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INSULIN DEPENDENT DIABETES MELLITUS;
CORRELATION BETWEEN SIALIC ACID AND DIABETIC RETINOPATHY

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ABSTRACT... Aims & Objectives: To test the hypothesis that an increased plasma concentration of sialic acid, a marker of the acute-phase response, is related to the presence of diabetic retinopathy in type 1 diabetes mellitus or Insulin Dependant Diabetes Mellitus (IDDM). **Research Design and Methods:** We investigated the relationship between plasma sialic acid concentration and diabetic retinopathy in a cross-sectional survey of 1,369 people with type 1 diabetes. Subjects were participants in the IDDM Complications Study, which involved diabetic centers of four different hospitals in Lahore. **Results:** There was a significantly increasing trend of plasma sialic acid with severity of retinopathy ($P < 0.001$ in men) and with degree of urinary albumin excretion ($P < 0.001$ men, $P < 0.01$ women). Elevated plasma sialic acid concentrations were also associated with several risk factors for diabetic vascular disease: diabetes duration, HbAlc, plasma triglyceride and cholesterol concentrations, waist-to-hip ratio, hypertension and smoking (in men), and low physical exercise (in women). In multiple logistic regression analysis, plasma sialic acid was independently related to proliferative retinopathy and urinary albumin excretion rate in men. **Conclusions:** We concluded that an elevated plasma sialic concentration is strongly related to the presence of microvascular complications in type 1 diabetes with retinopathy and nephropathy. Further study of acute-phase response markers and mediators as indicators or predictors of diabetic microvascular complications is therefore justified.

INTRODUCTION

The serum or plasma sialic acid (N-acetyl neuraminic acid) concentration is a marker of the acute-phase response, since many of the acute-phase proteins (e.g., α1-acid glycoprotein, fibrinogen, and haptoglobin) are glycoproteins, with sialic acid as the terminal sugar of the oligosaccharide chain[1]. An elevated serum sialic acid level is a strong predictor of cardiovascular mortality in the general population[2]. In type 2 diabetes, the circulating sialic acid concentration is elevated in comparison with non-diabetic subjects[3]specially in those with the metabolic syndrome[4]. This has led to the hypothesis that a cytokine-induced acute-phase response is an integral part of the pathophysiology of this type of diabetes[4,5].

In type 1 diabetes, however, serum sialic acid concentrations are not elevated in subjects without tissue complications, in comparison with non-diabetic subjects[3]. But in studies of relatively small numbers of type 1 diabetic subjects, circulating sialic acid concentrations have been found to increase progressively with the presence of microalbuminuria and clinical (dip-stick positive) proteinuria[6,7]. This finding suggests that sialic acid concentrations in the blood may be a useful marker of diabetic complications, but there have been no large-scale studies examining the link between sialic acid and complications in type 1 diabetes.

In the present study, we performed a clinical-based survey on the hypothesis that an increased plasma sialic

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acid concentration is associated with an increased prevalence of micro-and/or macrovascular disease in type 1 diabetic subjects, using the patients of type 1 diabetes and its complications attending diabetic centers of four different hospitals (Shaikh Zayed Hospital, Jinah Hospital, Sir Ganga Ram Hospital and Services Hospital) in Lahore, Pakistan.

RESEARCH DESIGN AND METHODS

Protocol

This Study is a cross-sectional survey of 3,250 people (51% male) with type 1 diabetes from 04 diabetic centers in Lahore. A random sample of all clinic attendees aged 15-60 years in one calendar year, stratified by age, sex, and diabetes duration, was taken. Their mean ± SD age was 32.7 ± 10.0 years and their mean diabetes duration was 14.7 ± 9.3 years. Type 1 diabetes was defined as diabetes diagnosed before the age of 40 years with continued need for insulin from within 1 year of diagnosis. Pregnant women, patients who were unrepresentative of local ethnic groups, and those with diabetes for <1 year were not recruited. Blood samples were available from 1,369 subjects for analysis of sialic acid and lipid cardiovascular risk factors. Venous samples were collected after an overnight fast and plasma was separated by centrifugation at 1,500 rpm for 10 min at ambient temperature. Aliquots of plasma and urine were stored at –20 ˚C at each center until transported to the Shaikh Zayed Hospital laboratory in ice box.

Height and weight were measured using a standard stadiometer and calibrated beam balance after the removal of heavy outer garments and shoes. The BMI (kg/m^2) was calculated using the formula $weight/height^2$. The waist circumference was measured at the midway level between the coastal margins and the iliac crests, and the hip circumference was measured at the level of the greater trochanter (if not palpable, the largest gluteal circumference was recorded). The waist-to-hip ratio was calculated. The frequency of mild physical activity (e.g., walking and general housework), moderate physical activity (e.g., leisurely swimming or cycling), and vigorous physical activity (e.g., hard swimming, running or playing games like hockey, football, tennis, etc.) was recorded, as were current and past smoking habits. Retinopathy was assessed from photographs of two retinal fields per eye, graded by a single observer.

Assays

Total plasma sialic acid was assayed using an enzymatic method (Boehringer Mannheim, Pak), adapted for use on a centrifugal analyzer (Cobas Bio; Roche, Pak)[8,11]. The between-batch coefficient of variation (CV) of this assay was 3.8%. Urinary albumin excretion rate (AER) was determined by an immunoturbimetric method using goat anti—human albumin antiserum and human albumin standards on a timed 24-h urine collection, after exclusion of urinary tract infection[9]. Microalbuminuria was defined as a urinary AER of 20-200 μg/min. HbAlc (reference range 2.9-4.8%) was also assayed in the laboratory using an enzyme immunoassay[10] with monoclonal anti—HbAlc antibody (Dako & Ely) and plasma creatinine was assayed by the Jaffé reaction (Boehringer Mannheim, Pak). The between-batch CV for these assays was <3%[5].

Statistical analysis

Sex differences between demographic and clinical characteristics were assessed using the t-test for continuous variables and the X^2 test for categorical variables. Analysis of covariance was used to calculate means adjusted for HbA_{lc} and diabetes duration and to assess differences between trends in adjusted means. To examine whether sialic acid was independently related to proliferative retinopathy, we used logistic regression. Variables entered into the initial model were plasma sialic acid, HbA_{lc}, diabetes duration, smoking status, exercise, BMI, waist-to-hip ratio, hypertension status and creatinine concentrations. Only factors with $P<0.05$ are displayed in the results table. Standardized relative risks were calculated for a continuous variable as the relative risk of the complication associated with an increase of 1 SD. For a categorical variable, it was the risk of the complication associated with the presence of the risk factor relative to the risk when it was absent.

To assess whether sialic acid was independently related to AER, we used linear regression. The variables that were entered into the initial model were those used for the logistic regression, and only factors with P<0.05 are displayed in the results table. Standardized regression coefficients were calculated for a continuous variable as the change in the complication with an increase of 1 SD. For a categorical variable, it was the change in the complication associated with the presence of the risk factor compared with that when it was absent. Because of the skewed distribution of AER and creatinine, these variables were log-transformed for statistical analysis.

RESULTS

The patient demographics and their clinical and biochemical features are shown in Table-I. The plasma sialic acid concentration was higher in female subjects than in male subjects with type 1 diabetes (P<0.001).

Tables II and III show the relationship between plasma sia lic acid concentration and risk factors for diabetic complications. Sialic acid was significantly associated with diabetes duration (correlation coefficient 0.10 [P < 0.001]) and with HbAlc (correlation coefficient 0.20 [P < 0.001]). After adjusting for diabetes duration and HbAlc, we found that plasma sialic acid was also significantly related to plasma triglyceride and cholesterol concentrations, waist-to-hip ratio, lack of exercise (women), and presence of smoking and hypertension (men). The women with the highest BMI also had high plasma sialic acid levels.

Table IV shows the relationships between plasma sialic acid concentrations and diabetic complications: nephropathy (as marked by AER and plasma creatinine concentration) and retinopathy. After adjusting for diabetes duration and HbAlc, we found the plasma sialic acid concentration to be significantly higher in men with retinopathy than in those without this complication, being greatest in those with proliferative retinopathy.

Table-I. Demography, clinical, and biochemical features of patients.

	Men	Women
N	737	632
Age (years)	32.5 ± 10.1	33.3 ± 10.5
Diabetes duration (years)	15.0 ± 9.5	16.0 ± 9.2
MBI (kg/m^2)	23.5 ± 27	23.7 ± 3.4
HbA_{10} (%)	6.7 ± 1.9	6.8 ± 1.9
Waist-to-hip ratio	0.89 ± 0.09	0.81 ± 0.13
Systolic PB (mmHg)	124.2 ± 17.6	119.8 ± 19.0
Diastolic BP (mmHg)	76.7 ± 11.7	73.9 ± 11.4
Total cholesterol (mmol/l)	5.2 ± 1.2	5.4 ± 1.1
HDL cholesterol (mmol/l)	1.4 ± 0.4	1.6 ± 0.4
Triglyceride (mmol/l)	1.1 (0.22 - 12.64)	1.0 (0.38 - 6.53)
Creatinine (µmol/l)	69.7 (6-451)	62.6 (15.5 - 496)
Sialic acid (mmol/l)	1.90 ± 0.39	2.02 ± 0.38*
Hypertension (%)	17	14
Ex-smoker (%)	20	14
Current smoker (%)	35	27
Retinopathy (%)	54	52
Neuropathy (%)	22	19
Microalbuminuria (%)	30	24
Macroalbuminuria (%)	11	11
CHD (%)	9	13

*Data are means ± SD or geometric means (range), unless otherwise indicated * P <0.001. BP, blood pressure*

Table-II. Plasma sialic acid (mmol/l) adjusted for HbA$_{Ic}$ and diabetes duration according to smoking habit, exercise, BMI, waist-to-hip ratio and hypertension

	Men	Women
Smoking status		
Non smoker	1.85 (1.81 - 1.89)	2.02 (1.98 - 2.06)
Ex-smoker	1.93 (1.87 - 1.99)	1.99 (1.92 - 2.07)
Current smoker	1.95 (1.90 - 1.99)	2.03 (1.97 - 2.08)
P value for trend	0.005	0.7
Exercise		
Vigorous weekly	1.87 (1.83 - 1.91)	1.97 (1.91 - 2.04)
Moderate weekly	1.90 (1.85 - 1.95)	2.06 (2.01 - 2.10)
Mild weekly	1.96 (1.90 - 2.01)	1.98 (1.94 - 2.03
Mild less than once a week	2.00 (1.85 - 2.16)	2.21 (2.05 - 2.36)
P value for trend	0.06	0.006
BMI		
< 21.5	1.90 (1.85 - 1.95)	1.97 (1.92 - 2.03)
21.6 - 23.3	1.89 (1.84 - 1.95)	2.03 (1.98 - 2.09)
23.4 - 25.1	1.87 (1.81 - 1.92)	1.98 (1.93 - 2.04)
> 25.2	1.94 (1.91 - 1.99)	2.08 (2.02 - 2.14)
P value for trend	0.4	0.04
Waist-to-hip ration		
< 0.83	1.85 (1.80 - 1.91)	1.97 (1.91 - 2.03)
0.84 - 0.87	1.83 (1.78 - 1.88)	2.00 (1.95 - 2.06)
0.88 - 0.92	1.94 (1.89 - 1.99)	2.04 (1.98 - 2.10)
> 0.93	1.98 (1.92 - 2.03)	2.06 (2.00 - 2.12)
P value for trend	0.0004	0.2
Hypertension		
Absent	1.88 (1.85 - 1.91)	2.01 (1.98 - 2.04)
Present	2.04 (1.97 - 2.10)	2.09 (2.00 - 2.17)
P value for difference	0.0001	0.1

*Data are means (95% CI) unless otherwise indicated *P<0.03, *P<0.01 (vs nonsmokers) *P <0.05, *P<0.01 (vs vigorous weekly exercise) : P<0.03, P<0.01 (vs lower quartile of BMI or waist-to-hip ratio).*

Plasma sialic acid was also higher in men and women with macroalbuminuria, compared with those with normoalbuminuria, and was highest in those with the highest plasma creatinine levels. Sialic acid was significantly higher in those male subjects with diabetic neuropathy than in those without this complication.

Table-III. Plasma sialic acid (mmol/l) adjusted for diabetes duration and HbA$_{Ic}$ according to plasma lipid concentration

	Men	Women
Total cholesterol (mmol/l)		
< 4.56	1.85 (1.80 - 1.89)	1.91 (1.85 - 1.98)
4.57 - 5.23	1.86 (1.81 - 1.92)	2.00 (1.94 - 2.05)
5.24 - 5.95	1.94 (1.88 - 1.99)*	2.06 (2.01 - 2.12)
>5.96	1.98 (1.92 - 2.04)	2.09 (2.03 - 2.14)
P value for trend	0.003	0.0004
HDL cholesterol (mmol/l)		
< 1.17	1.94 (1.89 - 1.99)	2.03 (1.96 - 2.11)
1.18 - 1.42	1.89 (1.83 - 1.94)	2.06 (1.99 - 2.12)
1.43 - 1.68	1.85 (1.79 - 1.91)*	2.00 (1.95 - 2.06)
> 1.69	1.90 (1.83 - 1.97)	2.01 (1.96 - 2.06)
P value for trend	0.1	0.5
Triglyceride (mmol/l)		
< 0.73	1.78 (1.72 - 1.85)	1.89 (1.83 - 1.96)
0.74 - 0.94	1.84 (1.77 - 1.91)	2.03 (1.96 - 2.09)
0.95 - 1.35	1.93 (1.87 - 1.98)	2.06 (1.98 - 2.13)
> 1.36	2.04 (1.98 - 2.10)	2.14 (2.06 - 2.22)
P value for trend	0.0001	0.0001

*Data are means (95% CI) unless otherwise indicated * P < 0.05, *P <0.001, *P <0.01 (all vs lower quartile*

Table-IV. Plasma sialic acid (mmol/l) adjusted for diabetes and HbAlc according to the presence of diabetic complications

	Men	Women
Retinopathy		
None	1.85 (1.80 - 1.89)	1.98 (1.93 - 2.03)
Background	1.91 (1.97 - 1.96)	2.05 (2.00 - 2.10)
Proliferative	2.09 (2.00 - 2.18)	2.09 (2.00 - 2.18)
P value for trend	0.0001	0.1
AER (µg/min)		
<20	1.86 (1.82 - 1.89)	1.99 (1.95 - 2.03)
20-200	1.90 (1.86 - 1.95)	2.05 (1.99 - 2.10)
>200	2.13 (2.05 - 2.21)	2.15 (2.06 - 2.24)
P value for trend	0.0001	0.004
Plasma creatinine(µmol/l)		
<57	1.95 (1.89 - 2.01)	2.03 (1.98 - 1.95)
58-65	1.87 (1.81 - 1.93)	1.98 (1.93 - 2.04)
66-75	1.83 (1.78 - 1.88)	1.93 (1.87 - 2.00)
>76	1.93 (1.88 - 1.98)	2.15 (2.07 - 2.22)
P value for trend	0.009	0.0002
Neuropathy		
Absent	1.88 (1.85 - 1.91)	2.00 (1.97 - 2.04)
Present	1.98 (1.92 - 2.04)	2.05 (1.98 - 2.11)
P value for difference	0.006	0.3
CHD		
Absent	1.89 (1.87 - 1.92)	2.02 (1.99 - 2.05)
Present	1.95 (1.86 - 2.04)	2.00 (1.92 - 2.08)
P value for trend	0.3	0.7

*Data are means (95% CI) unless otherwise indicated *P < 0.05 (vs no retinopathy), *P<0.001 (vs no retinopathy), *P <0.001 (vs AER <20 µg/min), *P <0.01 (vs AER <20 ug/min), P<0.01 (vs lower quartile), <0.05 (vs lower quartile)*

Tables V and VI show, the outcome of linear regression analysis of risk factors for AER, and logistic regression analysis for proliferative retinopathy. Plasma sialic acid was independently related to diabetic retinopathy in men (standardized relative risk 1.8) but not in women, and also independently related to AER in men but not in women.

Table-V. Logistic regression of risk factors for diabetes complications and linear regression of risk factors for AER in men.

Risk factor	Standardized relative risk (95% CI)	P
Proliferative retinopathy		
Diabetes duration	3.4 (2.1 to 5.4)	0.0001
Plasma sialic acid	1.8 (1.2 to 2.7)	0.008
Hypertension	7.4 (2.5 to 22.1)	0.0003
HDL cholesterol	0.5 (0.3 to 0.8)	0.008
Neuropathy		
HbA	1.4 (1.1 to 1.8)	0.01
Diabetes duration	1.5 (1.2 to 2.0)	0.0008
Waist-to-hip ratio	1.3 (1.0 to 1.6)	0.04
Triglyceride	1.2 (0.9 to 1.7)	0.03
CHD		
Diabetes duration	1.4 (1.0 to 1.8)	0.05
Hypertension	2.2 (1.0 to 4.9)	0.05
Plasma creatinine	0.7 (0.5 to 1.0)	0.03
HDL cholesterol	0.7 (0.5 to 1.0)	0.04
AER		
HbA	0.17 (0.04 to 0.30)	0.01
Plasma sialic acid	0.22 (0.10 to 0.34)	0.0006
Hypertension	1.08 (0.73 to 1.44)	0.0001
BMI	-0.15 (-0.27 to -0.02)	0.03
Total cholesterol	0.19 (0.04 to 0.33)	0.02
Plasma creatinine	0.27 (0.15 to 0.39)	0.0001
Triglyceride	0.25 (0.10 - 0.40)	0.002

Table-VI. Logistic regression of risk factors for diabetes complications and linear regression of risk factors for AER in women.

Risk factor	Standardized relative risk (95% CI)	P
Proliferative retinopathy		
HbA	1.6 (1.1 to 2.5)	0.03
Diabetes duration	4.1 (2.5 to 6.7)	0.0001
HDL cholesterol	0.6 (0.4 to 1.0)	0.05
BMI	0.6 (0.4 to 1.0)	0.05
Neuropathy		
Diabetes duration	2.2 (1.6 to 3.0)	0.0001
CHD		
BMI	1.5 (1.1 to 2.0)	0.008
Total cholesterol	0.6 (0.4 to 0.9)	0.006
AER		
HbA	0.20 (0.06 to 0.35)	0.007
Hypertension	1.25 (0.83 to 1.67)	0.0001
BMI	-0.17 (-0.31 to 0.03)	0.02
Plasma creatinine	0.41 (0.27 to 0.56)	0.0001

DISCUSSION

The main finding of this study is that elevated plasma sialic acid concentrations were associated with the presence of microvascular complications in this large group of type 1 diabetic subjects who attended diabetic center, Sir Ganga Ram Hospital. Significant associations were seen for retinopathy (men only), nephropathy, as indicated by urinary AER and plasma creatinine concentration, and neuropathy (in men only). Whereas in another study, we also established a significant relationship of total serum sialic acid (TSSA) concentrations with duration of diabetes mellitus and the degrees of retinal involvement[11]. However, in this study, we also found that plasma sialic acid concentration was significantly associated with several known risk factors for the development of diabetic micro- and macrovascular disease, i.e., diabetes duration, glycemic control (HbAlc), hyperlipidemia, waist-to-hip ratio, hypertension and smoking (men), and low level of physical exercise (women).

In this study, the association between sialic acid and HbAlc and diabetes duration most likely follows from the association between sialic acid and microvascular complications, which are well established to be related to glycemic control and diabetes duration. However, there was no association between plasma sialic acid concentration and CHD, as has been found previously in the general population[2,12,13] and in men with type 2 diabetes[14]. But this finding may be related to the young age of the patients (mean 32.5 years in the men, 33.3 years in the women) and the low prevalence of CHD (9% in men, 12% in women) in the present study.

A relationship between serum or plasma sialic acid levels and microvascular complications has been observed before in small-scale studies for microalbuminuria and clinical proteinuria in type 1[6,7] and type 2 diabetes[15], and for retinopathy in type 1[16] and type 2 diabetes[17]. Whereas, inflammatory markers have been related to the development of diabetes in adults[18] and in our previous study we also concluded that, increased total sialic acid (TSA) levels were associated with a previous diagnosis of GDM, with hyperglycemia and other elements of the metabolic syndrome[19]. An association with neuropathy has not been reported before. Whereas, links between sialic acid and risk factors for vascular disease, such as lipids[20,21], smoking[22], hyperfibrinogenemia[23,24], and lipoprotein(a)[25], have also been reported.

Plasma sialic acid is a marker of the acute-phase response[1,4], acute-phase glycoproteins with sialic acid as a component of the oligosaccharide side chain being produced by the liver, stimulated by pro-inflammatory cytokines such as interleukin-1, interleukin-6, and tumor necrosis factor-α[26,27]. However, higher TSA levels may also reflect increased levels of pro-inflammatory cytokines from adipose or other tissues[28], unrelated to exogenous infectious stimuli. The fact that correlations were slightly stronger for the 2-h than for the fasting values in our study could reflect that TSA elevation results, in part, from postprandial events such as cytokine release from adipose tissues[29] or even hyperglycemia[30,31].

Therefore, the two most likely explanations for the present findings are either or both of the following: 1) Tissue injury caused by diabetic vascular complications stimulates local cytokine secretion from cells involved in the complications such as endothelium and macrophages, which are known to be major sources of cytokine production[27], and this induces an acute-phase response. 2) The diabetic process stimulates cytokine production from cells throughout the body, and these cytokines play a direct role in the causation of vascular complications. The latter is supported, for example, by evidence that pro-inflammatory cytokines cause endothelial dysfunction by increasing capillary permeability, inducing prothrombotic properties, and promoting leukocyte recruitment by synthesis of adhesion molecules and chemoattractants[32,33], and play a role in macroangiopathy by promoting dyslipidemia[34]. The realization that microalbuminuria is a nonspecific marker of inflammation in the general population[35] further suggests that cytokinemia from a variety of causes leads to microvascular abnormalities. There is need for early predictors of diabetic complications such as nephropathy. Some patients with microalbuminuria, for example, have quite advanced renal structural changes, and microalbuminuria may here be a marker of microvascular damage that has already occurred. If circulating sialic acid increases before microangiopathy develops[36], it may be an early signal of processes such as hypercytokinemia that cause or drastically increase the risk of vasculopathy.

Plasma sialic acid is protein bound (to acute-phase proteins), with negligible free sialic acid in the circulation in either nondiabetic or diabetic subjects[37]. It is thus unlikely that increased circulating sialic acid is the result of desialylation of cell components and lipoprotein particles. There is evidence; however, that sialic acid is reduced in the endothelium in atherosclerosis[38] and in LDL[39] and erythrocytes[40] in diabetes, which may have pathophysiological significance in promoting vascular disease.

Our finding that plasma sialic acid concentrations were significantly elevated in women compared with men with type 1 diabetes confirms a sex difference that have also been found in type 2 diabetes$_{41}$. There is apparently no sex difference in serum sialic acid concentrations in nondiabetic subjects[42]. The significance of this sex difference is not clear, but one speculation is that a higher acute-phase response in women with diabetes may reflect the fact that women with diabetes lose the protection from cardiovascular disease enjoyed by nondiabetic women[43]. For several complications, the association with elevated plasma sialic acid concentrations was significant in men but not in women (retinopathy, neuropathy, and hypertension), even though the prevalence of complications and the baseline characteristics were similar in the sexes. Also, sialic acid emerged as an independent risk factor for AER in linear regression analysis in only the men[44]. Interestingly, it was found in a cross-sectional study of type 2 diabetic subjects[14] that, serum sialic acid concentration was related to CHD in men but not in women. In another group of type 2 diabetic subjects[41], serum sialic acid was significantly higher in men with diabetic complications than in those without, but in women it was similar in those with or without complications. The higher sialic acid in women in the present study probably similarly weakens the association with complications. It is not clear to us why plasma sialic acid is elevated in women with diabetes. Though we believe it likely that the association between sialic acid and complications is through the acute-phase response, we measured only one sample for sialic acid per subject, and caution must be exercised in inferring a cause-effect relationship between complications and the acute-phase reaction. One limitation of our study is that we did not measure circulating pro-inflammatory cytokines; had we done so, links between elevated cytokine concentrations and end organ damage, which we postulate, would be considerably strengthened.

CONCLUSIONS

In conclusion, we have found a significant association between plasma sialic acid concentrations and the presence of microvascular complications in a cross-sectional study of type 1 diabetes. It will now be important to investigate prospectively the relationship between sialic acid and/or other markers and mediators of the acute-phase response (e.g., pro-inflammatory cytokines) and the development or progression of diabetic microangiopathy. Such studies may lead to indicators of those individuals at risk of developing tissue complications.

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