

Lack of association between serum sialic acid levels and retinopathy in Type 2 diabetic patients

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Accepted 24 June 1998

Abstract

Sialic acid, an acetylated derivative of neuraminic acid, is reported to be a marker of micro- and macrovascular complications in diabetic subjects. The purpose of this study was to investigate the association of serum sialic acid and diabetic retinopathy. Serum sialic acid levels were measured in healthy non-diabetic control subjects, Type 2 diabetic patients without retinopathy (No DR), Type 2 diabetic patients with non-proliferative diabetic retinopathy (NPDR) and Type 2 diabetic patients with proliferative diabetic retinopathy (PDR). There was no significant difference in the serum sialic acid levels between the study groups (controls, 2.1 mmol/l; No DR, 2.5 mmol/l; NPDR, 2.2 mmol/l and PDR, 2.3 mmol/l). These results suggest that there is no association between serum sialic acid levels and diabetic retinopathy. © 1998 Elsevier Science Ireland Ltd. All rights reserved.

Keywords: Sialic acid; Acute phase reactants; Diabetic retinopathy; Type 2 diabetes

1. Introduction

Sialic acid is the terminal residue of the oligosaccharide side chain of glycoproteins and widely occurs in the exposed positions of molecules like hormones, enzymes and also on tissues [1]. Because of the electronegative charge, sialic acid is involved in capillary permeability, platelet aggregation, activity of enzymes, as anti-

genic determinants and as essential components of receptors [2].

Elevated levels of serum sialic acid are considered to be a good predictor of cardiovascular disease [3,4]. Asian Indians are reported to have increased levels of acute phase proteins in the diabetic state itself, even before the occurrence of any specific vascular complications [5]. Sialic acid is a constituent of acute phase reactants which have been associated with microvascular sequelae in diabetes [6,7]. Earlier studies have reported an association between serum sialic acid levels and

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diabetic nephropathy [8] but there are very little data on the association of sialic acid with diabetic retinopathy. This study reports on sialic acid concentrations in diabetic patients with and without retinopathy.

2. Research design and methods

Type 2 diabetic patients were recruited from the M.V. Diabetes Specialities Centre (MVDSC) at Chennai in Southern India. The following groups of subjects were studied:

Group 1: Twenty non-diabetic healthy controls were selected from the staff of MVDSC. They were matched for age and sex with diabetic patients.

Group 2: Comprised of 20 Type 2 diabetic subjects without any retinopathy. Type 2 diabetes was diagnosed according to the Report of the Expert Committee on the Diagnosis and Classification of Diabetes [9].

Group 3: Consisted of 20 Type 2 diabetic subjects with non-proliferative diabetic retinopathy (NPDR).

Group 4: Comprised of 20 Type 2 diabetic subjects with proliferative diabetic retinopathy (PDR).

Retinopathy was assessed by direct and indirect ophthalmoscopy and documented by colour photography. A modified version of the ETDRS grading system [10] was used to grade the photographs. Non-proliferative diabetic retinopathy was diagnosed if microaneurisms, dot haemorrhages, exudates or venous changes were present in any field. Proliferative retinopathy was diagnosed if new vessels were present on the disk or elsewhere on the retina.

Of the 60 diabetic patients, 15 patients were on oral hypoglycaemic drugs, 20 patients had once daily insulin along with oral hypoglycaemic drugs and the rest took insulin twice a day to keep their blood sugar under control. None of the study subjects had macroproteinuria as determined by dipstick analysis or ischaemic heart disease i.e. absence of angina or a history of myocardial infarction, normal resting 12-lead electrocardiograms (Minnesota Coded) and normal cardiac

stress tests using Bruce Protocol. None of the study groups had any other concurrent illness or acute infection.

Informed consent was obtained from all study subjects. Clinical examination included recording of height and weight with calculation of body mass index (BMI). Blood pressure was recorded in the sitting posture on the right hand. A fasting blood sample was taken, the plasma was separated and used for measuring fasting plasma glucose with a commercial kit (Boehringer Mannheim, Germany) on Opera Technicon Auto Analyser (Bayer). Serum was separated and total serum cholesterol and triglycerides were assayed with a commercial kit (Boehringer Mannheim, Germany) on the Opera Technicon Auto Analyser (Bayer Diagnostics). Glycosylated haemoglobin (HbA_{1c}) was measured on a dedicated HPLC system (Variant, Bio Rad, CA). A fasting serum sample was stored at -20°C until the serum sialic acid assays were performed. Total serum sialic acid was measured by enzymatic method using *Boehringer Mannheim Kit* (Mannheim, Germany). In brief, the glycoprotein bound sialic acid was hydrolysed by neuraminidase to release free sialic acid. This was followed by the cleavage of sialic acid by a second enzyme *N*-acetyl neuraminic acid aldolase to pyruvate. Oxidation of pyruvate in the presence of MgCl_2 , FAD and thiamine pyrophosphate by pyruvate oxidase yields hydrogen peroxide. H_2O_2 can then be determined colorimetrically by peroxidase in the presence of 4-amino antipyrine and *N*-ethyl N_2 hydroxy ethyl 1-3 toluidine [11]. The interassay coefficient of variation was 7.7% and the intra-assay coefficient of variation was 2.3%.

2.1. Statistical methods

Non-parametric tests were used to test significance. Kruskal–Wallis one-way ANOVA was employed to test the overall significance between the four study groups. If there was a significant difference between groups by Kruskal–Wallis test, then Mann–Whitney *U*-test was employed to identify the group of individuals in which the mean values were different, after adjusting for multiple comparison tests by Bonferroni's method of correction.

Table 1
Clinical characteristics of the study groups

Body mass index (kg/m ²)	24.3 (15.3–26.9)	24.3 (15.3–26.9)	24.3 (15.3–26.9)	24.3 (15.3–26.9)
Parameter	Controls	Type 2 diabetic patients		
	Group 1	Group 2, No DR	Group 3, NPDR	Group 4, PDR
Sex ratio (M/F)	10/10	10/10	10/10	10/10
Age* (years)	45 (27–66)	53 (45–65)	54 (44–63)	55 (42–70)
Body mass index (kg/m ²)	24.3 (15.3–26.9)	25.0 (19.6–28.6)	22.8 (15.3–28.0)	24.1 (18.7–30.9)
Duration of DM (years)	—	12 (6–18)	12 (5–21)	10 (5–28)
Systolic BP** (mmHg)	130 (110–160)	130 (120–160)	130 (120–160)	140 (130–180)
Diastolic BP (mmHg)	80 (74–90)	80 (60–94)	82 (70–100)	82 (70–100)

Data are expressed as median (range); DR, diabetic retinopathy; NPDR, non-proliferative diabetic retinopathy; PDR, proliferative diabetic retinopathy.

* Group 1 is significantly different from the other groups ($P < 0.05$).

** Group 4 is significantly different from the other groups ($P < 0.05$).

3. Results

Table 1 presents the clinical features of the study groups. The diabetic patients were older than the controls. No significant differences were seen either in the body mass index or duration of diabetes between the different diabetic groups, but the systolic blood pressures were higher in the diabetic patients.

Table 2 represents the biochemical parameters of the study groups. There were no significant differences in the fasting plasma glucose or glycosylated haemoglobin levels between the diabetic groups.

There were no significant differences in the sialic acid levels between the Type 2 diabetic patients without retinopathy compared to the patients with NPDR or PDR or indeed with the control groups.

4. Discussion

Circulating sialic acid levels have clinical significance as a biochemical marker of malignancy [12] and as a predictor of cardiovascular mortality in the general population [13]. This probably reflects the increase in acute phase proteins in response to the inflammatory process. Atherosclerosis is also associated with cytokine-induced acute phase response [14]. Diabetic nephropathy has also been

reported to increase acute phase proteins and in turn increase serum sialic acid levels [15]. NIDDM per se may also induce acute phase disease and increase haptoglobin and C Reactive Protein (CRP) [16]. It has been reported that there is an increase in several acute phase serum proteins like haptoglobin, and α -1 acid glycoprotein and α -1 antitrypsin in diabetic subjects with microvascular sequelae [6].

There are few studies on the association between sialic acid and diabetic retinopathy and the available data has produced conflicting results. A report by Crook et al. [17] suggests that sialic acid levels may be elevated in Type 2 diabetic patients with retinopathy. However the study included only nine patients with retinopathy of which six had non-proliferative diabetic retinopathy, three had maculopathy and none had proliferative diabetic retinopathy. Again, while total sialic acid levels were elevated in their patients with retinopathy, the lipid associated sialic acid levels were not. A recent report by Vijay et al. [18] showed that sialic acid levels had no association with diabetic retinopathy. However their study included patients with nephropathy and in the latter group, sialic acid levels were found to be elevated.

In the present study, where we excluded patients with clinical evidence of nephropathy and ischaemic heart disease, we found that total serum sialic acid concentrations did not differ in diabetic

Table 2

Biochemical characteristics of the study groups

Parameter	Controls		Type 2 diabetic patients	
	Group 1	Group 2, No DR	Group 3, NPDR	Group 4, PDR
FPG* (mmol/l)	5.10 (4.0–5.6)	10.6 (6.2–18.8)	9.3 (3.7–19.2)	8.4 (4.7–17.0)
HbA _{1c} * (%)	5.9 (4.9–6.4)	8.9 (5.1–12.5)	9.1 (5.7–13.4)	7.8 (5.6–12.4)
Serum cholesterol (mmol/l)	5.0 (3.2–6.3)	5.4 (4.0–9.6)	5.4 (3.3–7.0)	5.4 (3.1–7.3)
Serum triglycerides** (mmol/l)	1.3 (0.7–2.3)	2.0 (1.0–5.0)	1.5 (0.7–5.4)	1.6 (0.6–5.4)
Sialic acid (mmol/l)	2.1 (1.7–3.4)	2.5 (1.5–3.7)	2.2 (1.7–3.8)	2.3 (1.3–3.9)

Data are expressed as median (range); FPG, fasting plasma glucose; HbA_{1c}, glycosylated haemoglobin; DR, diabetic retinopathy; NPDR, non-proliferative diabetic retinopathy; PDR, proliferative diabetic retinopathy.

* Group 1 is significantly different from the other groups ($P < 0.05$).

** Groups 2 and 4 are significantly different from Group 1 ($P < 0.05$).

patients with or without diabetic retinopathy. Our earlier report [7] on acute phase proteins in diabetic retinopathy revealed elevated levels of haptoglobulin without any increase in α -1 antitrypsin or α -1 acid glycoprotein. It is known that α -1 antitrypsin and α -1 acid glycoprotein are believed to contain high concentrations of sialic acid [8]. These findings suggest that diabetic retinopathy appears to be related more to haptoglobulin and C Reactive Protein rather than α -1 antitrypsin and α -1 acid glycoprotein, and this may explain the lack of association with sialic acid.

Finally since Asian Indians already have higher sialic acid levels [5], it is possible that there is no further elevation in those with microangiopathy.

In summary, there seems to be no association between serum sialic acid levels and diabetic retinopathy in the South Indian Type 2 diabetic patients studied by us.

Acknowledgements

We thank K. Karkuzhali of M.V. Diabetes Specialities Centre, for her help in recruiting patients for the study and A.K. Mathai for the statistical analysis. We are also grateful to Boehringer Mannheim (Mannheim, Germany) for supplying the kits used for estimation of sialic acid.

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