ASSESSMENT OF OXIDIZED LOW DENSITY LIPOPROTEIN, AS ATHEROSCLEROSIS RISK MARKER IN TYPE 1 DIABETIC CHILDREN WITH SHORT HISTORY OF DIABETES MELLITUS

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Background: To evaluate the type-1 diabetic children for early atherosclerosis risk by measuring serum oxidized lipoprotein in relation with glycaemic control. Recent studies indicate that systemic markers of inflammation can identify subjects at high risk of cardiovascular disease (CVD). Oxidized low density lipoprotein (OxLDL) levels have been regarded as one of the independent determinants of atherosclerosis. **Methods:** This cross sectional study involved a total 79 subjects including 39 type 1 diabetics and 40 non-diabetic controls between the ages of 9 to 16 years. A detailed medical history was taken from each subject and the individuals with history of type-1 diabetes underwent clinical examination. Individuals with obesity, hypertension, smoking, and chronic infections, autoimmune and renal diseases were excluded. Serum concentrations of glucose and lipid profile were measured in duplicate by kits based on enzymatic methods. OxLDL was measured in duplicate by using standard enzyme linked immunosorbent assay (ELISA) method. Haemoglobin A_{1c} and Body mass index (BMI) were also measured. **Results**: Diabetic patients had significantly elevated levels of blood glucose (320.1vs 97) and HbA1c (10.3% vs 5.21%) as compared to controls (p<0.001) but the serum levels of OXLDL were not found to be significantly elevated in diabetic children (222.4 vs 140.2) as compared to controls (p>0.05). **Conclusion**: OxLDL is a strong independent risk marker for atherosclerosis observed in diagnosed old age patients of CVD but in present study we could not find statistically significant elevated levels of OxLDL in young diabetic subjects with short duration of diabetes.
Keywords: Type 1 diabetes, atherosclerosis, OxLDL

INTRODUCTION

Diabetes mellitus is a risk factor for atherosclerosis and asymptomatic low grade inflammation occurs prior to unconcealed vascular lesions in these patients.[1] Recently, there has been much interest in pro-oxidants in atherosclerosis - related vascular disease states and aging.[2] Atherosclerosis is considered a process involving the interplay of inflammation and oxidative stress. Oxidations of low-density lipoprotein (LDL), and the subsequent uptake by macrophages in the vascular wall, are important steps in the development of atherosclerosis. A small part of the oxidized LDL (OxLDL) particles escapes uptake by macrophages and returns to the blood stream or may leak from atherosclerotic plaques. Thus, measuring circulating levels of OxLDL may contribute to the estimation of cardiovascular disease (CVD) risk. In support of this notion, concentrations of OxLDL have been found to be elevated in patients with both stable and unstable angina and acute myocardial infarction. In apparently healthy middle-aged men, OxLDL was found to be a strong predictor for acute coronary heart disease events.[3]

A number of studies suggest the OxLDL is a more potent pro-atherosclerotic motivator than the native unmodified LDL. OxLDL has been observed to be increased in diabetic patients and this may contribute to the increased atherogenesis in diabetes, regardless of normal lipid levels, OxLDL levels may be elevated in diabetic patients and this may be explanation for the altered endothelial function.[4–6] Endothelium exposed to OxLDL develops alterations such as early signs of injury in the form of apoptosis.[7] The OxLDL decreases the gene expression of endothelial nitric oxide synthase and enhances production of reactive oxygen species.[8] The OxLDL itself activates inflammatory cells and potentiates the liberation of growth factors from monocytes/macrophage.[9,10] Most significantly, pathological studies have revealed build-up of OxLDL in the rupture–prone atherosclerotic plaque.[11] Given the understandable pathophysiologic significance, there has been extreme curiosity in the detection of OxLDL as predictor of coronary heart disease events.[12–14] In a nested case-control study in individuals with and without eminent LDL cholesterol levels, OxLDL levels were found to be increased in patients who afterward developed myocardial infarction (MI).[15,16]

Oxidized low density lipoprotein (OxLDL) levels have been regarded as one of the independent determinants of intima media thickness (IMT) of the common carotid artery, a surrogate marker of atherosclerosis.[17] OxLDL induces foam cell formation from macrophages that plays a key role in early atherogenesis.[18] Oxidation of LDL occurs primarily in the vessels wall, thus activating many inflammatory and atherogenic reactions.[19,20] During the last five years, the role of OxLDL in pre-clinical atherosclerosis has been investigated in a number of studies.[21] OxLDL is considered to encourage atherosclerosis through complex inflammatory and immunologic mechanisms that lead to lipid dysregulation and foam cell formation.

The present study was designed to assess serum OxLDL levels in children with short duration of type1 diabetes and healthy controls.

The finding that cardiovascular diseases may originate in childhood and adolescence leads to the need that these risk factors to be extensively investigated in these phases, with the purpose of planning earlier and more effective interventions.

SUBJECTS AND METHODS

This cross sectional study was carried out on 39 type-1 diabetic children (19 males and 20 female) between the ages of 9–16 years and 40 aged & sex matched healthy controls. Duration of diabetes was more than one year in diabetic children who were undergoing periodic follow-up examinations at the Diabetic Clinic of Children Hospital Lahore. The study was approved by the Ethical Committee and the Research Board of the University of Health Sciences (UHS), Lahore and conducted in the Department of Physiology, University of Health Sciences, Lahore. Written informed consent to participate in the study was obtained from each subject and/ his or her parents.

Children aged 9–16 years with Type1 diabetes having diabetes for more than one year were included in this study, while subjects taking medications known to affect body growth or lipid metabolism, antihypertensive, antiplatelet, or lipid-lowering medications, subjects with endocrinopathies (e.g., Cushing's syndrome, Down's syndrome, acromegaly, thyrotoxicosis, etc.) or any major illness since birth, any clinical evidence of infection, connective tissue disease, liver dysfunction, or angiopathy were excluded.

Body weight (BW) and height were recorded in all subjects by height and weight determining machine. Body mass index (BMI) was calculated by the following formula:

$$\text{BMI} = \frac{\text{BW (Kg)}}{[\text{Height (m)}]^2}$$

Arterial blood pressure (BP) was measured by the mercury sphygmomanometer (Certeza CR-2001) from the right arm in sitting position using an appropriate cuff size.

Serum OxLDL were determined by ELISA using commercial kits (OxLDL: Biomedia, Medizinprodukte, GmbH & Co, with an automated EIA analyzer (Bio-Rad Laboratories, Hercules, CA, USA).

Arithmetic mean and standard error of mean (SEM) of each parameter were determined. The significance of differences among the two groups was analyzed by student's *t*-test and $p<0.05$ was considered statistically significant. All calculations were carried out with the SPSS 15.

RESULTS

Table-1 summarizes the anthropometric characteristics of type1 diabetic subjects and non-diabetic controls. No significant differences were observed in the values of BMI ($p=0.09$) systolic blood pressure ($p>0.05$) and diastolic blood pressure ($p>0.05$) among the type1 diabetics and non-diabetic controls.

Table-2 summarizes the glycaemic parameters of type1 diabetic male subjects and respective non-diabetic controls. Mean blood glucose concentrations were significantly higher in type1 diabetic children than in controls. HbA_{1c} levels were significantly higher ($p<0.001$) in diabetic children as compared to the control group.

Table-3 depicts serum OxLDL levels in study groups. No significant differences were observed in serum levels of OxLDL in diabetic children and control groups (222.4±60 mg/dl vs 140.2±37 mg/dl, $p=0.25$).

Table-1: Anthropometric characteristics of type 1 diabetics and non-diabetic controls

Variable	Controls	Type 1 diabetics	*p*-value
Number of subjects (n)	40	39	
Age (years)	9±16	9±16	
Males	20	19	
Female	20	20	0.09*
Duration of diabetes	Nil	>1 year	>0.05*
BMI (Kg/m^2)	16.4±2	17.9±3	>0.05*
Systolic BP (mmHg)	100.7±13	99.7±10	
Diastolic BP (mmHg)	63.13±8	62.82±8	

*Non-significant

Table-2: Glycaemic parameters of type 1 diabetic subjects and non- diabetic controls.

Variables	Type 1 diabetic	Non-diabetic control	*p*-value
Plasma glucose level (mg/dl)	320.1±23.22	97.00±1.35	<0.001
HbA_{1C} %	10.3±0.55	5.21±0.08	<0.001

$p<0.001$=highly significant

Table-3: Serum OxLDL in type 1 diabetic subjects and non diabetic controls

Serum OxLDL (mg/dl)	Type 1 diabetics	Non-diabetic controls	*p*-value
Males	222.4±60	140.2±37	0.25*
Females	129.5±40	128.2±37	0.98*

*Non-significant

DISCUSSION

There are many studies about cardiovascular mortality and morbidity in diabetic adults and old age population with context to different risk factors like dyslipidemia, hypertension, metabolic disease, obesity and hyperglycaemia but the risk factors about the cardiovascular disease in type1 diabetic children have not been studied extensively.

HbA_{1c} is a marker of long term glycaemic control; increase in HbA_{1c} increases the relative risk for CVD events. Atherosclerosis in diabetes mellitus is more progressive in diabetic children than non diabetic controls. Both type-1 and type-2 diabetes are associated with abnormalities of lipid metabolism. Several researchers reported abnormal lipid metabolism in uncontrolled diabetic patients.

The present study is one of the few studies which have evaluated the serum level of oxidised low density lipoprotein (OxLDL) in type1 diabetic children at an early age group, i.e., 9 to 16 years. Serum Oxidised LDL levels were higher in type 1 diabetic children as compared to non-diabetic controls but the difference was not statistically significant (p>0.05). Matsumoto *et al* (2004) found that OxLDL levels were higher in diabetic patients as compared to healthy controls. Factors that may promote LDL oxidation in diabetic patients include antioxidant deficiencies, increased production of reactive oxygen species and protein glycation. The OxLDL correlated significantly with BMI but not with conventional lipid parameters, age, gender or smoking status.[22]

The clinical importance of circulating OxLDL has not been established, although OxLDL is involved in atherogenesis by inducing smooth muscle cell proliferation and smooth muscle cell foam cell generation.[23] Another study evaluates the relationship between age, inflammatory and oxidative stress-related markers with functional and structural changes of the arteries in the asymptomatic persons.[24] The results of a study suggested that elevated OxLDL and older age become more important determinants of structural changes of the arteries in asymptomatic person.[25]

The limitation of our study was that it mainly included type-1 children with short duration of disease, so it cannot be applied on type-2 patients or patients with longer duration of diabetes. Further studies need to be conducted in Pakistan with larger sample size.

CONCLUSION

Elevated levels of serum OxLDL in older age with longer duration of diabetes may be more important determinants of preclinical atherosclerosis.

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