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## **Sialic Acid and Vascular Cell Adhesion Molecule-1 as Early Markers in the First Degree Relatives of Type 2 Diabetes Mellitus Patients**

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Levels of sialic acid and Vascular Cell Adhesion Molecule-1 (VCAM-1) in the first degree relatives of type 2 diabetes mellitus patients and their possible role as an early marker of the prediabetic stage were studied in 74 controls and 150 first degree relatives. The total sialic acid concentration was significantly higher ( $p < 0.05$ ) in the first degree relatives compared to the control subjects. Amongst the offsprings, the total sialic acid concentration was significantly higher in the offspring with Normal Glucose Tolerance (NGT) than those with Impaired Glucose Tolerance (IGT). However the level of VCAM-1 did not differ amongst the controls and the first degree relatives. The total cholesterol and triglycerides were significantly higher ( $p < 0.05$ ) in the offspring with IGT when compared to the control subjects and the offspring with NGT. The above data suggests that desialylation of the vascular endothelium is an early event that precedes the expression of IGT or any lipid changes in asymptomatic offspring of one type 2 diabetic parent.

**Key words:** Sialic acid, type 2 diabetes, impaired glucose tolerance, lipids, vascular endothelium

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## INTRODUCTION

The first degree relatives of non-insulin dependant diabetes mellitus (Type 2) patients are at increased risk of accelerated atherosclerosis and microvascular diseases (Guerci *et al.*, 2001; Krolewski *et al.*, 1991). Many underlying factors appear to contribute to this outcome, including the presence of features of insulin resistance (Haffner *et al.*, 1988), abnormalities in plasma lipoproteins, blood pressure and renal functions (Schmidt *et al.*, 1995). However, a final common pathway in the development of vascular pathology is the expression of inducible adhesion molecules (Schmidt *et al.*, 1995). Recent experiments have reported the participation of the endothelium in metabolic, synthetic and regulatory pathways (Rubani, 1993). The earliest event in endothelial dysfunction is the accumulation of Low Density Lipoprotein (LDL) cholesterol in the affected arterial wall. The luminal surface of the endothelium is very rich in sialoglycated proteins and presents an anionic barrier that limits the passage of LDL (Smith and Staples, 1980). Thus, desialylation by changing the surface density of the sialic acid residues significantly modulates the uptake of LDL by the endothelium and increases the accumulation of LDL particles in the arterial wall (Gorog and Pearson, 1984). The accumulated LDL particles in the arterial wall become oxidized which in turn stimulates the production of adhesion molecules (Cominacini *et al.*, 1996). These adhesion molecules facilitate the attachment and transmigration of leukocytes through the endothelium (Ross, 1999). This leads to the accumulation of foam cells which in turn stimulate the growth factors and proinflammatory cytokines that causes an inflammatory process (Pickup and Crook, 1988).

In recent years, it has been reported that the sialic acid concentration is increased in the plasma of patients with diabetes mellitus (Chen *et al.*, 1996; Tomino *et al.*, 1998; Crook *et al.*, 1993). Vascular Cell Adhesion Molecule-1 (VCAM-1) which is an inducible cell-cell recognition protein on the endothelial cell surface (Schmidt *et al.*, 1995) is of interest because it has been linked to the early phase of experimental hypercholesterolemia-induced atherosclerosis (Cybulsky and Gimbrone, 1991; Li *et al.*, 1993). Elevated level of VCAM-1 was also observed in diabetic rabbits (Richardson *et al.*, 1994), human atherosclerotic lesions (O'Brien *et al.*, 1994) and plasma of diabetic patients (Schmidt *et al.*, 1995).

The aim of the present study was to investigate the relationship between sialic acid and VCAM-1 in prediabetic subjects and their possible role as an early marker of the prediabetic stage.

## MATERIALS AND METHODS

Healthy control subjects were chosen from Klang Valley, Kuala Lumpur, through the distribution of questionnaires in 2004. Any subject with the family history of diabetes, hypertension, coronary artery disease and a body mass index  $\pm 30 \text{ kg m}^{-2}$  was excluded from the study. The offspring of at least one parent with type 2 diabetes, with and without cardiovascular risk factors were randomly recruited through the diabetic clinic, University of Malaya Medical Center, Kuala Lumpur and through the distribution of questionnaires. The number of normal subjects who participated in this study consisted of 74 four subjects and the number of first degree relatives consisted of 150 subjects, out of which 35 subjects were classified as having Impaired Glucose Tolerance (IGT) and 115 subjects as having Normal Glucose Tolerance (NGT). The subjects were classified as having impaired glucose tolerance if the fasting glucose level was equal to or greater than  $6 \text{ mM L}^{-1}$  and if the 2 h plasma post glucose load value was between 7.8 and  $11 \text{ mM L}^{-1}$ , or as having normal glucose tolerance if the fasting level was less than  $6 \text{ mM L}^{-1}$  and the 2 h plasma glucose load value were less than  $7.8 \text{ mM L}^{-1}$  according to the World Health Organisation (Anonymous, 1979). None of the subjects received hypolipidemic drug therapy, or had any renal, hepatic or thyroid disease affecting glucose and lipid metabolism. Informed consent was obtained from all subjects and the study was approved by the institutional ethics committee.

Fasting blood was collected in bottles containing disodium Ethylene Diamine Tetraacetate Dehydrate (EDTA) and the plasma was separated immediately by centrifugation at 3000 rpm for 15 minutes at  $4^{\circ}\text{C}$ . Total cholesterol, triglycerides and high density lipoprotein was determined using the individual biochemical kits supplied with Dimension Clinical Chemistry System (Dode Behring, France) and low density lipoprotein was determined by Friedewald (1972) equation. Sialic acid was determined by the modification of the periodate resorcinol method as described by Jourdian *et al.* (1971). VCAM-1 was measured using hsVCAM-1 ELISA kit (Boehringer Mannheim GmbH, Germany) according to the manufacturer's instructions.

Data were expressed as mean  $\pm$  standard deviation. Continuous variables were analyzed using one way ANOVA. Two tailed  $p < 0.05$  was considered significant.

## RESULTS AND DISCUSSION

The two groups were of comparable age and did not differ significantly in their body mass index. The total

**Table 1: Total sialic acid concentration and plasma lipid levels**

Subjects	n	Total sialic acid (mM L <sup>-1</sup> )	Total Chol. (mM L <sup>-1</sup> )	LDL (mM L <sup>-1</sup> )	HDL (mM L <sup>-1</sup> )	Trig. (mM L <sup>-1</sup> )	p-values
Controls	74	1.97±0.25	5.20±0.90	3.22±0.91	1.36±0.35	1.04±0.5	* < 0.05
Offspring IGT	35	2.43±0.35	6.02±1.0	3.85±0.98	1.27±0.35	1.99±1.2	** < 0.05
Offspring non IGT	115	3.34±0.6	5.46±0.97	3.56±0.91	1.40±0.32	1.20±0.6	

Mean±SD; Offspring, first degree relative of one parent with type 2 diabetes; IGT, Impaired glucose tolerance; NGT, normal glucose tolerance.

\* offspring IGT versus controls; \*\* offspring Non IGT versus offspring IGT

**Table 2: VCAM-1 Concentrations in normal subjects and first degree relatives**

Subjects	n	Mean concentration (mM L <sup>-1</sup> )
Normal	45	440.38±189.42
Offspring IGT	46	386.22±171.59
Offspring non IGT	49	360.90±143.22

sialic acid concentration was significantly higher in the offspring of one parent with type 2 diabetes as compared to the control subjects (p<0.05). However the total sialic acid concentration was significantly higher (p<0.05) in the offspring with normal glucose tolerance as compared to the offspring with impaired glucose tolerance (Table 1). The total cholesterol and triglycerides were significantly higher (p<0.05) in the offspring with IGT as compared to the controls and the offspring with NGT (Table 2). However the concentration of VCAM-1 did not differ significantly amongst the three groups.

In this study, the plasma lipid status, plasma sialic acid and sVCAM-1 concentrations were evaluated in individuals who are genetically at high risk for developing diabetes, but who presently do not demonstrate clinical diabetes.

The steps by which type 2 diabetes causes atherosclerotic vascular disease are not entirely clear. Identification of diabetic patients at risk for accelerated development of vascular disease is a major challenge. In the majority of populations both genetic and environmental influences interact to determine individual risk of type 2 diabetes mellitus (Crook *et al.*, 1996). Subjects with type 2 diabetes mellitus are at increased risk for the development of both macrovascular and microvascular complications (Anonymous, 1985). At the time of diagnosis, the existence of atherosclerotic manifestations is already widespread in the patients with type 2 diabetes mellitus but the prevalence of Coronary Artery Disease (CAD) has no correlation with the duration of diabetes (Anonymous, 1985; Fuller *et al.*, 1980).

It is widely accepted that frank clinical type 2 diabetes is preceded by a long prediabetic stage (Lillioja *et al.*, 1988). Studies have shown that nondiabetic individuals with a positive family history of diabetes have elevated cardiovascular risk factors relative to nondiabetic individuals without such family history (Haffner *et al.*, 1989). Impaired Glucose Tolerance (IGT) is

commonly believed to represent the transitional state between normal and diabetic glucose tolerance. Although it has been proven to be a risk factor for cardiovascular disease, it is not a reliable marker (Stern *et al.*, 1985). Therefore, we decided to look at other markers. Since the emphasis is shifting from insulin resistance to endothelial dysfunction we decided to focus on some of the other components which could prove to be useful markers.

Atherosclerotic cardiovascular disease is an inflammatory process (Ross, 1979) associated with accumulation of cholesterol carrying LDL and fibrinogen/fibrin in the affected arterial wall (Playford and Watts, 1999). Therefore, it is important to understand the mechanisms which govern endothelial binding, uptake and transport of these macromolecules across the vessel wall as a prerequisite to the prevention of atherogenesis. The role of the luminal endothelial plasma membrane may be particularly relevant because it is the first interface between the vessel wall and circulating blood components. It has been shown that the removal of surface sialic acid from the luminal surface as well as the glycosaminoglycans increase the uptake of LDL (Gorog and Pearson, 1984). The internalized LDL is oxidized and taken up by the macrophages with the formation of foam cells, which is the first sign of demonstrable atherosclerosis causing a localized inflammatory process (Ross, 1979). Therefore, we hypothesize that desialylation at the endothelium could be an early event in the atherosclerotic process and could cause an increase in the sialic acid along with the acute phase proteins which results during the inflammatory process.

Desialylation is an early process in the vascular disease and since VCAM-1 are thought to play a role in the early stages of the disease, by facilitating the attachment of leucocytes to the endothelial wall we decided to look at the concentration of the sialic acid and VCAM-1 in the first degree relatives of the type 2 diabetic patients.

It has been reported that there is an increase in the concentration of VCAM-1 in type 2 diabetes (Cominacini *et al.*, 1996; Schmidt *et al.*, 1996). VCAM-1 also was shown to have a functional role for leukocyte adhesion in the microvasculopathy of diabetic retinopathy (Oslo *et al.*, 1997). It has also been demonstrated that levels of sVCAM-1 are elevated in the

plasma of diabetic patients with microalbuminuria compared to normoalbuminuric diabetic subjects. The patients with microalbuminuria demonstrated the presence of cardiovascular complications, suggesting the potential usefulness of microalbuminuria as a marker of diffuse vascular hyper permeability and measurement of plasma sVCAM-1 as a marker of ongoing vascular perturbation (Schmidt *et al.*, 1996).

It has been shown that the expression of adhesion molecules is up regulated by oxidized low density lipoprotein (Cominacini *et al.*, 1997) and by advanced glycation end products (Schmidt *et al.*, 1995).

Advanced glycation end products interact with their endothelial receptor to induce expression of vascular cell adhesion molecule (Schmidt *et al.*, 1995). Although the attachment and transmigration of leucocytes across the endothelial surface to the sub-intimal space is an early step in atherogenesis (Jang *et al.*, 1994) the increased expression of VCAM-1 is not visible in the first degree relatives of diabetics either in the group with normal or IGT. Bannan *et al.* (1998) also did not find a significant difference amongst the controls, the first degree relatives with non IGT and the first degree relatives with IGT. In agreement with that, our data also did not show any difference between the controls, the first degree relatives with IGT and the first degree relatives with NGT. Therefore it seems that the expression of sVCAM-1 cannot serve as a marker for the prediabetic phase but rather as a marker of the ongoing vascular perturbation.

The most important observation in the present study is that the total sialic acid was significantly higher ( $p < 0.05$ ) in the offspring of one parent with type 2 diabetes (Table 1). This is in accordance with other studies which have shown an increase in sialic acid in relation to cardiovascular disease and type 2 diabetes (Sarlund *et al.*, 1992).

The interesting finding in our study is that amongst the offspring the total sialic acid concentration was significantly higher ( $p < 0.05$ ) in the offspring with NGT than in the offspring with IGT (Table 1). One of the plausible explanations is that the offspring with IGT has a higher concentration of desialylated LDL. It is well documented that the LDL in diabetic patients are desialylated to a greater extent than in the normals and are responsible for the accumulation of LDL in the endothelium and the premature development of atherosclerosis in diabetic patients (Sobenin *et al.*, 1993). It has also been shown that the desialylated LDL is catabolized much more rapidly than the sialylated LDL (Malmendier *et al.*, 1985). Present study showed a significant increase in the total cholesterol and triglycerides in the offspring with IGT when compared with the offspring with NGT and control subjects

(Table 1). Despite the heterogeneity of atherosclerotic risk factors within type 2 diabetes, the offspring with NGT revealed virtually identical lipid profiles with the controls who had no family history of diabetes.

Other epidemiological studies have shown that the most common dyslipidemia in NIDDM and IGT is hypertriglyceridemia (Pyorala *et al.*, 1995). However the results concerning serum total and LDL cholesterol levels in patients has been conflicting. The lack of increase in the LDL, in the offspring with IGT could be due to the fact that compositional changes in LDL may be the first event in the process of atherosclerosis (Stewart *et al.*, 1993). Since the uptake of LDL and the oxidation of LDL is the first step in the inflammatory process of the endothelium, leading to atherosclerosis, we postulate that the desialylation of the endothelium may be an early event in the atherosclerotic process.

The present preliminary results show that the concentration of total sialic acid precedes any other metabolic disturbances in the subjects who are at high risk group for type 2 diabetes and subjects with asymptomatic hyperglycemia. In conclusion present results suggest that there is an increase in total sialic acid concentration in subjects who are genetically at high risk, for type 2 diabetes before any changes in the glucose tolerance and lipids. Further studies are in progress involving larger number of well characterized subjects and utilizing various inflammatory markers to confirm these findings.

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