Scholars Journal of Applied Medical Sciences
Abbreviated Key Title: Sch J App Med Sci
ISSN 2347-954X (Print) | ISSN 2320-6691 (Online)
Journal homepage: https://saspublisher.com/sjams/

OPEN ACCESS

Biochemistry

Dyslipidemia and Mediator of Inflammation in Diabetic Children of Delhi -NCR

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| **Received:** 13.04.2019 | **Accepted:** 22.04.2019 | **Published:** 30.04.2019
DOI: 10.21276/sjams.2019.7.4.45

Abstract **Original Research Article**

Background: In spite of continuous improvement in database of existing knowledge on T2DM in children from pathologic point of view, the intimate mechanisms involving systemic inflammation, hyperglycemia and dyslipidemia in T2DM young patients. ***Aim:*** The present study was designed to determine the extent of systemic inflammation, glycemic and lipid profile in T2DM children and to determine their role in future cardiac complication. ***Materials & Methods:*** Fasting blood was collected and serum C – reactive protein (CRP) along with blood glucose, serum total cholesterol, triglycerides, HDL, LDL and VLDL levels were estimated by using standard methods in 20 children of either sex (07-16 years age group) suffering from T2DM (Group II) and in 20 normal healthy individuals, served as healthy controls (Group I). The observed values were expressed as Mean ± SD and data from patients and controls were compared using students't' test. ***Result:*** Marked elevated levels of serum CRP (significantly high; $p<0.05$) was observed in T2DM children as compared to healthy controls. Similarly, 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. Due to existence of systemic inflammation in combination with dyslipidemia and hyperglycemia, these metabolic alterations have been implicated in the development of future cardiovascular complication in young T2DM patients. ***Conclusion:*** Thus, regular screening of metabolic profiles, cardiac markers and systemic inflammation along with life style modifications are predictive and preventive measures which may help in reduction of T2DM complications and its burden in the growing young population.

Keywords: Systemic inflammation, dyslipidemia, cardiac complication, adolescent.

INTRODUCTION

Type 2 diabetes mellitus (T2DM) is an endocrine disorder and have found to be associated with serious complications such as cardiac complications, hypothyroidism and renal diseases.[1] T2DM has evolved into an epidemic in India and it is estimated that diabetics affect about 285 million population worldwide and this figure is expected to be 439 million in next decade [2] With increase in life expectancy, as older population increases simultaneously incidence of T2DM in younger population increases dramatically because of increasing obesity and reduced body activity levels due to more industrialization and sedentary mode of life style. In general, T2DM is now emerging in epidemic proportions among pediatric group [3]. Moreover, family history, as one of the important genetic factor, has been implicated to predispose the younger population to develop T2DM.

Inflammation is potentially a key mechanism that appears to act through alteration of cytokine profiles, which occurs secondary to aging of the immune system or increase in body weight [4]. Contrary to aging process, previous studies have demonstrated an association between Type 1 diabetes mellitus and inflammation as measured by plasma C-reactive protein (CRP, a marker of systemic inflammation) levels in children [5]. In addition, a plethora of studies have evidenced an array of complex intertwining between obesity, inflammation and altered metabolic profile in age related disease complication [6, 7]. However, there is a paucity of data in relation to the development of T2DM and its related complication in young population.

Advancing of age with passage of time multifold enhances the cardiovascular disease (CVD) risk due to complex intertwining biochemical, genetic, and abnormal metabolic profile [8]. In general, presence of systemic inflammation exerts their culprit effect in the favor of disease complexity irrespective of age. However, evidences related to assessment of systemic

inflammation in combination with altered metabolic profile in younger population to predict CVD risk are scanty. Interestingly, it is conceivable that young T2DM population may exert dyslipidemia in combination with hyperglycemia and elevated systemic inflammation, as an important factor in future CVD risk. Therefore, the present study was carried out to ascertain the extent of systemic inflammation along with estimation blood glucose and serum lipid profile content in young patients of T2DM and to determine the relationship of systemic inflammation and altered metabolic profile with disease complexity as an effective approach in the prediction of cardiovascular complication.

MATERIALS AND METHODS

20 young patients with Type 2 diabetes mellitus (12 males and 08 females) of either sex belonging to age group 07 to 16 years were recruited as patient group (Group II), after taking their informed consent and approval of protocol by ethics committee of college. 20 age and sex matched healthy subjects with fasting blood glucose less than 100 mg/dl were recruited as controls (Group I). All patients had T2DM, defined as per revised American Diabetic Association criteria (ADA 2013)[9].

Inclusion criteria

Children and adolescents belonged to 07 to 16 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. 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 after checking their fulfillment with inclusion criteria.

Exclusion criteria

Young T2DM patients above 16 and below 07 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 anticubital vein of the study group subjects and divided into three parts. First part was collected in fluoride vial for glucose estimation; second part was kept in a syringe for half an hour for proper coagulation followed by serum separation at 2000 rpm to estimate serum CRP and lipid profile.

Serum C-reactive protein levels were measured using commercially available ELISA kits (R&D Systems, USA), according to manufacturer's instructions. 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].

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. H_2O_2 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 phosphates by glycerol kinase. In addition, glycerol phosphate oxidase converts glycerol 3 phosphate into dihydroxy aceteone 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/Mg^{2+} 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 ± SD. The significance of mean difference between groups was compared by using Student's t-test and distribution of probability (P).

RESULTS

In the present study, general information pertaining to mean age, height, weight and BMI of the study group subject's i.e. demographic indices of younger T2DM subjects and healthy controls are represented in Table 1. Patients with T2DM have insignificant variation ($p<0.1$) with respect to age as compared to healthy controls. Younger subjects with and without diabetes belonged to age group 07- 16 years i.e. 11.5 ± 2.25 and 13.2 ± 2.8 years in Group I and Group II respectively, as represented in Table 1. The recruited younger population with diabetes had positive family history of T2DM i.e. in 76%. Group I subjects were overweight as they had significantly high ($p<0.05$) BMI with respect to healthy controls which reflect the role of increased body weight in T2DM development as one of the important risk factor. However, incidence of T2DM in male are more than female HT subjects as 12 males and 08 females were recruited as younger T2DM patients. However, waist hip ratio in the patients group subjects was insignificantly increased ($p<0.1$) of as compared to healthy controls.

Markers of inflammation and metabolic profile are presented in Table 2. Serum CRP and fasting blood glucose levels were found to be significantly high ($p<0.001$; 47.5% and 44.74% high) in patient group as compared to healthy controls which reflect the role of inflammation in association with hyperglycemia in etiopathology of T2DM young patients.

As compared to normal healthy controls, abnormalities in lipid profile were observed in study group subjects with diabetes, as represented in Table 2. In the Group II subjects, dyslipidemia was highly prevalent and believed to be significantly associated with future CVD complication in T2DM children and adolescents. Patients with T2DM showed significantly increased level of total cholesterol ($P< 0.05$; 16.31% high), triglycerides ($P< 0.05$; 17.49% high), LDL-cholesterol ($P< 0.001$; 28.92% high) and VLDL ($P< 0.05$; 21.51% high) whereas HDL-cholesterol levels were significantly reduced in Group II ($P< 0.05$; 15.86% low) subjects as compared to non-diabetic healthy younger controls.

Table-1: Demographic profile of the study group subjects (Mean ± SD)

S.No.	Parameters	Group I (n=20)	Group II (n=20)
1	Age (years)	11.5 ± 2.2	13.2 ± 2.8
2	Males/Females	12/08	15/11
3	Family history	-	76 %
4	Height (meter)	151.2 ± 9.5	147.75 ± 10.6
5	Weight (Kg)	40.6 ± 3.2	48.2 ± 4.1
6	BMI (Kg/m^2)	17.88 ± 1.21	23.15 ± 1.32**
7	Waist: Hip ratio	0.78 ± 0.04	0.97 ± 0.03*

Where, *p<0.1: Non-significant, **p<0.05: Significant; *** p<0.001: Highly Significant

Table-2: Marker of inflammation and metabolic profile in study group subjects (Mean ± SD)

S.No.	Parameters	Group I(n=20)	Group II(n=20)
1	CRP (mg/L)	4.10 ± 0.57	6.05 ± 0.52**
2	Fasting Blood Glucose (mg/dl)	76.42± 10.2	138.30± 12.45***
3	Total cholesterol (mg/dl)	145.52± 9.36	169.26± 10.20**
4	Triglyceride (mg/dl)	97.3± 10.12	114.32± 9.65**
5	HDL-cholesterol (mg/dl)	41.18± 2.64	34.65± 2.42**
6	LDL-cholesterol (mg/dl)	80.32± 11.20	103.55± 12.42***
7	VLDL-cholesterol (mg/dl)	27.24± 2.15	33.10± 2.85**

Where, *P<0.1: Non significant, **P<0.05: Significant, ***P<0.001: Highly significant

DISCUSSION

Contrary to common belief type 2 diabetes mellitus (T2DM) is not a trivial illness but a major medical condition that affects the quality of human life of not only middle aged and older population but also of younger population as well [2,15]. T2DM owes its pathological origin to inappropriate secretion of insulin, due to defective islet cell function or beta cell mass. The incidence of diabetes and its complications are increasing at alarming pace with advancing of medical science. However, T2DM is frequently asymptomatic and may go undiagnosed in children. Massive research

have been carried out to come across the etiology of diabetes in children and adolescents, it needs more efforts to reach at concrete decision. It is well documented in previous studies on diabetic population irrespective of age that the disease process begins with reduced glucose absorption from gastrointestinal tract accompanied by prolonged peripheral glucose accumulation, gluconeogenesis, reduced disposal of glucose and diminished hepatic glucose output. These factors collectively contribute in the development of T2DM and considered as hallmark [16].

In addition to family history of T2DM and increased body weight, continuous consumption of calories-rich meals, junk food and sedentary lifestyle have culminated into an epidemic of diabetes [17, 18]. 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 in younger diabetic population, as compared to non-diabetic subjects. 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 and overproduction of hepatic glucose. Our findings are in concordance with that of Arslanian *et al.* who 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 [19].

It has been well documented that abnormal lipid profile or dyslipidemia in T2DM patients is an alarming condition of future health complications predominantly cardiovascular diseases (CVD) such as myocardial infarction, atherosclerosis etc [20]. Moreover, 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 younger diabetic subjects as compared to healthy controls. 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. Amusingly, our findings are quite similar with the recently documented findings of Nandkeoliar *et al.* who observed a characteristic altered serum lipid profile in children and adolescent of T2DM patients [21].

Interestingly, the presence of inflammation further enhances the frequency to develop CVD. C-reactive protein, an acute phase reactants, synthesized in liver and raised by many folds following acute inflammation, is a marker of systemic inflammation in several conditions including hypertension, rheumatoid arthritis, psoriasis, cancer and pre-eclampsia [22, 23]. The present study also showed significant increase in the serum CRP levels along with dyslipidemia and hyperglycemia in the younger diabetic subjects as compared to healthy controls. Our findings were in congruence with the findings of previous investigators where association of T2DM with increased inflammation, characterized by elevated CRP levels, are well documented [25]. Enhanced systemic inflammation in combination with altered metabolic such as dyslipidemia and hyperglycemia may be implicated as the main patho-physiological basis attributed to future CVD risk in younger T2DM population. The present study had certain limitations which include relatively small sample size. Therefore, a large-scale study is required to validate our findings.

CONCLUSION

Thus, on the basis of present observations and findings, it is obvious that the incidence of T2DM is not related to aging factor and it affects not only in middle age and elderly but also in the children and adolescents. Early identification of T2DM in children and adolescents plays a crucial role in order to reduce the morbidity and mortality caused by T2DM and its related future cardiovascular disease. In addition to regulate the dietary pattern of food stuff, maintaining of normal body weight and inclusion of light exercises as life style modification, regular screening of cardio-metabolic profile including glycemic and lipid profile estimation along with marker of systemic inflammation, are required to prevent the development of childhood diabetic population in general, and in children with positive family history of T2DM. Furthermore, in order to detect early the incidence of future cardiac complication in children, study group parameters are reliable and affordable diagnostic markers even for children belonging to low socioeconomic status as well and thereby, help in reducing the burden of diabetes along with cardiovascular complications.

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