Dipeptidyl peptidase-4 activity might be a link between tumour necrosis factor alpha and insulin resistance in type 1 diabetes

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Abstract

PURPOSE: Tumor necrosis factor alpha (TNF α) leads to β cell damage in type 1 diabetes (T1DM) but also causes insulin resistance (IR). It modulates dipeptidyl peptidase-4 (DPP-4) activity, adipokine linked with both IR and T1DM. We were interested if there is an association of TNF α in conjunction with DPP-4 and IR in T1DM.

METHODS: DPP-4 activity, TNF α concentration measurements and insulin sensitivity calculation using estimated glucose disposal rate (eGDR) equation were performed in 70 T1DM patients. They were divided into two groups according to eGDR median.

RESULTS: The group with higher IR had higher value of DPP-4 activity (27.57±1.77 *vs* 18.33±1.14, p<0.001) and TNF α concentration (12.91±0.83 *vs* 6.72±0.36, p<0.001). TNF α concentration and DPP-4 activity negatively correlated with eGDR (r=-0.616, p<0.001 and r=-0.643, p<0.001), while positively with each other (r=0.422; p=0.001). The linear regression showed that eGDR decreases for 0.166 mg kg−1min−1 by TNF α concentration increase of 1 pg/mL (p<0.001) and for 0.090 mg kg−1 min−1 by DPP-4 activity increase of 1 U/L (p=0.001) when adjusted for age, gender disease duration, glycated haemoglobin, body mass index and waist-to-hip ratio. eGDR decreased by additional 0.60 mg kg−1 min−1 (B=-0.150, p<0.001) when DPP-4 activity was additionally adjusted for TNF α.

CONCLUSION: TNF α concentration is associated with IR, correlates with its severity and increases the drop in insulin sensitivity modulated by DPP-4 activity. Whether TNF α involvement in the insulin signalling pathway is mediated by DPP-4 activity needs to be further evaluated.

Key words: tumor necrosis factor alpha, dipeptidyl peptidase-4, insulin sensitivity, type 1 diabetes

Introduction

Type 1 diabetes mellitus (T1DM) is an inflammatory disease of the pancreatic islets that results in absolute insulin deficiency and the consecutive hyperglycaemia due to immune mediated β cell destruction [1]. Although β cell death is the patophysiological core of T1DM, recent data suggest that the disease associated metabolic disturbances represent a common feature of autoimmunity and insulin resistance (IR) caused by adipocyte derived proinflammatory factors, chronic activation of innate immune system and low grade inflammation [2, 3]. Out of recently identified adipocytokines, dipeptidyl peptidase-4 (DPP-4) and tumor necrosis factor-alpha (TNF α) appear to be important in the pathogenesis of IR [4, 5].

DPP-4 is a serine exopeptidase that cleaves X-proline dipeptides from the polypeptide N-terminus. A fraction of soluble DPP-4 originates from the immune system cells which explains its altered abundance and the circulating activity in various immune mediated conditions [6] including T1DM [7]. Recent data suggest that DPP-4 activity is higher in patients with T1DM compared to healthy controls independently of islet-cell antibody status, C-peptide concentration, disease duration or glycated hemoglobin (HbA1c) level [8] and in an inverse correlation with body mass index (BMI) and insulin sensitivity [8, 9].

TNF α is closely related to metabolic disorders and diabetes. It was originally identified as cytokine associated with weight loss, hypermetabolism and energy expenditure in infectious diseases or malignancies [10, 11]. The observation that visceral adipocytes of obese animals over express TNF α provided evidence that it might be involved in the pathogenesis of IR [12]. Because of its interaction with the insulin signalling cascade, a major role in the pathogenesis of obesity-linked IR and T2DM was postulated for TNF α [13]. In T1DM, TNF-α was found to be higher compared to age-matched healthy controls but also associated with glycaemic control and dyslipidaemia independent of BMI [14]. Despite, the mechanism whereby TNF α modulates insulin sensitivity remain unresolved. However, our recent finding that DPP-4 activity might link adiposity to IR in T1DM patients [9] and the fact that DPP-4 activity is partially modulated by TNF α [15] leads to the speculation that TNF α might modulate insulin sensitivity via DPP-4 activity.

Thus, with respect to the relationship between TNF α to IR and glycaemic control in T2DM, as well as its involvement in the β cell apoptosis in T1DM, it was the aim of our study to investigate the association of TNF α plasma concentrations in conjunction with serum DPP-4 activity with IR in cohort of T1DM patients.

Materials and methods

Seventy patients with T1DM were recruited from the In-patient Clinic for Diabetes, endocrinology and metabolic diseases Vuk Vrhovac, Zagreb, Croatia. Histories, complete physical examination and laboratory tests were performed in all subjects in order to exclude diseases other than T1DM or medications that might affect insulin sensitivity. Type 1 diabetes was defined by undetectable meal stimulated C-peptide concentrations (C-peptide <0.2 ng/mL) and positive islet cell and glutamic acid autoantibodies (at least from the previous medical record if the measurement was performed in our Clinic laboratory, respectively). All of the patients were non-smokers and were not using any medication except insulin which was administered by a basal-bolus regimen. Furthermore, patients with macrovascular complications such as significant arterial obliteration detected by imaging methods, an acute cardiovascular event (e.g. myocardial infarction), unstable angina or stroke within six months prior to enrolment, impaired glomerular filtration rate (<60 ml/min/1.73m2) and urine albumin excretion (UAE) rate >300 mg/24h were not included because higher TNF α concentration was described in those individuals [16] . The study was conducted according to the guidelines laid down in the Declaration of Helsinki and approved by local Ethics committee. Written informed consent was obtained from and signed by all patients.

Insulin sensitivity was calculated using the estimated glucose disposal rate equation (eGDR): 24.31−12.2X(WHR)−3.29X(AHT)−0.57X(HbA1c), where WHR indicates the waist to hip ratio, AHT indicate blood pressure, and is expressed as: 0 = no, 1 = yes. Those on blood pressure medications or with blood pressure>140/90 mmHg were considered to have hypertension; the equation was derived from a substudy of 24 EDC (Epidemiology of Diabetes Complications) participants who underwent euglycemic–hyperinsulinemic clamp studies [17]. Lower GDR levels the units expressed in mg kg−1 min−1 indicate greater IR.

Fasting venous blood samples were collected for the determination of lipid profile status, glycated haemoglobin A1c (HbA1c), TNF α and serum DPP-4 activity.

Urine albumin excretion (UAE) was measured from two 24-h urine samples and determined as the mean of 24-h urine collections expressed as mg/24 h. Data on serum creatinine levels, age, sex and race were used to calculate the estimated GFR (eGFR) using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) formula, which has been shown to be accurate in determining renal function in diabetic patients with normal renal function [18]. DPP-4 activity was measured by a colorimetric assay procured from Sigma, St. Louis, MO, USA in a microplate reader (CaryEclipseVarian, Agilent Technologies) at 460 nm, 37 °C in a continuous monitoring for 35 min. In this assay, DPP4 cleaves H-Gly-Pro-AMC to release a fluorescent product, 7-Amino-4-Methyl Coumarin (AMC) which can be measured spectrophotometrically as previously described [9]. One unit of activity was defined as the amount of enzyme which will hydrolise the DPP-4 substrate to yield

1.0 μmol of AMC per minute at 37 °C. TNF α was determined by a solid phase enzyme amplified sensitivity immunoassay (TNF α ELISA, Invitrogen Corporation 542 Flynn Road, Camarillo, CA 93012). This sandwich ELISA, with a lower detection limit of 1.7 pg/mL detects both free TNF α and TNF α bound to its soluble receptor. The intra-assay coefficient of variation for analysis in serum is <8%. The correlation coefficient for duplicate determination of the same sample was 0.92.

The data distribution was assessed by Shapiro–Wilk test. All the continuous variables were log-transformed and reported as mean values and standard error of mean (S.E.M), whereas categorical variables were reported as numbers and percentages. The differences between the two study groups were tested by Student's t-test while the categorical variables were analysed by the $\chi 2$ test. Correlations between fasting serum DPP4 activity with anthropometric and metabolic variables were determined using Pearson's correlation coefficient. All the tests were two-sided. The association between fasting serum DPP4 activity, TNF α and eGDR value was further evaluated in multivariate linear regression. Adjustments were performed for age, gender, disease duration, eGFR and UAE since it might have an effect on insulin sensitivity as well as on serum DPP4 activity. Level of statistical significance was chosen to be 0.05.

Results

The mean age of our T1DM study population was 50.85±1.57 years with a mean duration of diabetes 26.36±1.97 years. Fourty seven (67.1%) were male. Patients were divided into two groups according to the median eGDR value (6.57 mg kg^{-1} min^{-1}). The detailed clinical and laboratory findings and the difference between them are given in Table 1. Lower insulin sensitivity was recorded more frequently in male subjects, corresponding to literature [19], as well as its association with higher BMI, waist circumference, blood pressure (BP) values and lower HDL concentration. Furthermore, the group of patients with lower insulin sensitivity showed higher values of serum DPP-4 activity ($p<0.001$) and TNF α concentration ($p<0.001$). Both TNF α concentration and DPP-4 activity showed negative correlation with eGDR ($r=-0.616$, $p<0.001$ and $r=-0.643$, $p<0.001$), while positive correlation with each other ($r=0.422$; $p=0.001$), respectively (Figure 1. a-c). Furthermore, DPP-4 activity significantly correlated with WHR and BMI while TNF α concentration correlated with WHR, BMI and HbA1c (Table 2.). The simple linear regression with eGDR as dependent variable has shown that eGDR significantly decreases by 0.166 mg kg−1 min−1 by each increase of TNF α concentration of 1 pg/mL ($p<0.001$) by 0.090 mg kg−1 min−1 by each increase of DPP-4 activity of 1 U/L ($p=0.001$) in the model adjusted for age, gender, disease duration, WHR, BMI as well as HbA1c. Furthermore, when DPP-4 activity was additionally adjusted for TNF α concentration, it revealed that eGDR decreases by additional 0.060 mg kg−1 min−1 when compared to the model unadjusted for the same variable (B=-0.150 (95%CI, -0.225-(0.074=), $p<0.001$), Table 3.

Discussion

In the present study, we examined whether circulating TNF α levels are increased in T1DM patients with higher degree of IR and, if so, whether they are related to the DPP-4 activity which isalready described as a link between central adiposity and IR in normal glucose tolerant, T2DM and T1DM individuals [9, 20, 21]. Our results demonstrate that T1DM subjects with higher degree of IR had significantly higher circulating TNF α concentrations. The elevated TNF α concentration was correlated with DPP-4 activity and insulin sensitivity and further, the drop in insulin sensitivity followed by increase in DPP-4 activity is increased by 60% when adjusted for the TNF α concentration. The inverse correlation between TNF α levels and insulin sensitivity in T1DM patients, independent of obesity/age/gender/HbA1c, is consistent with a potential role of circulating TNF α in the regulation of insulin action by its interference with the insulin-signalling pathway [22]. This multifunctional cytokine seems to be involved in the pathogenesis of obesity-linked IR; even short-term incubations of muscle cells and of adipocytes with TNF α resulted in a significant impairment of insulin action [23].

Previous studies reporting an association between serum TNF α and IR have employed a combined analysis of diabetic and nondiabetic subjects. In all of them, a significant correlation between TNF α and IR was observed [24, 25, 26]. So far, there is only one study accessing the correlation between TNF α concentrations and metabolic parameters [14]. It revealed a significant correlation of TNF α with long-term parameters of metabolic control: HbA1c and fructosamine for glycaemic control, and HDL cholesterol for lipid metabolism, as well as thiobarbituric acid reacting substances as oxidative stress measurement, also linked to IR. However, the underlying mechanism explaining the potential role of TNF α in energy metabolism is poorly understood.

Ever since Lamers et al. (2011) [4] performed a comprehensive proteomic profiling of the media derived from primary human adipocytes and proposed DPP-4 as a novel adipokine linking adipose tissue to IR, the number of studies linking DPP-4 activity with IR in non diabetic population as well as in T2DM and T1DM individuals has rapidly increased [8, 9, 21, 27]. It was also recently demonstrated that the DPP-4 inhibition in human adipocytes, skeletal and smooth muscle cells increases insulin-stimulated Akt phosphorylation in a dose-dependent manner and thus might result in insulin sensitivity increase [28]. Since DPP-4 activity might be modulated by TNF α in proinflammatory states [15, 29], it seems rationale to believe that plasma TNF α concentration might cause increase in IR at least partially by increasing the level of DPP-4 activity and thereby affecting insulin signalling pathway.

The cross-sectional design of this study, sample size and the lack of healthy controls limits the possibility to derive any general conclusion. Further, the insulin sensitivity was not assessed by a euglycemic clamp which is a gold standard in insulin sensitivity determination Despite, we can conclude that serum DPP-4 activity may be affected by TNF α concentration, and that it might play an important role in TNF α mediated insulin signalling pathway interference. However, it is possible that elevated DPP-4 activity may be secondary to low grade inflammation already existing in the IR state. Further investigation with comprehensive evaluation of insulin sensitivity in a larger sample are warranted to elucidate the role of TNF α on soluble DPP-4 activity and IR development.

In summary, the present results demonstrate that: (i) an increase in circulating TNF α concentration is associated with peripheral insulin resistance; (ii) the increased serum TNF α concentration in type 1 diabetic individuals is correlated with the severity of insulin resistance; (iii) the increased serum TNF α concentration increases the drop in insulin sensitivity modulated by DPP-4 activity. This study emphasises the importance in determination of cytokines possibly involved in the IR pathogenesis in T1DM which might reveal a subgroup of patients that require more intensified and multidisciplinary treatment approach in which the use of DPP-4 inhibitors might have a an important role .

Conflict of interest

None declared.

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Table 1. Characteristics of the study participants according to the median eGDR value of 6.57 mg kg^{-1} min^{-1}

	eGDR>6.57 mg kg^{-1} min^{-1} N=35	eGDR<6.57 mg kg^{-1} min^{-1} N=35	p
Age (yrs)	50.34±1.52	51.37±1.77	0.448
Gender (F/M)	16/19	7/28	0.020
Diabetesduration (yrs)	25.44±1.71	27.25±1.69	0.471
BMI (kg/m^2)	23.23±0.45	26.71±0.65	<0.001
Waist circumference (cm)	85.65±1.54	97.37±1.64	<0.001
HbA1c (%)	7.1±0.3	7.5±0.2	0.032
Systolic BP (mmHg)	124.6±2.6	129.4±2.1	0.048
Diastolic BP (mmHg)	76.6±1.3	79.7±1.2	0.055
hsCRP (mg/L	1.7±0.4	3.4±1.1	0.031
Total serum cholesterol (mmHg)	5.27±0.21	4.89±0.16	0.083
HDL cholesterol (mmol/L)	1.74±0.07	1.45±0.05	0.003
LDL cholesterol (mmol/L)	2.86±0.14	3.01±0.18	0.792
Triglycerides (mmol/L)	1.32±0.13	1.13±0.07	0.262
TNF α (pg/mL)	6.72±0.36	12.91±0.83	<0.001
DPP-4 activity (U/L)	18.33±1.14	27.57±1.77	<0.001

NOTE: values are expressed as mean±S.E.M.

Table 2. Pearson correlation coefficient for the association of DPP4 activity (U/L), TNF α concentration (pg/mL) with blood pressure, BMI, WHR and HbA1c

Variable	TNF α (pg/mL)	p	DPP-4 activity (U/L)	p
Systolic BP (mmHg)	0.010	0.936	0.059	0.652
Diastolic BP (mmHg)	0.018	0.866	0.050	0.705
BMI (kg/m^2)	0.345	0.003	0.350	0.006
Waist to Hip ratio	0.447	0.001	0.313	0.014
HbA1c (mmol/L)	0.384	0.001	0.107	0.413

Table 3. Linear regression analysis for the serum DPP-4 activity (U/L), TNF α concentration (pg/mL) and the eGDR(mg kg^{-1} min^{-1}) derived from three separate models

Model	*B*	*p* value	95,0 % confidence interval for *B*	
			Lower bound	Upper bound
Plasma TNF α concentration (pg/mL) adjusted for age, gender, disease duration, BMI, WHR and HbA1c	−0.166	0.001	−0.239	−0.0.093
Serum DPP-4 activity (U/L) adjusted for age, gender, disease duration, BMI, WHR and HbA1c	−0.0.090	0.001	−0.132	−0.048
Serum DPP-4 activity (U/L) adjusted for age, gender, disease duration, disease duration, BMI, WHR, HbA1 and plasma TNF α concentration (pg/mL)	−0.150	<0.001	−0.225	−0.074

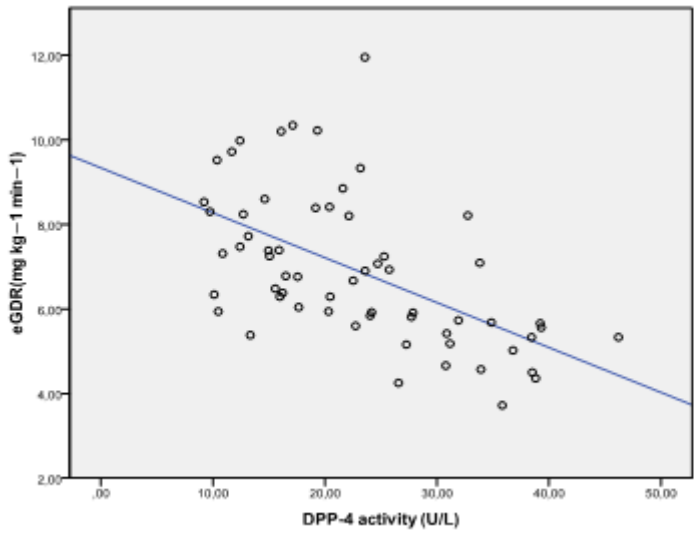
Figure legend:

Figure 1. Correlation analysis of the serumDPP4 activity (U/L), TNF α concentration (pg/mL) and the eGDR(mg kg−1 min−1)

a) DPP-4 activity inversely correlates with eGDR (r=-0.643, p<0.001)

b) TNF α concentration inversely correlates with eGDR (r=-0.616, p<0.001)

c) TNF α concentration correlates with DPP-4 activity (r=0.422; p=0.001)


eGDR(mg kg−1 min−1)
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10,00
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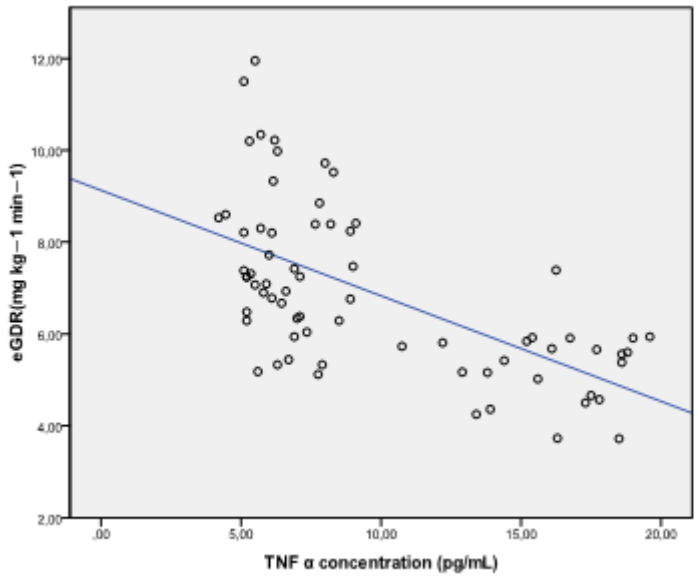
DPP-4 activity (U/L)


eGDR(mg kg−1 min−1)
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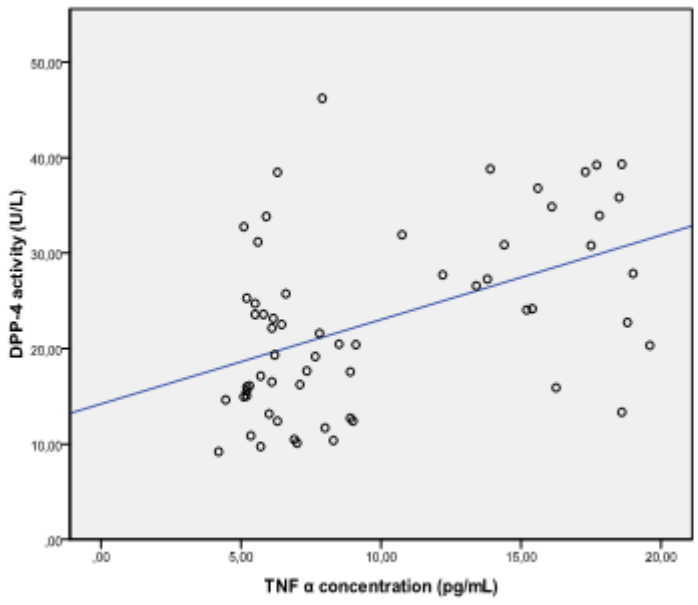
TNF α concentration (pg/mL)


DPP-4 activity (U/L)
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TNF α concentration (pg/mL)