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β-N-Acetylglucosaminidase and β-Glucuronidase Activities in Insulin-dependent Diabetic Subjects with Retinopathy

E. Pitkänen, M. Kyllästinen, T. Koivula, and P. Hormila[1]

Fourth and [1]Third Department of Medicine, University Central Hospital, Helsinki, Finland

Summary. The serum activities of two lysosomal enzymes, β-N-acetylglucosaminidase (EC 3.2.1.30, NAG) and β-glucuronidase (EC 3.2.1.31, GLU), were determined in 41 insulin-dependent diabetics, 27 age-matched non-diabetic first-degree relatives of the diabetics and 103 age-matched non-diabetic blood-donors. The diabetics were divided into three groups on the basis of ophthalmoscopy: (1) no retinal abnormalities; (2) non-proliferative retinopathy; and (3) proliferative retinopathy. The activities of both serum enzymes were higher in diabetics (NAG 21.39 ± 5.99; GLU 2.19 ± 1.01) than in their relatives (NAG 17.22 ± 3.99; GLU 1.62 ± 0.61). The diabetics with non-proliferative retinopathy had higher serum enzyme levels (NAG 24.05 ± 6.26; GLU 2.60 ± 1.06) than diabetics without retinopathy (NAG 17.88 ± 3.00; GLU 1.69 ± 0.64), whereas no statistically significant difference was found in patients with the proliferative form of retinopathy (NAG 18.67 ± 6.28; GLU 1.99 ± 1.04). In diabetics a positive correlation was found between serum β-N-acetylglucosaminidase activity and blood glucose ($p < 0.01$), but not between β-glucuronidase and blood glucose. Furthermore, the activities of both enzymes in diabetics correlated with the plasma triglyceride level ($p < 0.05$ for both correlations). No correlation was found between the enzyme levels and signs of other diabetic late complications.

Key words: β-N-acetylglucosaminidase, β-glucuronidase, lysosomal enzymes, blood serum, insulin-dependent diabetes, diabetic retinopathy, background retinopathy, proliferative retinopathy, blood glucose, serum triglyceride.

Activities of the lysosomal enzymes β-N-acetylglucosaminidase (EC 3.2.1.30, NAG) and β-glucuronidase (EC 3.2.1.31, GLU) have been shown to be elevated in the sera of diabetics [2, 4, 6, 7, 11, 18, 25] and a close correlation between the enzyme activities and the blood glucose level of diabetics has been documented [2, 4, 5, 25]. The serum lysosomal enzyme changes may also be associated with the pathogenesis of microangiopathy [2, 4, 11], although the relation is not yet clear and more data are needed. In the present study, serum NAG and GLU have been determined in patients with insulin-dependent diabetes of long duration and with different degrees of diabetic retinopathy and the levels compared with those in two non-diabetic reference groups of subjects.

Materials and Methods

Details of the patients are shown in Table 1. Forty-one diabetic subjects, (21 men and 20 women), aged 40 ± 6 years, who had been treated with insulin for 10 years or more were studied. The patients were taken from the register of the local diabetes association and from health centres in Helsinki. The patients were ambulatory and capable of carrying out normal daily activities. Three patients were handicapped by a severe loss of vision. The diabetics were studied after pupillary dilatation with an ophthalmoscope by one of the investigators. The presence of microaneurysms, haemorrhages, exudates, and new vessels was taken into account. Fluorescein angiography was performed on those patients who showed marked retinal changes by ophthalmoscopy. The diabetics were divided into three groups on the basis of retinal vascular changes: those without any retinal changes, those with non-proliferative changes (one or more of the following changes: microaneurysms, haemorrhages, exudates), and those with proliferative retinopathy (neovascularization). The absence of knee and/or ankle reflexes and the absence of vibration sense tested on the middle part of the tibia were regarded as signs of neuropathy. Nephropathy was presumed following the detection and semi-quantitative determination of proteinuria with Labstix (Ames Company, England). The serum creatinine concentration was also determined.

The investigation included two different control groups. The first consisted of 27 non-diabetic relatives (siblings) of the diabetics, 11 men and 16 women (Table 1). The second involved determination of the activities of serum NAG and GLU in non-diabetic blood donors (Finnish Red Cross). The group consisted of 45 male and 58 female subjects (mean age 36 ± 6 years).

Three diabetics with normal retinae, two diabetics with non-proliferative retinal changes, two patients with proliferative retinal

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Table 1. Clinical characteristics of the diabetics and their relatives (Mean ± SD)

	Diabetics			Relatives
	without retinopathy	with non-proliferative retinopathy	with proliferative retinopathy	
n	13	22	6	27
Age (years)	42 ± 8	40 ± 6	35 ± 5[a, d]	42 ± 5
Duration of diabetes (years)	16 ± 6	21 ± 6	23 ± 7[a]	–
Daily insulin dose (units)	39 ± 11	49 ± 17	41 ± 17	–
% ideal body weight	103 ± 12	106 ± 10	99 ± 11[c]	111 ± 11
Systolic blood pressure	125 ± 9	138 ± 14[b, d]	134 ± 22	125 ± 14
Diastolic blood pressure	85 ± 9	92 ± 11	90 ± 11	85 ± 10

The values in diabetics with non-proliferative or proliferative retinopathy are compared with those in diabetics without retinopathy (A) and with those in their relatives (B). Statistical significance of the differences: [a] and [c] $p < 0.05$; [b] and [d] $p < 0.01$

Table 2. Activities of serum β-N-acetylglucosaminidase and β-glucuronidase in diabetic patients, their relatives and non-diabetic blood donors (Mean ± SD)

	Diabetics (A)	Relatives (B)	Non-diabetic blood donors (C)	Significance of the difference between		
				A and B	A and C	B and C
S-β-N-acetyl-glucosaminidase	21.39 ± 5.99	17.22 ± 3.99	19.93 ± 5.19	< 0.005	NS	< 0.005
S-β-glucuronidase	2.19 ± 1.01	1.62 ± 0.61	1.74 ± 0.69	< 0.01	< 0.025	NS

changes and one of the relatives were receiving antihypertensive drugs. There was no history of liver disease in the diabetics or in the relatives. One patient with non-proliferative retinopathy had a history of glaucoma and recurrent urinary tract infection.

The body weight of the subjects is expressed as a percentage of the "ideal body weight", which is taken as the midpoint of the ideal body weight range for subjects of medium build from the Metropolitan Life Insurance Company Tables [24].

Venous blood samples were taken from the diabetics and their relatives in the forenoon after overnight fasting for the determination of serum NAG, GLU, triglycerides and cholesterol. At the same time capillary blood samples were taken for whole blood glucose determination. Venous blood samples (without anticoagulant) were also taken from the blood donors in the forenoon, at the beginning of the blood donation. The samples were brought for enzyme determination with a delay not exceeding two hours. Routine laboratory methods were used for the determination of serum triglyceride [16], cholesterol [14], serum creatinine (Technicon, USA, Autoanalyzer method M-11b) and blood glucose (Kabi, Sweden, Glox novum, Autoanalyzer II) concentrations.

Serum NAG was assayed according to the method of Levvy and Conchie [17]. The activity of serum GLU was determined by the method of Fishman [9]. The substrates of the enzymes, p-nitrophenyl-β-D-glucuronide and p-nitrophenyl-2-acetamido-2-deoxy-β-D-glucopyranoside, were obtained from Koch-Light Laboratories, Colnbrook, England. The amount of p-nitrophenol liberated was measured with a system Olli 3000 Analyzer (Kone Oy, Espoo, Finland). The results of the enzyme activities are expressed as substrate hydrolysis rate (μmol/min) at 37 °C. The within-run coefficients of variation, estimated from the differences of duplicates were 3.6 per cent (NAG) and 7.8 per cent (GLU). The day-to-day variations were 6.0 per cent (NAG) and 6.2 per cent (GLU). After separation from the blood cells it was possible to store the samples at +4 °C overnight and at −20 °C for at least one month without loss of enzyme activity. The statistical significance of the differences was calculated using the non-parametric Mann Whitney test.

Results

Data for male and female subjects (Table 1) have been combined because no significant differences were found between males and females. The diabetic subjects with proliferative retinopathy were younger than both the diabetics without retinopathy and their relatives. The duration of diabetes was longer in diabetics with retinal changes than in patients with normal retinae. Systolic blood pressure was higher in the diabetic group with non-proliferative retinopathy than in diabetics without retinopathy or their relatives.

The serum NAG and GLU activities in the diabetics and their relatives are shown in Table 2. The values of both enzymes in diabetics exceeded those in their relatives. There was also a statistically significant difference in the GLU values between diabetics and blood donors. The NAG values, but not the GLU values, were significantly higher in blood donors than in the relatives of diabetics.

The NAG and GLU activities in diabetics with different retinal findings are shown in Table 3. The serum activities of both enzymes were higher in diabetic patients with non-proliferative retinopathy than in diabetics without retinal changes ($p < 0.05$ for both). The difference was statistically more significant in women ($p < 0.01$). No differences in enzyme activities were detected between diabetics without retinopathy and diabetics with proliferative retinopathy.

The blood glucose, serum triglyceride and serum cholesterol values in the diabetics and in their relatives are shown in Table 4. The mean blood glucose level was similar in diabetics with no retinopathy and with non-proliferative retinopathy. The serum triglyceride and cholesterol concentrations were higher in diabetics with non-proliferative retinopathy than in patients with normal retinae.

In diabetic subjects, there was a positive correlation between serum NAG activity and blood glucose level ($p < 0.01$; $r = 0.42$), but not between GLU and blood glucose. Furthermore, the activities of both NAG and GLU correlated with the concentration of plasma triglycerides in diabetics (NAG: $p < 0.05$, $r = 0.36$; GLU: $p < 0.05$, $r = 0.40$), but not with the plasma cholesterol level.

There were more signs of other late diabetic sequelae in diabetic patients with than in those without retinal abnormalities. However, when the diabetics were divided into groups on the basis of the presence or absence of the renal or neural complications, no differences were found in the serum NAG or GLU activities between those groups.

Discussion

The present results are in accordance with those of earlier studies, which have shown an elevation of serum GLU [4, 8, 25] and NAG [2, 6, 11, 25] in patients with diabetes mellitus. Further, in the present study the levels of the two lysosomal enzymes in the diabetics were significantly higher in subjects with non-proliferative retinopathy ($p < 0.05$ for both enzymes). In previous studies an association has been reported between serum GLU [4] and NAG [2] and the presence of microangiopathy and large vessel lesions in diabetic subjects, and also between serum NAG level and retinopathy [11].

In contrast to the finding in patients with non-proliferative retinopathy, the changes in lysosomal enzyme levels in the group with the proliferative form were slight. However, the group comprised only six patients. The mean age, weight and several other data of the group also differed from those of the other groups in the study.

A close correlation has been observed between the serum levels of both GLU and NAG and the level of blood glucose [5, 25] in diabetics. Very high enzyme levels have been encountered in diabetics with a severely deranged metabolic balance [2, 4, 10]. Although a similar correlation was also detected in the present study, all of the patients were clinically in fairly good glycaemic control and the blood glucose level was similar in the groups with and without non-proliferative retinopathy. This suggests that the difference in serum enzyme levels was not due to a difference in the degree of derangement of carbohydrate metabolism at the time the blood sample was collected.

A larger difference was seen between the diabetic patients and their close non-diabetic relatives (NAG: $p < 0.005$; GLU: $p < 0.01$) than between the patients and non-diabetic blood donors (NAG: NS; GLU: $p < 0.025$), although the groups were matched for age and sex. The marked influence of various physiological factors on the reference values of serum enzymes [21, 22] has been pointed out previously. Fasting is one of the factors which have been shown to alter the level of lysosomal enzymes in rat liver [8]. The fact that the samples from the blood donors were not taken after overnight fasting may have had an influence on the difference between the two reference groups.

Table 3. Activities of serum β-N-acetylglucosaminidase and β-glucuronidase in relation to retinopathy in diabetic patients (Mean ± SD)

	No retinopathy	Retinopathy	
		non-proliferative	pro-liferative
S-β-N-acetyl-glucosaminidase	17.88±3.00	24.05±6.26[a]	18.67±6.28
S-β-glucuronidase	1.69±0.64	2.60±1.06[a]	1.99±1.04

Statistical significance of the difference between groups with retinopathy vs. no retinopathy [a] $p < 0.05$

Table 4. Blood glucose, serum triglyceride and cholesterol levels in diabetic subjects and their relatives

	Diabetics			Relatives
	no retinopathy	with non-proliferative retinopathy	with proliferative retinopathy	
Blood glucose (mmol/l)	9.5 ± 5.1	9.8 ± 4.1	12.2 ± 6.6	4.1 ± 0.4
Serum triglycerides (mmol/l)	0.91 ± 0.27	1.49 ± 0.80[a]	1.18 ± 0.56	1.02 ± 0.50
Serum cholesterol (mmol/l)	6.1 ± 1.2	7.4 ± 1.7[a, b]	6.4 ± 2.1	6.0 ± 1.1

The results are means ± SD

The triglyceride and cholesterol values in diabetics with non-proliferative or proliferative retinopathy are compared with those in diabetics without retinopathy (A) and with those in the relatives (B). Statistical significance of the differences: [a] $p < 0.05$; [b] $p < 0.01$

A positive correlation was found between the serum triglyceride concentration and the activities of lysosomal enzymes in diabetics. Further, both serum triglyceride and cholesterol levels were significantly higher in diabetics with non-proliferative retinopathy than in patients without retinal changes. This latter finding may, of course, indicate poorer pre-existing metabolic control of diabetics with non-proliferative retinopathy. On the other hand, an association between serum triglyceride and cholesterol elevation and large vessel disease in diabetic subjects has been noted [20]. In non-diabetics the increased level of GLU has been associated with both coronary artery disease [19] and a response in the glucose tolerance test [12] in atherosclerotic persons. Also, an association between increased serum NAG activity and atherosclerosis has been noted [3]. The possibility that large vessel disease may contribute to the increase in enzyme levels in our diabetic subjects cannot be excluded.

The changes in the activity of lysosomal enzymes in tissues may play an essential role in the retention of high molecular weight glycoproteins in the vascular bed in diabetes [5]. This has been further substantiated by the observation of a marked decrease in lysosomal enzymes in rat kidney in experimental diabetes [10, 15], while serum enzyme levels were increased. Another possible mechanism for the elevation of lysosomal enzymes is afforded by the increase in the prevalence of soluble immune complexes, which is reported to occur in diabetic subjects, especially if microangiopathy is present [1]. It is of interest in this context that an association has been reported between the levels of soluble immune complexes and NAG in serum [13] in systemic lupus erythematosus. On the other hand, the possibility that the elevation of lysosomal enzymes was due to liver enzyme induction cannot be excluded. Data obtained from studies on experimental diabetes [23] suggest that the liver may be the source of the increase in serum lysosomal enzyme levels.

The present results demonstrate a close correlation between non-proliferative retinopathy and serum levels of lysosomal enzymes. This is consistent with the postulation that the enzymes are somehow involved in the process leading to diabetic microangiopathy.

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Dr. E. Pitkänen
Laboratory, Fourth Department of Medicine
University Central Hospital
Unioninkatu 38
SF-00170 Helsinki 17, Finland