Plasma sialic acid levels in the offspring of one parent with Type 2 diabetes mellitus

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Abstact

Sialic acid, oral glucose and plasma lipids were determined in 70 control subjects age range 20-60 and 150 individuals of age range 20-60 who were offspring of one type 2 diabetic parent. The number of first degree relatives who had impaired glucose tolerance were 50. The total sialic acid concentration was significantly higher ($p<0.05$) in the first degree relatives when compared to the control subjects. However the total sialic acid concentration was significantly higher in the offspring with normal glucose tolerance than those with impaired glucose tolerance. There was no significant difference in the lipids between the control subjects and the offspring with normal glucose tolerance. But the total cholesterol and triglycerides were significantly higher in the offspring with impaired glucose tolerance when compared to the control subjects and the offspring with normal glucose tolerance. Our study shows that the desialylation of the vascular endothelium is an early event that precedes the expression of impaired glucose tolerance or any lipid changes in asymptomatic offspring of one type 2 diabetic parent.

Introduction

Identification of diabetic patients at risk for accelerated development of vascular disease is a major challenge. In the majority of the populations, both genetic and envi-ronmental

influences interact to determine individual risk of type 2 diabetes mellitus [1]. Subjects with type 2 diabe-tes mellitus are at increased risk for the development of both macrovascular and microvascular complications [2]. 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 [2,3]

It is widely accepted that frank clinical type 2 diabetes is preceded by a long prediabetic stage [4]. Impaired glucose tolerance (IGT) is a widely accepted entity of the pre-diabetic stage and is associated with hypertension, obesity, insulin resistance and dyslipoproteinemia commonly known as metabolic syndrome X [5]. It is even possible that type 2 diabetes and CAD may share common antecedents [6,7] and the so called insulin resistance syndrome could be such a common factor. More complex abnormalities include changes in the composition of lipoproteins leading to increased production of atherogenic "remnant particles" [8], increased non enzymatic glycosylation and desialylation [9]. Studies have shown that non diabetic individuals with a positive family history of diabetes have elevated cardiovascular risk factors relative to nondiabetic individuals without such family history [10]. Therefore it is possible that the prediabetic phase could be a period of enhanced cardiovascular risk. Impaired glucose tolerance is commonly believed to represent the transitional stage 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 [11]. Therefore it is of paramount importance to determine some definitive early markers to identify the people at high risk.

The steps by which type 2 diabetes causes atherosclerotic vascular disease is not clear. Emphasis is shifting from elucidation of risk factors such as insulin resistance to an understanding of the process occurring at the vasculature [12,13]. Increase in the concentration of serum sialic acid has been shown to be a possible cardiovascular risk factor in patients with non insulin dependent diabetes [14]. The earliest event associated with atherosclerosis is the accumulation of low density lipoprotein (LDL) cholesterol and fibrinogen/fibrin in the affected arterial wall [15]. It is therefore important to understand the mechanisms, which govern the 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. The luminal sur-face of the endothelium is rich in sialoglycated proteins and thus provides an anionic barrier for the receptor mediated uptake of LDL. It has been shown that the removal of the sialic acid as well as the glycosaminoglycans, increases the internalization of LDL by 20 fold [16]. Therefore, desialylation of the endothelium could be an early event in the atherosclerotic process in cardiovascular disease and in type 2 diabetes mellitus. The accumulated LDL particles in the arterial walls are oxidized which in turn stimulates the production of adhesion molecules [17]. The adhesion molecules play a role in the early stages of vascular disease by facilitating the attachment and the transmigration of the leucocytes through the endothelium [18] which leads to the accumulation of foam cells and the stimulation of growth factors and proinflammatory cytokines that causes an inflammatory process [19]. The inflammatory process brings

about an acute phase response with the increase in the acute phase proteins which are sialylated. Concentrations of acute phase response markers and mediators of inflammation such as alpha tumor necrosis factor (TNFα) and interleukin–6 are raised in people with type 2 diabetes [20]. If this be the case, then the raised concentration of proinflammatory cytokines and the resultant acute phase response may underlie much of the metabolic clustering including glucose tolerance [19]. Therefore an increase in the acute phase pro-teins may partly explain the elevation of sialic acid in type 2 diabetes mellitus.

In this study we have shown that the concentration of sialic acid precedes the manifestation of IGT and lipid abnormalities in people in the high risk group for type 2 diabetes.

Methodology

Subjects

Healthy control subjects were chosen from Klang valley, Kuala Lumpur, through the distribution of questionnaires. Any subject with the family history of diabetes, hyperten-sion, coronary artery disease and a body mass index of more than 30kg/m2 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 Centre, Kuala Lumpur, and through the distribu-tion of questionnaires. The number of normal subjects who participated in this study consisted of seventy four subjects and the number of first degree relatives consisted of one hundred and fifty subjects, out of which thirty five subjects were classified as having impaired glucose tolerance (IGT) and one hundred and fifteen subjects as having normal glucose tolerance (NGT). The subjects were classified as having impaired glucose tolerance if the fast-ing glucose level was equal to or greater than 6mmol/l and if the two hour plasma post glucose load value was between 7.8 and 11 mmol/l, or as having normal glucose tolerance if the fasting level was less than 6 mmol /l and the two hour plasma glucose load value were less than 7.8 mmol/l according to the World Health Organisation [21]. 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.

Methods

Fasting blood was collected in bottles containing disodium ethylene diamine tetraacetate Dehydrate (EDTA) and the plasma was separated immediately by centrifuga-tion at 3000 rpm for 15 minutes at 40 C. Total cholesterol, triglycerides and high density lipoprotein was determined using the individual biochemical kits supplied with dimension R clinical chemistry system (Dode Behring France) and low density lipoprotein was determined by Friedewald equation [22]. Sialic acid was determined by the modification of the periodate resorcinol method as described by Jourdian et al [23].

Statistical Analysis

Data were expressed as mean ± standard deviation. Continuous variables were analysed using one way ANOVA. Two tailed p value of less than 0.05 was considered sig-nificant.

Results

The total sialic acid concentration of the controls, offspring of one parent with type 2 diabetes with normal glucose tolerance and offspring of one parent with type 2 diabetes with impaired glucose tolerance are shown in Table 1. The two groups were of comparable age group and did not differ significantly in their body mass index. The total 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.

The values of total cholesterol, triglycerides, high density lipoprotein and low density lipoprotein of the normals, offspring with NGT and offspring with IGT are shown in Table 2. 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 1: Sialic acid concentrations

Subjects	n	Total sialic acid	p Concentration
Controls	74	1.97±0.25 m.mole/l	50.0>*
Offspring IGT	35	2.43±0.35 m.mole/l	50.0>**
Offspring NGT	115	3.34±0.6 m.mole/l	

Offspring, first degree relative of one parent with type 2 diabetes; IGT, impaired glucose tolerance ; NGT, normal glucose tolerance. $p<0.05$ offspring versus controls; $p < 0.05$ NGT vs IGT

Table 2: Plasma lipid levels

Subjects	n	Total Chol m.mole/l	LDL m.mole/l	HDL m.mole/l	Trig m.mole/l	p
Controls	74	5.20±0.90	3.22±0.91	1.36±0.35	1.04±0.5	
Offspring IGT	35	0.1±20.6*	3.85±0.98	1.27±0.35	2.1±99.1*	p<0.05
Offspring Non IGT	115	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.

The total cholesterol and triglycerides were significantly higher in the offspring with impaired glucose tolerance ($p<0.05$) as compared to the offspring with normal glucose tolerance, and the controls.

Discussion

In this study , the plasma lipid status and plasma sialic acid 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 clear.

Emphasis is shifting from elucidation of specific metabolic risk factors such as Insulin resistance to an understanding of the processes occuring at the level of the vas-culature and in particular the endothelial cell. Atherosclerotic cardiovascular disease is an inflammatory process [18], associated with accumulation of cholesterol carrying LDL and fibrinogen/fibrin in the affected arterial wall [24]. 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 the surface sialic acid from the luminal surface as well as the glycosami-noglycans increases LDL internalization. Thus the LDL uptake is inversely correlated with the sialic acid content of the luminal surface [15]. The internalized LDL is oxidized and is taken up by the macrophages with the formation of foam cells, which is the first sign of demonstrable atherosclerosis causing a localized inflammatory process [18]. 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.

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 type2 diabetes [25].

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 explanation 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 [9]. It has also been shown that the desialylated LDL is catabolised much more rapidly than the sialylated LDL [26].

Our 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 2. 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 (Table 2).

Other epidemiological studies have shown that the most common dyslipidemia in NIDDM and IGT is hyper-triglyceridemia [27]. However the results concerning serum total and LDL cholesterol levels in patients with NIDDM have 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 atheroscle-rosis [28] 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.

Our preliminary results, which need to be confirmed with a larger number of patients 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 our results suggest that there is an increase in total sialic acid concentration in the 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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