chem. 1972; 18 : 499 – 502.
 13. Karar T, Alhammad RSI, Fattah MA, Alanazi A, Qureshi S. Relation between glycosylated hemoglobin and lipid and thyroid hormone among patients with type 2 diabetes mellitus at King Abdulaziz Medical City, Riyadh. J Nat Sci Biol Med. 2015; 6(1): S75–S79.
 14. Wang C. The Relationship between Type 2 Diabetes Mellitus and Related Thyroid Diseases. J Diabetes Res 2013; 39: 534-538.
 15. Arslanian SA, Saad R, Bacha E, Gungor N. Family history of type 2 diabetes is associated with decreased insulin sensitivity and an impaired balance between insulin sensitivity and insulin secretion in White youth. Diabetes Care. 2005; 28; 127-131.
 16. Ramchandran A, Sivasankari S, Snehalatha C, Vijay V, Satyavani K. Type 2 diabetes in Asian Indian urban children. Diabetes Care. 2003; 26: 1022-1025.
 17. Ten S, Maclaren N. Insulin resistance syndrome in children. J Clin Endocrinol Metab. 2004; 2526-2539.
 18. Singh BM, Goswami B, Mallika V. Association between Insulin Resistance and Hypothyroidism in Females Attending a Tertiary Care Hospital. Indian J Clin Biochem. 2010; 25: 141–145.
 19. Prasad RS, Nandkeoliar MK, Rai G, Srivastava S, Saxena R. Assessment of insulin resistance in diabetic hypothyroidism patients – A clinical insight. SJAMS. 2017; 5(2C): 531-535.
 20. Nandkeoliar MK, Dharmalingam M, Marcus SR. Diabetes mellitus in Asian Indian children and adolescents. J PediatrEndocrinol Metab. 2007; 20: 1109-1114.