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Transforming growth factor β (TGF-β) levels in otherwise healthy subjects with impaired glucose tolerance

Stężenia transformującego czynnika wzrostu β (TGF-β) u zdrowych osób z nieprawidłową tolerancją glukozy

Halil Genc[1], Nuri Karadurmus[1], Ucler Kisa[2], Serkan Tapan[3], Ilkin Naharci[1], Alper Sonmez[4], Teoman Dogru[1]

[1]*Department of Internal Medicine, Gulhane Military Medical Academy, Etlik, Ankara, Turkey*
[2]*Department of Clinical Biochemistry, Faculty of Medicine, Kirikkale University, Kirikkale, Turkey*
[3]*Department of Biochemistry, Gulhane Military Medical Academy, Etlik, Ankara, Turkey*
[4]*Department of Endocrinology, Gulhane Military Medical Academy, Etlik, Ankara, Turkey*

Abstract

Introduction: Serum transforming growth factor beta (TGF-β) level is increased in type-2 diabetes mellitus (T2DM) and certain diabetic complications are mediated by this cytokine. Impaired glucose tolerance (IGT) is a prediabetic condition, and confers a risk for the development of certain diabetes-specific complications. However, no data is available regarding the alteration of TGF-β in IGT subjects. Therefore, we aimed to investigate TGF-β levels in otherwise healthy subjects with IGT.

Material and methods: Thirty IGT subjects and 30 subjects relatively matched for age, sex and body mass index with normal glucose tolerance were enrolled. Subjects with overt diabetes, cardiovascular, renal or inflammatory disease, or on any medication were excluded. Relevant laboratory examinations were performed by routine methods. Assessment of TGF-β was made by a commercially available enzyme-linked immunosorbent assay kit. IGT and control subjects were compared for their clinical and laboratory parameters.

Results: Serum TGF-β levels were found to be similar in IGT and normal glucose tolerance subjects ($p < 0.05$). No statistically significant correlation was found between TGF-β and other laboratory parameters, either in IGT subjects or in the whole study population.

Conclusions: Serum TGF-β is not elevated in otherwise healthy subjects with IGT. The results of our study imply that the presence of IGT alone is not sufficient to induce TGF-β elevation; and for the alteration of TGF-β, worsening of metabolic risk factors may be required.
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Keywords: *TGF-β, impaired glucose tolerance, cytokine*

Streszczenie

Wstęp: U chorych na cukrzycę typu 2 zwiększa się stężenie transformującego czynnika wzrostu beta (TGF-β, *transforming growth factor beta*). Cytokina ta pośredniczy w rozwoju wielu powikłań cukrzycowych. Nieprawidłową tolerancję glukozy (IGT, *impaired glucose tolerance*) uważa się za stan przedcukrzycowy. Zaburzenie to wiąże się ze zwiększonym ryzykiem niektórych powikłań charakterystycznych dla cukrzycy. Brakuje danych dotyczących zmian stężenia TGF-β u osób z IGT. Dlatego też autorzy postanowili zbadać osoczowe stężenia tego czynnika u zdrowych osób z IGT.

Materiał i metody: Do badania włączono 30 osób z IGT i 30 osób z prawidłową tolerancją glukozy dobranych pod względem wieku, płci i wskaźnika masy ciała. Kryteria wykluczenia obejmowały jawną cukrzycę, choroby sercowo-naczyniowe, choroby nerek i choroby zapalne oraz przyjmowanie jakichkolwiek leków. Odpowiednie badania laboratoryjne przeprowadzono rutynowymi metodami. Stężenia TGF-β zmierzono, stosując komercyjny zestaw immunoenzymatyczny. Porównano parametry kliniczne i laboratoryjne w grupie osób z IGT i grupie kontrolnej.

Wyniki: Osoczowe stężenia TGF-β były podobne u osób z IGT i z prawidłową tolerancją glukozy ($p < 0,05$). Nie stwierdzono istotnych statystycznie korelacji pomiędzy stężeniem TGF-β i innymi parametrami laboratoryjnymi ani w grupie osób z IGT, ani w całej badanej populacji.

Wnioski: U zdrowych osób z IGT stężenie TGF-β w osoczu nie było podwyższone. Wyniki przeprowadzonego przez autorów badania wskazują, że do wywołania zmian stężenia TGF-β nie wystarcza sama IGT, lecz konieczne jest współwystępowanie innych metabolicznych czynników ryzyka. **(Endokrynol Pol 2010; 61 (6): 691–694)**

Słowa kluczowe: *TGF-β, nieprawidłowa tolerancja glukozy,cukrzyca, cytokina*

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Nuri Karadurmus MD, Department of Internal Medicine Gulhane Military Medical Academy Asagieglence, Etlik, Ankara, Turkey, fax: +90 312 304 40 00; email: drnkaradurmus@yahoo.com

Introduction

In the course of type 2 diabetes mellitus (T2DM), several perturbations in the immune system take place. Some of these disturbances are related to cytokines [1–3]. Transforming growth factor β (TGF-β) is such a cytokine and has a pivotal role in regulation of many biological processes, including cellular proliferation and differentiation, modulation of immune response, extracellular matrix deposition, angiogenesis and tissue repair [4]. Serum TGF-β is demonstrated to be elevated in complicated and uncomplicated T2DM patients [5–7]. TGF-β elevation has been linked to diabetic complications, particularly to diabetic nephropathy, retinopathy and cardiovascular disease [8–10]. At present, there is convincing evidence regarding the pathogenetic role of TGF-β in the development of diabetic nephropathy and cardiovascular disease [8, 10]. What triggers TGF-β elevation in T2DM patients is not completely understood. However, hyperglycaemia, hyperinsulinaemia and advanced glycation end-products have been shown to induce TGF-β production [11–13]. Another question is when TGF-β elevation occurs in diabetic patients. A previous study reported increased levels of TGF-β in patients with recent onset T2DM, which remained elevated throughout the disease course [6]. IGT is a pre-diabetic condition with a 10% annual risk of progressing to overt diabetes. Although metabolic disturbances in IGT are mild, as compared to T2DM, these subjects are at risk of developing certain diabetes-specific complications, like cardiovascular disease and retinopathy [14]. However, whether TGF-β is altered in pre-diabetic subjects, and whether diabetes-specific complications in these subjects are mediated through this cytokine, remain to be elucidated. Our study investigated TGF-β levels in otherwise healthy subjects with IGT to evaluate the alteration of this cytokine in pre-diabetic subjects.

Material and methods

Participants

All subjects were randomly recruited from our outpatient clinic and underwent a glucose tolerance test with 75-g glucose. Impaired glucose tolerance was defined according to the recommendations of the American Diabetes Association (ADA) [15]. The inclusion criteria were: nonsmokers; normal resting 12-lead electrocardiogram; normal renal function and absence of hypertension or overt diabetes or angina or myocardial infarction, or history of any known vascular, infectious, or inflammatory diseases; and not on statins, antihypertensives or aspirin. Written informed consent was obtained from all participants and the local ethics committee approved the study.

Blood samples

Blood was withdrawn between 8.00–8.30 a.m. and two hours later later for basal glucose and 2-hglucose, put into fluoride-containing tubes and immediately analysed. Glucose, total cholesterol, high-density lipoprotein (HDL)-cholesterol and triglyceride (TG) levels were measured by the enzymatic colorimetric method with Olympus AU600 auto analyser using reagents from Olympus Diagnostics (GmbH, Hamburg, Germany). Low-density lipoprotein (LDL) cholesterol was calculated by the Fridewald's formula [16]. Serum high-sensitive C-reactive protein (hs-CRP) was determined by turbimetric fixed rate method by an automated analyser (Olympus AU-2700, Mishima, Japan) [17]. Serum insulin value was determined in duplicate by the coated tube method (DPC, Los Angeles, CA, USA). Homeostasis model assessment of insulin resistance (HOMA-IR) was calculated by using the formula HOMA-IR = fasting insulin (U/mL) × fasting glucose (mg/dL)/405, and a high HOMA-IR score denoted low insulin sensitivity [18]. Blood samples for TGF-β were collected at 8 a.m. and immediately centrifuged at 4°C and at 1,000 g for 10 min, then all sera were stored at –80°C until the time of analysis. Serum TGF-β concentration was determined using a commercially available quantitative enzyme-linked immunosorbent assay kit (Human TGF-β1, KAC1688/KC1689, Biosource International, Camarillo, CA, USA). Intra- and inter-assay coefficients of variation for TGF-β at 183 pg/mL were 5.5 and 7.5% respectively.

Statistical analyses

All analyses were performed using SPSS version 15.0 (SPSS Inc., Chicago, IL, USA). Categorical variables of IGT and control groups were compared by chi square test. Continuous variables were expressed as mean ± ± standard deviation (SD) or median (interquartile range) for the non-normal data. Numerical data were analysed by unpaired *t* or Mann Whitney U tests. Correlations were assessed by Spearman's correlation. All reported p values in analyses were two-tailed and a p value of < 0.05 was considered statistically significant.

Results

Thirty IGT subjects and 30 relatively age-, sex- and body mass index (BMI) matched subjects with normal glucose tolerance were enrolled. IGT and control groups were comparable for clinical and demographic characteristics (Table I). There was a remarkable difference between groups regarding fasting and 2-h glucose, as well as for HOMA-IR scores. Serum TGF-β concentrations were found to be similar between IGT and control subjects. No significant correlation was observed be-

Table I. *Demographic, anthropometric, clinical and laboratory variables of IGT and control subjects*
Tabela I. *Dane demograficzne, antropometryczne, kliniczne i laboratoryjne osób z IGT i osób z grupy kontrolnej*

Variable	IGT (n = 30)	Control (n = 30)	p
Age (years)	44.3 ± 4.9	44.9 ± 10.4	ns
Sex (n, female/male)	8/22	12/18	ns
Body Mass Index [kg/m^2]	27.5 ± 2.4	26.9 ± 2.8	ns
Fasting glucose [mg/dL]	112.5 ± 8.2	89.2 ± 6.3	< 0.001
2-h glucose [mg/dL]	163.9 ± 18.5	94.7 ± 15.7	< 0.001
Total cholesterol [mg/dL]	206.9 ± 38.0	186.2 ± 35.1	0.032
HDL-cholesterol [mg/dL]	49.1 ± 8.6	49.8 ± 11.1	ns
LDL-cholesterol [mg/dL]	124.1 ± 32.2	108.9 ± 32.2	= 0.07
Triglycerides [mg/dL]	135.5 (85.5)	93.0 (59.7)	0.01
TGF-β [pg/mL]	16.7 ± 7.1	16.1 ± 6.4	ns
HOMA-IR score	1.6 (1.6)	0.9 (1.0)	0.001
hs-CRP [mg/dL]	2.40 ± 1.98	2.63 ± 1.99	ns

Values are expressed as mean ± SD, unless indicatedl; IGT — impaired glucose tolerance; HDL — high-density lipoprotein; LDL — low density lipoprotein; TGF-β — transforming growth factor β; hs-CRP — high sensitive C-reactive protein; ns — non-significant

tween TGF-β and other clinical or laboratory parameters, either in IGT subjects or in the whole study population.

Discussion

Several lines of studies demonstrated elevated levels of TGF-β in both complicated and uncomplicated T2DM individuals [5–7]. Moreover, some of the disease-specific complications that arise in the course of diabetes have been linked to TGF-β elevation previously [8–10]. IGT is a prediabetic condition and confers an increased risk for the development of diabetes-specific complications and cardiovascular disease [14]. However, no data is available regarding TGF-β levels in prediabetic individuals. A previous study on gestational diabetic subjects suggested that elevation of TGF-β is probable in prediabetic subjects [19]. Glucose, insulin and advanced glycation end-products are reported to induce TGF-β production in diabetic subjects [11–13]. In our study, although there was a significant difference between IGT and healthy subjects regarding fasting- or 2-h glucose, TGF-β concentrations were found to be similar. Therefore, we suggest that for the induction of TGF-β, a higher blood glucose threshold or more severe metabolic disturbances may be required, as seen in diabetic individuals. In support of this theory, Meigs et al. previously reported worsening of metabolic risk factors by the worsening of glucose tolerance in IGT subjects, and worsening of metabolic risk factors increase complication rates in IGT subjects [20]. In the absence of other metabolic risk factors, lone IGT may not be able to stimulate TGF-β as observed in our study population. Alternatively, TGF-β induction may be a result of subclinical inflammation seen in insulin resistance conditions. It is well recognised that activity of subclinical inflammation increases by the severity of metabolic disturbances on the course of diabetes [21]. In our study, we included IGT subjects without any confounding factors, which may explain similar inflammatory activity between IGT and control subjects. Therefore, the magnitude of inflammation in our IGT subjects may not be able to stimulate TGF-β.

Conclusions

In conclusion, IGT patients have similar TGF-β levels to healthy subjects. However, the sample size of our study is relatively small due to strict selection criteria, so our results need to be confirmed in studies with more IGT subjects. Furthermore, looking to the future, investigation of TGF-β in IGT subjects with diabetes-specific complications may better establish the role of this cytokine in the pathogenesis of diabetic complications.

References

1. Pickup JC. Inflammation and activated innate immunity in the pathogenesis of type 2 diabetes. Diabetes Care 2004; 27: 813–823.
2. King GL. The role of inflammatory cytokines in diabetes and its complications. J Periodontol 2008; 79 (Suppl.): 1527–1534.
3. Chiarelli F, Santilli F, Mohn A. Role of growth factors in the development of diabetic complications. Horm Res 2000; 53: 53–67.
4. Kim IY, Kim MM, Kim SJ. Transforming growth factor-beta: biology and clinical relevance. J Biochem Mol Biol 2005; 38: 1–8.
5. Pfeiffer A, Middelberg-Bisping K, Drewes C et al. Elevated plasma levels of transforming growth factor-beta 1 in NIDDM. Diabetes Care 1996; 19: 1113–1117.

6. Azar ST, Salti I, Zantout MS et al. Alterations in plasma transforming growth factor beta in normoalbuminuric type 1 and type 2 diabetic patients. J Clin Endocrinol Metab 2000; 85: 4680–4682.
7. Yener S, Comlekci A, Akinci B et al. Serum transforming growth factor--beta 1 levels in normoalbuminuric and normotensive patients with type 2 diabetes. Effect of metformin and rosiglitazone. Hormones (Athens) 2008; 7: 70–76.
8. Chen S, Jim B, Ziyadeh FN. Diabetic nephropathy and transforming growth factor-beta: transforming our view of glomerulosclerosis and fibrosis build-up. Semin Nephrol 2003; 23: 532–543.
9. Matsumoto Y, Takahashi M, Ogata M. Relationship between glycoxidation and cytokines in the vitreous of eyes with diabetic retinopathy. Jpn J Ophthalmol 2002; 46: 406–412.
10. Singh NN, Ramji DP. The role of transforming growth factor-beta in atherosclerosis. Cytokine Growth Factor Rev 2006; 17: 487–499.
11. Rocco MV, Chen Y, Goldfarb S et al. Elevated glucose stimulates TGF-beta gene expression and bioactivity in proximal tubule. Kidney Int 1992; 41: 107–114.
12. Yang C-W, Vlasara H, Peten EP et al. Advanced glycation end products up-regulate gene expression found in diabetic glomerular disease. Proc Natl Acad Sci USA 1994; 91: 9436–9440.
13. Morrisey K, Evans RA, Wakefiled L et al. Translational regulation of renal proximal tubular epithelial cell transforming growth factor-beta 1 generation by insulin. Am J Pathol 2001; 159: 1905–1915.
14. Garber AJ, Handelsman Y, Einhorn D et al. Diagnosis and management of prediabetes in the continium of hyperglycemia — When do the risk of diabetes begin? A consessus statement from the American College of endocrinology and the American association of clinical endocrinologists. Endocr Pract 2008; 14: 933–946.
15. American Diabetes Association. Diagnosis and classification of diabetes mellitus. Diabetes Care 2008; 31 (Suppl. 1): 55–60.
16. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem 1972; 18: 499–502.
17. Wasunna A, Whitelaw A, Gallimore JR et al. C-reactive protein and bacterial infection in preterm infants. Eur J Pediatr 1990; 149: 424–427.
18. Matthews DR, Hosker JP, Rudenski AS et al. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia1985; 28: 412–419.
19. Yener S, Demir T, Akinci B et al. Transforming growth factor-beta 1 levels in women with prior history of gestational diabetes mellitus. Diabetes Res Clin Pract 2007; 76: 193–198.
20. Meigs JB, Nathan DM, Wilson PW et al. Metabolic risk factors worsen continuously across the spectrum of nondiabetic glucose tolerance. The Framingham Offspring Study. Ann Intern Med 1998; 128: 524––533.
21. Deepa R, Velmurugan K, Arvind K et al. Serum levels of interleukin 6, C-reactive protein, vascular cell adhesion molecule 1, and monocyte chemotactic protein 1 in relation to insulin resistance and glucose intolerance — the Chennai Urban Rural Epidemiology Study (CURES). Metabolism 2006; 55: 1232–1238.