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Association of reduced glyoxalase 1 activity and painful peripheral diabetic neuropathy in type 1 and 2 diabetes mellitus patients ☆,☆☆

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ABSTRACT

Aims: The present study was undertaken to investigate the relationship between glyoxalase 1 (Glo1) enzyme activity and painful diabetic neuropathy (DN) in patients with diabetes mellitus.
Methods: Glo1 activity and biochemical markers were determined in blood samples from 108 patients with type 1 diabetes, 109 patients with type 2 diabetes, and 132 individuals without diabetes as a control. Painful and painless peripheral DN was assessed and multivariate regression analysis was used to determine independent association of Glo1 activity with occurrence of painful DN.
Results: In patients with type 1 and type 2 diabetes mellitus and painful DN compared to patients with painless DN, Glo1 activity was significantly reduced by 12 and 14%, respectively. The increase in Glo1 activity was significantly associated with reduced occurrence of painful DN after adjusting for confounders by multivariate analysis.
Conclusions: Our results demonstrate for the first time that Glo1 activity is lower in patients with both types of diabetes mellitus who were diagnosed with painful DN. These data support the hypothesis that Glo1 activity modulates the phenotype of DN and warrant further investigation into the role of Glo1 in DN.

1. Introduction

Diabetic complications are classically associated with a prolonged exposure to high glucose concentrations, thus establishing the duration and severity of hyperglycemia as the key determinants of hyperglycemia-induced tissue damage (The Diabetes Control & Complications Trial Research Group, 1993; UK Prospective Diabetes Study (UKPDS) Group, 1998). Peripheral neuropathy is the most common and intractable microvascular complication of diabetes (Boulton et al., 2005), but serious limitations exist in the current knowledge regarding the causes of neuropathic pain. Although exact pathophysiological mechanisms are unknown, interaction of both metabolic and vascular factors has been proposed to play a role in the pathogenesis of the painful symptoms (Tesfaye & Selvarajah, 2012). Several hyperglycemia-related biochemical pathways investigated in experimental diabetic models such as increased oxidative-nitrosative stress and aldose reductase activity (Dobretsov, Hastings, Romanovsky, Stimers, & Zhang, 2003; Obrosova et al., 2007), activation of protein kinase C (Cotter, Jack, & Cameron, 2002) and poly(ADP-ribose) polymerase (PARP) (Obrosova et al., 2008), as well as increased proinflammatory response (Ramos, Jiang, Svensson, & Calcutt, 2007) have been implicated in neuropathic hyperalgesia and abnormal sensation. The role of increased aldose reductase activity and enhanced oxidative stress in pathogenesis of peripheral neuropathy has been confirmed in clinical trials (Hotta et al., 2004; Sharma & Sharma, 2008; Ziegler, Nowak, Kempler, Vargha, & Low, 2004). Moreover these biochemical changes lead to impaired skin microvascular reactivity, increased peripheral nerve epineurial blood flow and autonomic dysfunction recognized in patients with painful diabetic neuropathy (Eaton et al., 2003; Quattrini, Harris, Malik, & Tesfaye, 2007; Selvarajah et al., 2008). The molecular events behind the hyperglycemia-induced neuronal dysfunction are extensively

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investigated to discover new pathways that may prevent or relieve the DN.

Among other molecular mechanisms capable of stimulating or delaying the development of microvascular complications, the metabolism of reactive dicarbonyls (such as methylglyoxal, glyoxal and other alpha-oxoaldehydes) by the glyoxalase enzyme system is one of the most studied (Rabbani & Thornalley, 2012; Thornalley, 2008). Glyoxalase 1 (Glo1) (EC 4.4.1.5) and glyoxalase 2 (Glo2) (EC 3.1.2.6) enzymes present an enzymatic defense system against glycation that suppresses glycation-mediated cell damage (Thornalley, 1998; Thornalley, 2008). Altered Glo1 activity is associated with late diabetic complications (McLellan, Thornalley, Benn, & Sonksen, 1994; Ratliff, Vander Jagt, Eaton, & Vander Jagt, 1996; Thornalley et al., 1989). It has been shown experimentally that the increased formation of methylglyoxal in diabetes-associated hyperglycaemia leads to a 2–4 fold increase in modifications of proteins by methylglyoxal to form AGEs at the sites of vascular complications (Duran-Jimenez et al., 2009; Karachalias, Babaei-Jadidi, Rabbani, & Thornalley, 2010). Recently, Bierhaus with colleagues found that methylglyoxal plasma levels were increased in type 2 diabetes mellitus patients with painful diabetic neuropathy. Further experiments demonstrated that methylglyoxal induces primary hyperalgesia in animal models and could be the cause of pain in patients with diabetes mellitus (Bierhaus et al., 2012). A recent study showed that the expression of *GLO1* varies in the peptidergic sensory neurons from two inbred strains of mice that display significant differences in mechanical algesia, suggesting that Glo1 directly contributes to the phenotype of neuropathy (Jack, Ryals, & Wright, 2011). However, there are limited data regarding the association of peripheral DN with glyoxalase enzyme activities and the data concerning the activities of glyoxalase enzymes in DM patient samples are inconsistent (McLellan et al., 1994; Thornalley et al., 1989; Thornalley, McLellan, Lo, Benn, & Sonksen, 1996). The present study was undertaken to investigate the relationship between Glo1 activity and painful peripheral DN in patients with type 1 and type 2 diabetes mellitus. We hypothesised that decreased Glo1 activity could be associated with the painful phenotype of DN.

2. Materials and methods

2.1. Subjects

The study included patients with type 1 (n = 108) and type 2 (n = 109) diabetes mellitus diagnosed according to the American Diabetes Association (2007) and treated at the Riga East Clinical University Hospital, Clinic "Gailezers", Riga, Latvia. For a control group, 132 non-diabetic individuals were recruited. All control participants and patients were Caucasians. Exclusion criteria were: chronic renal failure (GFR<60 ml/min), chronic liver disease, an active inflammatory disorder (a white blood cell count>8.5×10^3/mm^3, an erythrocyte sedimentation rate>20 mm/h, or high-sensitivity C-reactive protein (hs-CRP)>15 mg/l because high levels may indicate a latent chronic inflammatory condition), anemia, current evidence or a history of malignancy in the last 5 years, a history of drug or alcohol abuse and causes of neuropathy other than diabetes. Data collected at study entry included age, medical history, age at diagnosis, medication, smoking history and alcohol intake. The BMI was calculated and late complications of diabetes mellitus were assessed. A smoker was defined as anyone who presented a history of smoking. The study was carried out in accordance with the Declaration of Helsinki after approval by the Central Medical Ethics Committee of Latvia (Resolution A-9, 29th May 2006). A written informed consent was obtained from all subjects.

2.2. Biochemical assays

Venous blood samples were obtained from all subjects in the morning after an overnight fast and collected in EDTA-containing tubes (VACUETTE® EDTA K3). Plasma total cholesterol, LDL-cholesterol, HDL-cholesterol and triglycerides were measured shortly after sampling directly with a commercially available kit by enzymatic methods in an accredited diagnostic laboratory of the hospital. Plasma glucose levels were assessed using the hexokinase method (Cobas 6000, Roche Diagnostics, Mannheim, Germany), HbA_{1c} levels were measured by high-pressure liquid chromatography (Primus Corporation, Kansas City, USA), hs-CRP tests were performed quantitatively by the immunoturbidimetry method (Cobas Integra 700, Roche Diagnostics, Mannheim, Germany) and C-peptide levels were measured by the electrochemiluminescence immunoassay (ECLIA, Roche Diagnostics, Mannheim, Germany).

2.3. Assay of Glo1 activity in blood samples

Glo1 activity was determined in whole blood samples on the same day that the blood was collected according to a previously described method (Skapare et al., 2011; Thornalley, 1988). Briefly, Glo1 activity was determined spectrophotometrically (μQuant™, BioTek, Winooski, VT, USA) by measuring the formation of *S*-D-lactoylglutathione from hemithioacetal in hemolysates at 240 nm for 5 min in a 96-well UV-transparent plate (Greiner Bio-One, Germany). The blood samples

Table 1
The demographic and clinical characteristics of the control and groups of patients with diabetes mellitus.

	Control	Type 1 diabetes mellitus	Type 2 diabetes mellitus
n	132	108	109
Age, years	42.0 (33.0–52.8)	34.5 (27.0–50.0)	58.0 (51.5–64.0)
Male sex, *n* (%)	50 (38)	52 (48)	44 (40)
Duration of diabetes, years	–	11.0 (6.0–21.0)	7.0 (4.0–13.8)
Fasting plasma glucose, mmol/L	5.0 (4.7–5.4)	8.6 (6.6–11.6)	7.8 (6.9–9.6)
HbA_{1C}, %	5.1 (4.9–5.3)	8.6 (7.7–10.3)	7.9 (7.2–9.9)
BMI, kg/m^2	24.9 (23.1–28.7)	24.7 (22.4–27.0)	31.6 (28.4–36.1)
CRP, mg/L	0.9 (0.5–1.7)	2.2 (1.2–3.3)	2.0 (1.1–3.7)
Total cholesterol, mmol/L	5.4 (4.8–6.1)	5.5 (4.6–6.1)	5.8 (5.1–6.4)
Triglycerides, mmol/L	1.2 (0.8–1.8)	1.1 (0.9–1.8)	2.1 (1.6–3.2)
HDL-cholesterol, mmol/L	1.4 (1.2–1.9)	1.5 (1.3–2.0)	1.3 (1.0–1.6)
LDL-cholesterol, mmol/L	3.7 (3.0–4.3)	3.4 (2.6–3.9)	3.4 (2.8–4.2)
C-peptide, ng/mL	2.1 (1.7–3.2)	0.4 (0.2–0.5)	2.8 (1.8–4.1)
Haemolysate Glo1 activity, U/g Hb	162.7 (146.7–187.3)	178.1 (151.9–210.7)	182.1 (148.4–219.2)
Current smoker, *n* (%)	27 (21)	46 (43)	27 (25)
Alcohol intake (≥1 consumptions/week), *n* (%)	28 (22)	12 (11)	6 (6)
Statin therapy, *n* (%)	13 (10)	29 (27)	63 (58)
ACE-inhibitor/AT1 receptor antagonist therapy, *n* (%)	21 (17)	53 (50)	86 (79)

HbA_{1C} – glycated haemoglobin; BMI – body mass index; CRP – C-reactive protein; Glo1 – glyoxalase 1; Values are presented as medians (interquartile ranges) or *n* (%).

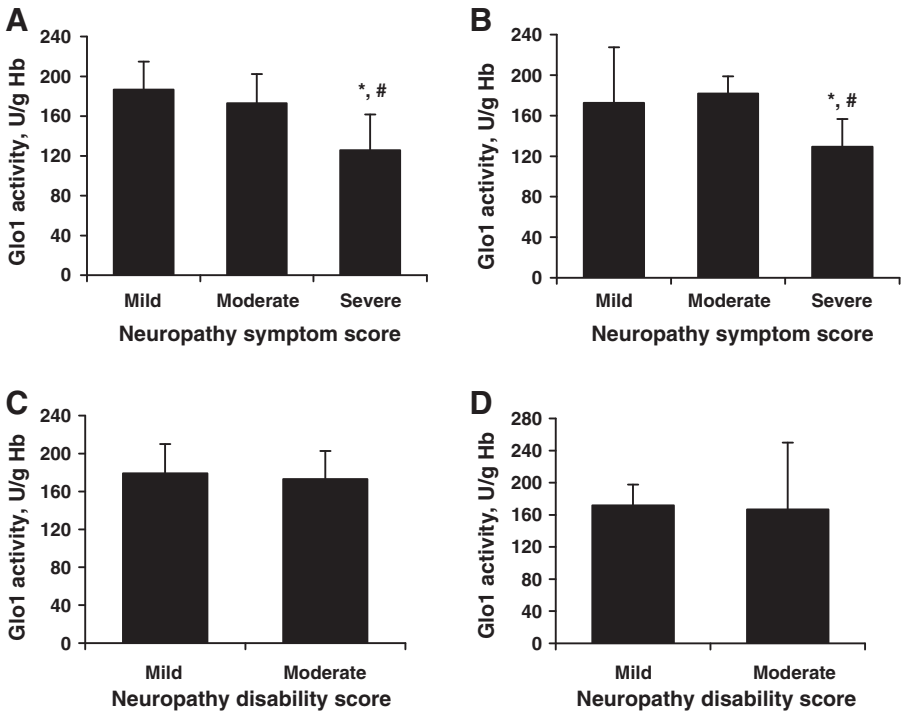

Fig. 1. The association of haemolysate's Glo1 activity with severity of neuropathy. The association of haemolysate's Glo1 activity with severity of neuropathy symptom score (NSS) (A, B) and neuropathy disability score (NDS) (C, B) in type 1 ($n=88$) (A, C) and type 2 ($n=74$) (B, D) diabetes mellitus patients. Data are presented as medians (interquartile ranges) of Glo1 activity in diabetic patients characterized by their level of NSS (mild (1–4), moderate (4–5), severe (7–9)) or NDS (mild (3–4), moderate (5–8)). *Significantly different from mild NSS symptoms (a one way ANOVA with Dunn's Multiple Comparison post-hoc test, $p<0.05$). #Significantly different from moderate NSS symptoms (a one way ANOVA with Dunn's Multiple Comparison post-hoc test, $p<0.05$).

were lysed 1:40 (v/v) in ice-cold water for 10 min, and after centrifugation at 20000×g at +4°C for 10 min, the supernatant was used for the determination of hemoglobin and glyoxalase activity. The final assay mixture in the 96-well plate contained 20 μl of lysate and 180 μl of hemithioacetal solution. The hemithioacetal was prepared fresh for each measurement by pre-incubating 1.5 mmol/L methylglyoxal (Sigma-Aldrich, Germany, Cat.No.M0252) and 2 mmol/L reduced glutathione (Sigma-Aldrich, Germany, ≥98%, Cat.No.G4251) in 50 mmol/L sodium phosphate buffer, pH 6.6, for 10 min at +37 °C immediately before the use. The enzyme activity was determined in four parallel measurements for each blood sample and calculated using the change in extinction coefficient Δ ε240 = 2.84. The Glo1 activity was expressed in μmol of *S*-D-lactoylglutathione formed per min per g of hemoglobin (U/g Hb).

2.4. Evaluation of peripheral DN

Assessment of peripheral neuropathy was evaluated using the neuropathy disability score (NDS) that included tests for Achilles tendon reflexes, vibration perception, pain perception, and

Table 2
The clinical characteristics of patients with diabetes mellitus with painless and painful diabetic neuropathy (DN).

	Type 1 diabetes mellitus			Type 2 diabetes mellitus		
	Painless DN	Painful DN	*p* value	Painless DN	Painful DN	*p* value
n	43	51		45	48	
Age, years	32.0 (24.0–47.0)	45.0 (29.0–57.0)	0.03	56.0 (50.5–62.0)	59.0 (54.2–68.0)	0.03
Male sex, *n* (%)	21 (49)	24 (47)	ns	20 (44)	15 (31)	ns
Duration of diabetes, years	10.5 (6.0–16.5)	19.0 (7.0–26.0)	0.01	5.5 (3.0–11.0)	11.0 (7.0–17.7)	<0.0001
Haemolysate Glo1 activity, U/g Hb	188.4 (165.6–222.7)	165.0 (131.3–199.8)	0.01	186.1 (148.4–228.4)	166.7 (137.0–199.8)	0.04
Fasting plasma glucose, mmol/L	7.8 (6.1–10.4)	8.9 (7.0–11.5)	ns	7.8 (6.9–9.6)	8.1 (7.0–9.6)	ns
HbA_{1C}, %	8.4 (7.6–10.4)	9.1 (7.8–10.0)	ns	8.2 (7.1–11.2)	7.8 (7.3–9.0)	ns
BMI, kg/m^2	24.0 (21.3–25.2)	25.8 (22.6–28.4)	0.01	31.9 (28.3–37.4)	31.2 (28.1–34.0)	ns
CRP, mg/L	2.4 (1.0–3.2)	2.3 (1.2–3.3)	ns	2.0 (1–3.7)	1.8 (1.1–3.5)	ns
Total cholesterol, mmol/L	5.5 (4.4–6.4)	5.6 (4.7–6.0)	ns	5.7 (4.9–6.4)	5.9 (5.2–6.5)	ns
Triglycerides, mmol/L	1.2 (0.9–1.9)	1.1 (0.9–1.9)	ns	2.1 (1.5–2.7)	2.1 (1.6–3.4)	ns
HDL-cholesterol, mmol/L	1.5 (1.3–1.9)	1.6 (1.3–2.1)	ns	1.2 (1–1.6)	1.4 (1.1–1.7)	ns
LDL-cholesterol, mmol/L	3.2 (2.7–4.1)	3.5 (2.6–3.9)	ns	3.4 (3.0–4.2)	3.4 (2.4–4.2)	ns
C-peptide, ng/mL	0.5 (0.2–0.5)	0.3 (0.1–0.5)	ns	2.9 (2–4.1)	2.3 (1.4–4.5)	ns
Current smoker, *n* (%)	17 (40)	24 (47)	ns	12 (27)	7 (15)	ns
Alcohol intake (≥1 cons./month), *n* (%)	7 (16)	4 (8)	ns	4 (9)	1 (2)	ns
Statin therapy, *n* (%)	8 (19)	17 (35)	ns	24 (53)	29 (60)	ns
ACE-inhibitor/AT1 receptor antagonist therapy, *n* (%)	22 (51)	28 (56)	ns	39 (87)	38 (79)	ns
NDS, score	4.0 (3.0–4.0)	4.0 (3.0–4.0)	ns	4.0 (3.0–4.0)	4.0 (3.3–4.0)	ns

HbA_{1C} – glycated haemoglobin; BMI – body mass index; CRP – C-reactive protein; Glo1 – glyoxalase 1; NDS – neuropathy disability score; Values are presented as medians (interquartile ranges) or *n* (%).

temperature threshold on both feet and a neuropathy symptom score (NSS) that included anamnestic questions concerning the quality and localization of symptoms, any exacerbation during the night or improvements by physical activity, as described previously by Young et al. (Young, Boulton, MacLeod, Williams, & Sonksen, 1993). An NSS of four or more points was considered to be abnormal and a diagnosis of painful DN was made. Standardized current perception threshold (CPT) measurements using a constant alternating current sinusoid waveform stimulus at 5, 250, and 2,000 Hz were obtained using a Neurometer current perception threshold device (Neurotron Inc, Baltimore, USA). The diagnosis of peripheral neuropathy was made when at least two of the three tests (NSS, NDS and CPT) were abnormal. The severity of symptoms was graded according to the NSS as follows: mild (1–4), moderate (5–6), and severe (7–9). The severity of NDS was graded as mild (3–4) and moderate (5–8).

2.5. Statistical analyses

Results are expressed as a median (interquartile ranges) or count (%). Differences in the continuous variables between the studied groups were tested using a Student's t or a Mann Whitney U-test according to the specific indications, whereas a χ^2 test was used to compare categorical data. Spearman's correlation analysis was used to examine the relationship of Glo1 activity with the measured variables from all groups. To compare Glo1 activity within different groups of patients according to severity of DN, a one-way ANOVA with the Dunn's Multiple Comparison post-hoc test was performed. A multivariate regression analysis was used to determine independent association of Glo1 activity with painful DN after an adjustment for variables associated with painful DN in a univariate analysis, such as age, the duration of diabetes and BMI. p values of <0.05 were considered statistically significant. The data were analysed using the SPSS 19.0 statistical software (SPSS, Chicago, IL, USA).

3. Results

The demographic and clinical characteristics of the participants are presented in Table 1. As seen in the table, patients with type 2 diabetes mellitus were older, smoked less, had significantly higher BMIs, triglycerides and fasting C-peptide levels, and they received anti-hypertension therapy more often compared to patients with type 1 diabetes mellitus (Table 1). The average Glo1 activity in patients with type 1 and type 2 diabetes mellitus was 178.1 (IQR 151.9–210.7) and 182.1 (IQR 148.4–219.2) U/g Hb, respectively. The Glo1 activity in the blood of non-diabetic individuals was 162.7 (IQR 146.7–187.3) U/g Hb and was significantly different from diabetic patients.

Univariate correlation analysis of the study data showed that the haemolysate's Glo1 activity negatively correlated with the duration of diabetes ($r=-0.261$, $p=0.007$) in patients with type 1 diabetes mellitus (data not shown). Glo1 activity was significantly reduced in patients with severe painful neuropathy symptoms compared to mild or moderate NSS in both diabetes mellitus groups (Fig. 1A and B). Glo1 activity did not differ between diabetic patients with mild and moderate NDS (Fig. 1C and D). No correlations of Glo1 activity with age, gender, glucose levels, HbA_{1C}, CRP results, lipid levels, BMI, alcohol consumption, smoking or the use of medications in both types of diabetes mellitus were found (data not shown). In the control group, there were no significant associations of Glo1 with the different clinical variables.

Because NSS showed the strongest relationship with Glo1 activity in univariate analysis, we compared patients with painless or painful DN. Patients with painless and painful neuropathy had similar degree of nerve dysfunction reflected by NDS score (Table 2). In patients with type 1 and type 2 diabetes mellitus and painful DN compared to patients with painless DN in groups, the Glo1 activity was significantly reduced by 12 and 14%, respectively (Table 2). As shown in Table 2, patients with type 1 and type 2 diabetes mellitus and painful DN were significantly older by 19 and 7%, respectively, and had about a 2-fold longer duration of diabetes. Patients with painful DN had a slightly increased BMI (by 10%) in the type 1 diabetes mellitus group. There were no significant differences between patients with diabetes mellitus and painless or painful DN for other parameters (Table 2). The increase in Glo1 activity per one unit was significantly associated with reduced occurrence of painful DN after adjusting for age, the duration of diabetes and BMI by multivariate regression analysis (Table 3). In patients with type 2 diabetes mellitus, the increase in duration of diabetes was significantly associated with increased occurrence of painful DN (ExpB = 1.12 [1.04–1.21], p = 0.003). There was no significant association of occurrence of painful DN with age in both diabetes mellitus groups (Table 3).

Table 3
A multivariate regression analysis of association between the occurrence of painful DN and Glo1 activity after an adjustment for other risk factors.

	ExpB (95% CI)	p value
Type 1 diabetes mellitus		
Haemolysate Glo1 activity, U/g Hb	0.93 (0.87–1.00)	0.04
Duration of diabetes, years	1.04 (0.99–1.10)	ns
Age, years	1.00 (0.97–1.04)	ns
BMI, kg/m^2	1.11 (0.96–1.28)	ns
Type 2 diabetes mellitus		
Haemolysate Glo1 activity, U/g Hb	0.92 (0.86–0.98)	0.01
Duration of diabetes, years	1.12 (1.04–1.21)	0.003
Age, years	1.02 (0.97–1.07)	ns

DN – diabetic neuropathy; BMI – body mass index; Glo1 – glyoxalase 1; Values are presented as odds ratio (95% confidence interval).

4. Discussion

The pathophysiology of painful DN is not well understood with regard to the complexity of its aetiology and manifestations. The present study is the first to investigate the association of Glo1 activity in patients with painful DN and phenotypical factors by analyzing the largest amount of clinical material thus far (187 patients with diabetes mellitus with peripheral DN and 132 control individuals without diabetes). Our results demonstrate that decreased Glo1 enzyme activity in haemolysates from both type 1 and type 2 diabetes mellitus patients is associated with painful DN. The multivariate regression analysis, which was adjusted for confounders, showed an inverse significant association of Glo1 activity with occurrence of painful DN, independent of age, diabetes duration and BMI. In our study, we assessed both NSS and NDS as measures of subjective pain perception and objective neuropathic deficits (Doupis et al., 2009; McIntosh et al., 2003). Glo1 activity was significantly reduced in patients with severe painful neuropathy symptoms for both type 1 and type 2 diabetes mellitus patients, but was not different between different NDS groups. Previous studies in patients with painful neuropathy found elevated CRP values, which suggested that inflammatory processes and endothelial dysfunction are linked to the peripheral sensitization resulting in pain sensation and DN (Doupis et al., 2009; Herder et al., 2009). The results of the present study however did not confirm any correlations with CRP. This difference could be explained by the slightly elevated CRP values in both the painful and painless DN groups due to poor metabolic control, which had been associated with an elevated CRP in both type 1 (Jenkins et al., 2008) and type 2 (Arnalich et al., 2000) diabetes mellitus. It should be pointed out that the pathogenesis of type 2 diabetes mellitus is considerably heterogeneous and both glycation stress and inflammation-related processes could be cooperatively driving forces in the development of late complications of diabetes mellitus.

In the present study, the haemolysate Glo1 activity in patients with diabetes mellitus was increased compared to healthy individuals. The previous investigations of Glo1 activity in blood samples from patients with diabetes mellitus have provided inconsistent results (McLellan et al., 1994; Thornalley et al., 1989). Interestingly, studies that included patients with diabetes mellitus with poor metabolic control found an association between a higher Glo1 activity and the development of microvascular complications but no correlation between Glo1 activity and HbA_{1C} (McLellan et al., 1994; Ratliff et al., 1996). Thus, the authors of the latter study concluded that glucose control was not a factor in the level of Glo1 expression (Ratliff et al., 1996). This is substantiated by the results presented here, since the relationships of glucose, HbA_{1C} and lipid levels with activity of Glo1 were not observed. There was no significant association of Glo1 activity with other clinical variables in healthy controls or patients with type 2 diabetes mellitus. Although age-dependent changes in Glo1 activity have been reported (Sharma-Luthra & Kale, 1994; Thornalley, 2003), our study did not find any significant correlation of Glo1 activity with age in the control or diabetes mellitus groups. Instead, our study provides evidence for correlation of decreased Glo1 activity with the duration of diabetes in patients with type 1 diabetes mellitus. However, in multivariate regression analysis Glo1 activities of patients with type 1 and type 2 diabetes mellitus were significantly associated with occurrence of painful DN, independent of age and diabetes duration. The regulatory mechanisms behind this observation will be a topic for future investigations.

As a limitation of the present study it should be noted that the groups of patients with diabetes mellitus and painless or painful DN were not matched for age or the duration of diabetes, therefore the observed differences in Glo1 activity might be additionally influenced by these factors. However, the multivariate regression analysis showed that age and duration of diabetes do not associate with the occurrence of painful DN in both types of diabetes mellitus. Another limitation is too small number for statistical analysis of the participants in diabetes patient subgroups that did not present neuropathy. Although our study shows that the decreased activity of Glo1 is related to an increased pain perception, the molecular mechanisms that are linked to the altered activity of Glo1 deserve further investigations.

In summary, the present study provides evidence that the activity of the Glo1 enzyme is lower in blood samples from patients with both types of diabetes mellitus diagnosed for painful DN. In addition, in patients with type 1 diabetes mellitus, Glo1 activity negatively correlates with the duration of diabetes. These findings support the hypothesis that Glo1 activity modulates the phenotype of DN and warrant further investigation into the role of Glo1 in DN.

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