



A comparison of the levels of hepatocyte growth factor in serum in patients with type 1 diabetes mellitus with different stages of diabetic retinopathy

Ocena stężenia wątrobowego czynnika wzrostu w surowicy krwi u chorych na cukrzycę typu 1 z różnym stopniem rozwoju retinopatii cukrzycowej

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Abstract

Introduction: The aim of this study was to evaluate the blood concentration of hepatocyte growth factor (HGF) in patients at various stages of retinopathy. We hypothesised that the high level of HGF found in diabetic patients may be an important marker of retinopathy progression and that HGF level may be an index of the risk of proliferative retinopathy.

Material and methods: The participants in the study were 76 patients with type 1 diabetes mellitus. Of these, 35 patients were without retinopathy and formed Group 1. Of the remaining 41 patients with retinopathy, 20 patients had non-proliferative diabetic retinopathy (NPDR) and formed Group 2, while 21 patients had proliferative diabetic retinopathy (PDR) and formed Group 3. We evaluated the concentration of HGF in the peripheral blood by an enzyme-linked immunosorbent assay.

Results: Mean serum concentrations of HGF in the control group were significantly lower than in the type 1 diabetic patients. We found a significant increase in HGF serum concentrations in diabetic patients with PDR compared with the control group. Mean serum HGF concentrations were significantly higher in diabetic subjects with PDR than in diabetic patients without retinopathy.

Conclusion: HGF concentration is increased in patients with type 1 diabetes mellitus with proliferative retinopathy, and concentrations increase with the progression of retinopathy, suggesting that HGF plays a role in the pathogenesis of proliferative diabetic retinopathy. (Pol J Endocrinol 2008; 59 (1): 2-5)

Key words: hepatocyte growth factor; type 1 diabetes mellitus; diabetic retinopathy

Streszczenie

Wstęp: Celem pracy była ocena stężenia wątrobowego czynnika wzrostu (HGF, *hepatocyte growth factor*) w surowicy krwi chorych na cukrzycę typu 1 z różnym stopniem rozwoju retinopatii cukrzycowej oraz próba oceny, czy podwyższone stężenie HGF może być biochemicznym markerem oceny progresji retinopatii cukrzycowej.

Materiał i metody: Badania przeprowadzono w grupie 76 chorych na cukrzycę typu 1: 35 osób bez retinopatii — grupa 1 oraz 41 osób ze stwierdzoną klinicznie retinopatią cukrzycową: 20 osób z retinopatią nieproliferacyjną (NPDR, *non-proliferative diabetic retinopathy*) — grupa 2 oraz 21 osób z retinopatią proliferacyjną (PDR *proliferative diabetic retinopathy*) — grupa 3. W surowicy krwi oznaczano stężenie HGF z zastosowaniem metody immunoenzymatycznej.

Wyniki: Stwierdzono znamienne statystycznie wzrost HGF w surowicy krwi chorych z retinopatią proliferacyjną w porównaniu z grupą kontrolną oraz chorymi na cukrzycę bez objawów retinopatii.

Wnioski: U chorych na cukrzycę typu 1 powikłaną rozwojem proliferacyjnej retinopatii cukrzycowej wzrasta stężenie HGF w surowicy krwi. Stężenie HGF wzrasta wraz z progresją retinopatii, sugerując udział HGF w jej patogenezie. (Endokrynol Pol 2008; 59 (1): 2-5)

Słowa kluczowe: wątrobowy czynnik wzrostu; cukrzyca typu 1, retinopatia cukrzycowa

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Introduction

Type 1 diabetes mellitus accounts for only 5–10% of all cases of diabetes but represents an important health problem, since this disorder begins early in life and leads to long-term complications. Diabetic retinopathy is a microvascular complication of diabetes and is closely correlated with chronic long-standing hyperglycaemia [1]. The other pathogenetic risk factors for microvascular damage in diabetes are polyol accumulation, free radical damage and non-enzymatic glycation of several proteins [2, 3].

Medical treatment of diabetic microangiopathy is based on control of glycaemia, lipaemia and blood pressure using glytazones, ACE-inhibitors, angiotensin II receptor antagonists and statins. New knowledge of the pathogenesis of microangiopathy suggests potential drugs for its therapy (ruboxistaurin, AGE-inhibitors, angiopoietin-1 and anti-vascular endothelial growth factor [4, 5].

Diabetes mellitus leads to endothelium dysfunction and an accelerated progression of atherosclerosis. Diabetic retinal neovascularisation is considered to be a consequence of retinal ischaemia caused by capillary occlusion. Capillary occlusion is the result of microvascular thrombi in which erythrocytes, platelets and leucocytes may each play a role. An ischaemic retina acts as a source of production of upregulated growth factor, particularly vascular endothelial growth factor (VEGF) and fibroblast growth factors, thereby inducing new vessel formation in the surrounding tissue [6]. Numerous angiogenic factors have been reported to be involved in development of proliferative diabetic retinopathy (PDR), including insulin-like growth factors, transforming growth factor- β , fibroblast growth factor, tumour necrosis factor and VEGF, as well as hepatocyte growth factor (HGF) [7–10].

Hepatocyte growth factor is produced mainly by the liver, but this immunoreactive peptide can be found in many tissues, including the kidney and lung, in the endothelial cells and the vascular smooth muscle cells [11, 12]. Recent studies have demonstrated that serum levels of HGF are powerful in inducing angiogenesis [13]. Thus it is possible that HGF plays a role in retinal neovascularisation in association with other factors.

The aim of this study was to evaluate the blood concentration of HGF in patients with different stage of retinopathy. We hypothesised that the high level of HGF found in diabetic patients may be an important marker of retinopathy progression and that HGF level may be an index of the risk of proliferative retinopathy.

Material and methods

A total of 76 type 1 diabetic patients (male/female 30/46) aged 20 to 65 years (mean \pm SD 40.7 \pm 11.58) who

had been treated for diabetes for more than 10 years were recruited to the study. After ophthalmological examination the patients were divided into three groups:

- group 1 consisted of 35 patients aged 20 to 57 years (mean age \pm SD 38.94 \pm 12.06 years) without diabetic retinopathy; the mean duration of diabetes was 17.66 \pm 12.2 years and haemoglobin A1C was 6.4% \pm 1.3%;
- group 2 consisted of 20 patients aged from 28 to 62 years (mean age \pm SD 42.17 \pm 11.26) with non-proliferative diabetic retinopathy (NPDR); the mean duration of diabetes was 19.37 \pm 12.05 years haemoglobin A1C was 6.9% \pm 0.9%;
- group 3 consisted of 21 patients aged from 27 to 65 years (mean age \pm SD 43.57 \pm 10.8) with PDR; the mean duration of diabetes was 24.37 \pm 8.05 years and haemoglobin A1C was 6.7% \pm 1.3%.

There was no significant difference between the studied groups in systolic and diastolic blood pressure and in the frequency of antihypertensive drug use.

The eye examination, performed by a specialised ophthalmologist, included visual acuity, biomicroscopy and ophthalmoscopy, using a 90-diopter lens or direct ophthalmoscopy after pupil dilatation, photography or fluorescein angiography of the retina.

Exclusion criteria were duration of diabetes less than ten years, other than diabetes endocrine disease, hepatitis and rheumatological or neoplastic diseases. The control group consisted of 35 non-diabetic age-matched healthy volunteers aged from 30 to 60 years mean age \pm SD 40.3 \pm 9.88 years.

Blood was collected after an overnight fast, and serum was obtained by centrifugation and then stored at -70°C . The concentration of HGF in the serum samples was measured by an enzyme-linked immunosorbent assay (ELISA) for human HGF (R&D Systems), according to the manufacturer's standard protocol. Intra-assay and inter-assay variations were 2.9% and 2.6%, respectively. The statistical analysis was carried out using the "Statistical 5.0 PI" programme with Student's *t* test for normal distribution of the results in the study groups or Mann-Whitney's *U* test in other than normal distribution, assuming the levels $p < 0.05$ as statistically significant.

All subjects had given their formal consent before participating in the study, and the research followed the tenets of the Declaration of Helsinki.

Results

The results of study are presented in Tables I and II. Mean serum concentrations of HGF in the control group were significantly lower than in the type 1 diabetic patients (930.5 \pm 285.8 vs. 1098.2 \pm 273.7 pg/ml, $p < 0.05$)

Table I. Blood concentration of hepatocyte growth factor (HGF) in type 1 diabetes: patients and control group

Tabela I. Stężenie wątrobowego czynnika wzrostu (HGF) w surowicy krwi chorych na cukrzycę typu 1 oraz w grupie kontrolnej

	Means and standard deviation		p (Mann-Whitney U)
	Diabetes type 1 (n=76)	Control (n=35)	
HGFpg/ml	1098.2 ± 273.7	930.5 ± 285.8	p < 0.05

(Tab. I). No significant difference was found between serum HGF concentrations in the control patients and in the diabetic patients without retinopathy and with presence of NPDR. We found a significant increase in HGF serum concentrations in diabetic patients with PDR (Group 3) compared with the control group 1409.3 ± 309.2 vs. 930.5 ± 285.8 pg/ml, $p < 0.005$) (Tab. II). The mean serum HGF concentration was significantly higher in diabetic subjects with PDR than in diabetic patients without retinopathy (1409.3 ± 309.2 vs. 906.5 ± 174.7 pg/ml, $p < 0.05$) and diabetic patients with NPDR (962.9 ± 210.9 vs. 1409.3 ± 309.2 pg/ml, $p < 0.05$) (Tab. II).

Discussion

In the retinas of patients with diabetes the earliest pathophysiological changes include selective loss of capillary pericytes, proliferation of endothelial cells, impairment of retinal autoregulation and, in consequence, failure of the capillary circulation. These changes lead to increased retinal vascular permeability and chronic retinal hypoxia, resulting in retinal neovascularisation. These processes are mediated by various growth factors, such as hepatocyte growth factor. Blood and in-

traocular concentrations of VEGF have been studied [14–16] and the authors concerned have reported increased concentrations of VEGF in PDR patients [14, 15]. This growth factor has a potent angiogenic action and it is expected that increased VEGF would be involved in neovascularisation in PDR. HGF also has an angiogenic action as well as an endothelium-specific growth action and the effect of HGF is reported to be stronger than that of VEGF [17]. This finding supports the idea that HGF, together with VEGF, may be involved in retinal neovascularisation in PDR. HGF, which is identical to scatter factor [18, 19], is a disulfide-linked heterodimeric molecule composed of a 69-kDa kringle-containing α -chain and a 34-kDa β -chain [20, 21]. Although HGF has been well characterised as a hepatotrophic [22, 23] and renotropic factor [24, 25] in liver and kidney regeneration, the presence of the local HGF system (HGF and its receptor c-met) has been demonstrated in both endothelial cells and vascular smooth muscle cells *in vivo* and *in vitro* [26].

Shinoda et al. studied concentrations of HGF and VEGF in the peripheral blood and aqueous fluid of patients with diabetic retinopathy [16]. In contrast to our results these authors found no significant difference in serum concentrations of the growth factors studied between non-diabetic patients, diabetic patients without retinopathy and diabetic patients with different stages of retinopathy (NPDR and PDR). On the other hand, the aqueous HGF level increased with the stage of retinopathy. The aqueous VEGF level in PDR was significantly higher than in non-diabetic patients and diabetic patients without retinopathy. Nishimura et al. reported that the mean HGF concentration in the vitreous is higher in diabetic subjects with PDR than in either non-diabetic subjects or diabetic subjects without PDR. The vitreous HGF concentration in diabetic subjects without PDR was almost the same as in the non-diabetic control subjects and was much lower than in the PDR

Table II. Blood concentration of hepatocyte growth factor (HGF) in type 1 diabetes at different stages in the development of diabetic retinopathy

Tabela II. Stężenie wątrobowego czynnika wzrostu (HGF) w surowicy krwi chorych na cukrzycę typu 1 z różnym stopniem rozwoju retinopatii cukrzycowej

	Means and standard deviation				p (Mann-Whitney U)
	Group 1 (n=35)	Group 2 (n=20)	Group 3 (n=21)	Control (n=35)	
HGF pg/ml	906.5 ± 174.7	962.9 ± 210.9	1409.3 ± 309.2	930.5 ± 285.8	p=0.09 (NS) p* = 0.197 (NS) p** < 0.005 p*** < 0.05 p**** < 0.05

Group 1 — patients without diabetic retinopathy; Group 2 — patients with non-proliferative diabetic retinopathy (NPDR); Group 3 — patients with proliferative diabetic retinopathy (PDR); p — control group vs. Group 1; p* — control group vs. Group 2; p** — control group vs. Group 3; p*** — group 1 vs. Group 3; p**** — group 2 vs. Group 3

subjects [14]. In the other study Nishimura et al. [15] found a high concentration of HGF not only in the original vitreous but also in the artificial vitreous after vitrectomy of patients with PDR. The authors concluded that vitreous HGF plays a role not only in the occurrence of PDR but also in its recurrence after vitrectomy. In a previous study Nishimura et al. reported that serum concentrations of HGF were lower in diabetic patients without retinopathy. The mean serum HGF concentration did not differ between diabetic patients with and those without the presence of NPDR and pre-PDR. Serum concentrations of HGF increased in patients with PDR who had not undergone photocoagulation, which is consistent with our results [27].

Canton et al. [28] found a significantly elevated intravitreal concentration of HGF in diabetic patients with PDR compared with non-diabetic patients. Intravitreal HGF concentrations were strikingly higher than serum HGF concentrations both in diabetic patients and in the control group. No correlation was found between serum and vitreous levels of HGF and the authors concluded that intraocular synthesis of HGF in PDR rather than serum diffusion is directly involved in the neovascularisation process [28]. These findings suggest that HGF could play a role in neovascularisation in PDR.

Conclusion

Concentrations of hepatocyte growth factor are increased in patients with type 1 diabetes mellitus with proliferative retinopathy, and the concentration increases with the progression of the retinopathy. This suggests that hepatocyte growth factor plays a role in the pathogenesis of diabetic proliferative retinopathy.

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