Journal of Diabetes & Metabolic Disorders (2021) 20:1521–1527
https://doi.org/10.1007/s40200-021-00895-z

RESEARCH ARTICLE

Association of serum kynurenine/tryptophan ratio with poor glycemic control in patients with type2 diabetes

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Received: 20 March 2021 / Accepted: 2 September 2021 / Published online: 12 September 2021

Abstract

Purpose The role of indoleamine 2,3-dioxygenase (IDO) has been shown in insulin resistance and metabolic syndrome. The present study aimed to measure serum IDO activity in patients with type 2 diabetes (T2DM) and to determine its association with glycemic control, oxidative stress, and insulin resistance.

Methods Seventy-four patients with T2DM and 74 healthy subjects were selected to participate in this study. Fasting serum biochemical parameters including fasting blood sugar (FBS), HbA1c, insulin, uric acid, albumin, tryptophan, kynurenine, and total antioxidant capacity (TAC) were measured. HOMA-IR, QUICKI, and HOMA-B were calculated using serum FBS and insulin values. IDO activity was estimated using kynurenine/tryptophan ratio (KTR). Data were analyzed using SPSS software (Version 15) and $p < 0.05$ was considered as a significant difference.

Results The findings showed higher levels of FBS, HbA1c, HOMA-IR, and KTR in the patients compared to the controls**.** TAC and HOMA-B were significantly lowered in the T2DM patients compared to controls. KTR was significantly correlated with the level of HbA1c, and T2DM patients with poor glycemic control (HbA1c ≤ 8) had significantly higher level of KTR. HOMA-B was significantly correlated with serum tryptophan and inversely correlated with HbA1c.

Conclusion Serum KTR is increased in T2DM patients with poor glycemic control. Potential clinical implications and possible pathogenic roles of IDO in T2DM development should be further elucidated.

Keywords Type 2 diabetes mellitus · Insulin resistance · Indoleamine 2 · 3 dioxygenase · Oxidative stress

Introduction

Type 2 diabetes mellitus (T2DM) is the most common type and accounts for 90% of the patients with diabetes [1]. The main features of T2DM include impaired insulin secretion and insulin resistance (IR). Sedentary life style [2], obesity [3], oxidative stress (OS) [4], genetic and epigenetic factors [5], and low-grade chronic inflammation [6] are among causal factors in the development of IR and beta cell dysfunction. The effects of chronic inflammation in inducing IR and beta cell dysfunction and thereby T2DM are mediated through inflammatory cytokines such as interleukin 6 (IL6) and tumor necrosis factor-α(TNF-α) [6]. Increased serum TNF-α and IL6 levels [7–9] and their association with the development of T2DM complications such endothelial dysfunction [10], diabetic nephropathy [11], and retinopathy [12] have been shown previously. It has been proposed that the effects of IL6 and TNF-α on inducing IR could be mediated through direct impairing of insulin signaling pathway. In addition, IL6 could trigger IR

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through increased expression of interferon-gamma (INF-γ). INF-γ induces upregulation of indoleamine 2,3 dioxygenase (IDO) expression [13], a rate limiting enzyme in the conversion of tryptophan (Trp) to kynurenine (Kyn) [14]. Kyn can inhibit production and biological activity of insulin and cause IR. Therefore, monitoring Trp metabolism might contribute to identifying the individuals at risk of IR [15, 16]. Obesity is related to chronic low-grade inflammation in the adipose tissue [17]. A higher level of IDO expression has been shown in the subcutaneous adipose tissue and liver of obese compared to lean individuals. Furthermore, a significant higher serum Kyn/Trp ratio (KTR), an index of IDO activity, has been reported in overweight compared to the subjects with normal body mass index (BMI). Oxenkrug et al. have revealed a positive association between IR and IDO activity, suggesting a possible role of Trp metabolism in the development of IR [18]. The association of IDO activity with diabetic complications including coronary heart disease [19], atherosclerosis risk factors [20],nephropathy [21], and retinopathy [22] has been demonstrated. Moreover, Kyn is transformed to quinolinic acid (QA) that has neurotoxic effects. Increased retina level of QA was shown in diabetic patients with retinopathy [23].

Despite the fact that some studies have been conducted on the significance of tryptophan metabolism in the pathogenies T2DM, there are scanty results which are controversial. It was hypothesized that these controversies might be associated with difference in the degree of hyperglycemia, oxidative stress, insulin resistance, and metabolic syndrome (Mets) in the subjects under study. Thus, this study aimed to compare serum KTR between patients with T2DM and healthy controls. We also determined the relationship of KTR with the glycemic control status, IR, beta cell activity, total antioxidant capacity (TAC), and Mets.

Materials and methods

This case-control study was approved in the ethics committee of Islamic Azad University - Shiraz branch with the ethics ID IR.IAU.SHIRAZ.REC.1399.036. Seventy-four patients that were previously diagnosed with T2DM based on American Diabetes Association guidelines [24] and 74 age and gender- matched healthy control subjects were enrolled in the present study. Having normal fasting blood sugar (FBS) and HbA1c, as well as absences of any major diseases were inclusion criteria for recruiting healthy control individuals. We selected the subjects from those who referred to the Clinical Laboratory at Shahid Motahari Clinic of Shiraz University of Medical Sciences. Written informed constant was obtained from the patients and control subjects. Patients with T2DM on anti-inflammatory drugs, T1DM patients, and those who had any major diseases including known endocrine, liver, kidney, and inflammatory ones were excluded from the study.

Biochemical tests

Fasting serum biochemical parameters including FBS, uric acid, albumin, triglyceride (TG), total cholesterol (TC), and high-density lipoprotein cholesterol (HDL-C) were measured using commercial kits (Pars Azmoon, Iran). Low density lipoprotein cholesterol(LDL-C) values were calculated using Firedewald equation [25]. HbA1c was measured using direct enzymatic method (Diazyme, USA). Serum insulin levels were estimated using a human insulin kit (Monobind, USA). Homeostatic model assessment of insulin resistance (HOMA-IR), quantitative insulin sensitivity check index (QUICKI), and homeostatic model assessment to quantify beta-cell function (HOMA-B) were calculated using serum FBS and insulin values [26]. The subjects were diagnosed with Mtes in case they had at least three out of the five characteristics features (waist circumference > 102 cm for men or > 88 cm for women; TG ≥ 150 mg/dl; HDL-C < 40 mg/dl for men or < 50 mg/dl for women; systolic blood pressure ≥ 130 mmHg or diastolic ≥85 mmHg; FBS ≥ 100 mg/dl) [27].

Measurement of kynurenine and tryptophan

IDO activity was estimated using serum KTR [28]. High performance liquid chromatography (HPLC, Knauer, Germany) with ultraviolet detector, sodium acetate- acetonitrile (5%) buffer, and reverse phase C-18 column (Spherisorb column; 4.6 mm × 250 mm) were used to separate and quantify serum Kyn and Trp. In brief, 13 µl of perchloric acid (40%) was added to 100 µl of serum and centrifuged at 15000 g and 4 °C for 15 min. Twenty µl of the supernatant or standard solution were injected to the column and the amount of serum Kyn and Trp were quantified by comparing the chromatographic peak areas of Kyn and Trp of the serum and the standard solution [28].

Total antioxidant capacity assay

TAC was measured using cupric reducing antioxidant capacity (CUPRAC) method based on reduction of Cu^{2+} to Cu^{+1} by the serum antioxidants [29]. In brief, 30 µl of diluted serum sample were added to 150 µl of chromogen working solution, mixed, and incubated for 30-min at room temperature. We measured the absorbance of the solution against blank at 450 nm through a spectrophotometer. Trolox (analogue of vitamin E) was used as a standard, and the assay results were expressed in mmol of Trolox equivalent/L.

Statistical analyses

Data were analyzed using SPSS software (Version 15). We evaluated the normal distribution of the data through Kolmogorov-Smirnov test. Parametric variables were analyzed using Student-T test or one-way ANOVA. Mann-Whitney and Kruskal-Wallis tests were used to compare non-parametric data between groups. Spearman correlation test was used to determine the relationship between the studied variables. Multivariate regression analysis was used to determine the predictive indices. Data were represented as mean ± standard deviation. $p < 0.05$ was considered as a significant difference.

Results

Demographic characteristics of study population

One hundred and forty-eight age and gender-matched individuals including 74 healthy control and 74 patients with T2DM were enrolled in this study. There were no significant differences in the age and BMI of the control and T2DM groups. The mean of systolic and diastolic pressures was significantly higher in the patients compared to healthy controls ($p = 0.01$ and $p < 0.001$, respectively). In addition, patients with T2DM had higher values of waist/hip ratio compared to the control subjects($p < 0.001$) (Table 1). Table 1 also shows the values of serum FBS, HbA1c, insulin, HOMA-IR, QUICKI, and HOMA-B in the control and patients with T2DM.The mean of FBS, HbA1C, and HOMA-IR were significantly higher in the T2DM patients compared to the controls, while the mean of QUICKI and HOMA-B were lower in the patients compared to the healthy controls. No significant difference was observed in the mean of insulin concentration between T2DM and control groups. The mean of serum TG, HDL-C, and TG/HDL-C ratio did not show significant difference between T2DM and control subjects (Table 1). However, our data revealed significant lower values of TC and LDL-C in the patients with T2DM compared to the control group (Table 1). For comparison of serum oxidant/antioxidant status between T2DM and control groups, we measured several markers including serum albumin, uric acid, and TAC (Table 1). The findings indicated lower values of TAC and serum albumin in patients with T2DM as compared to the controls. There was no significant difference between the mean value of uric acid in the patients and control groups. The controls and T2DM patients revealed no significant difference in Trp concentration. The concentration of serum Kyn was higher in the T2DM patients as compared to the controls. There was also a significantly higher level of KTR in the T2DM group compared to the control group (Table 1). The prevalence of Mets in the controls and patients with T2DM was shown to be 30 and 58%, respectively**.** Spearman correlation analyses revealed that HOMA-IR was positively correlated with HbA1c and inversely associated with TAC (Table 2). In stepwise regression model both of HbA1c ($\beta = 0.304$, $p = 0.01$) and TAC ($\beta = -0.237$, $p = 0.043$) remained the independent predictive of IR in the patients with T2DM. HOMA-B showed positive significant correlation with serum Trp concentration and BMI of the patients. An inverse correlation was observed between TAC and HbA1c as well as age of the patients (Table 2). These variables entered stepwise regression analyses and the data showed that HbA1c ($\beta = -0.704$, $p = 0.001$) was the only factor that independently predicts HOMA-B in patients with T2DM. No significant associations were observed between Kyn and KTR and HOMA-IR, HOMA-B, as well as Mets and Trp metabolism indices in patients with T2DM and controls.

Table 1 Comparison of demographic characteristics between the T2DM and control groups

Characteristics	Control(n = 74)	T2DM(n = 74)	p values
Age(years)	46.2 ± 6.1	48.1 ± 6.5	0.66
Gender (Male/female)	31/38	33/41	0.511
BMI(Kg/m^2)	27.9 ± 4.0	29.3 ± 5.1	0.082
Waist/hip ratio	0.88 ± 0.07	1.1 ± 0.1	<0.001
Systolic BP (mmHg)	119 ± 14	125 ± 14	0.01
Diastolic BP (mmHg)	75 ± 13	83 ± 10	<0.001
FBS (mg/dl)	93.0 ± 12.5	163.3 ± 80.7	<0.001
HbA1c (%)	4.6 ± 0.66	7.4 ± 2.3	<0.001
Insulin (μIU/dl)	7.0 ± 5.2	8.2 ± 7.8	0.248
HOMA-IR	1.7 ± 1.4	3.8 ± 3.3	0.002
HOMA-B (%)	91.4 ± 64.4	47.4 ± 53.3	<0.001
QUICKI	0.375 ± 0.048	0.342 ± 0.037	<0.001
TG (mg/dl)	155 ± 83	166 ± 65	0.61
TC (mg/dl)	189 ± 38	166 ± 44	0.002
LDL-C (mg/dl)	107 ± 26	92 ± 29	0.002
HDL-C (mg/dl)	47 ± 10	46 ± 11	0.372
TG/HDL-C ratio	3.5 ± 2.2	3.9 ± 1.7	0.279
Albumin (g/dl)	4.6 ± 0.5	4.4 ± 0.4	0.027
Uric acid (mg/dl)	5.3 ± 1.2	5.2 ± 1.5	0.181
TAC (nmol/lit)	1155± 1255	999 ±256	<0.001
Trp (mmol/lit)	69.5 ± 17.8	62.3 ± 28.1	0.168
Kyn (mmol/lit)	0.88 ± 0.56	1.15 ± 0.44	0.08
KTR	0.013 ± 0.006	0.022 ±0.014	0.028

BMI Body mass index, *BP* blood pressure, *FBS* fasting blood sugar, *HOMA-IR* Homeostatic model assessment of insulin resistance, *QUICKI* quantitative insulin sensitivity check, *HOMA-B* homeostatic model assessment to quantify beta-cell function, *TG* Triglyceride, *TC* Total cholesterol, *LDL-C* Low density lipoprotein cholesterol, *HDL-C* High density lipoprotein cholesterol, *Trp* Tryptophan, *Kyn* Kynurenine, *KTR* Kynurenine/Tryptophan ratio. Data were analyzed using Student T test or Mann-Whitney-U test. $p < 0.05$ was considered as significant difference among groups

Table 2 Correlation of HOMA-IR and HOMA-B with various variables in the T2DM group

Variable	HOMA-IR		HOMA-B (%)	
	r	p	r	p
Age (Years)	−0.200	0.104	−0.275	0.028
BMI(Kg/m^2)	0.056	0.660	0.345	0.006
HbA1c (%)	0.253	0.039	−0.644	<0.001
Trp (mmol/lit)	0.242	0.304	0.326	0.161
Kyn (mmol/lit)	−0.119	0.618	0.621	0.003
KTR	0.083	0.729	−0.289	0.128
TAC (nmol/lit)	−0.302	0.013	−0.336	0.007

HOMA-IR Homeostatic model assessment of insulin resistance, *QUICKI* quantitative insulin sensitivity check, *HOMA-B* homeostatic model assessment to quantify beta-cell function. Data were analyzed using Spearman correlation test. $p<0.05$ was considered as significant difference

Table 3 Correlation of serum TAC levels with various variables in the control and T2DM groups

Characteristics	Control group		T2DM group	
	r	p	r	p
Albumin (g/dl)	0.740	<0.001	0.523	<0.001
Uric acid (mg/ml)	0.476	<0.001	0.643	<0.001
HOMA-IR	−0.245	0.047	−0.322	0.013

TAC Total antioxidant capacity. Data were analyzed using Spearman correlation test. $p<0.05$ was considered as significant difference

Comparison of serum oxidant parameters in T2DM and control groups

In the T2DM patients, Spearman correlation analyses revealed a significant positive correlation between TAC and uric acid (r = 0.476, $p<0.001$) and albumin (r = 0.740, $p<0.001$), while it was inversely correlated with HOMA-IR (r = −0.322, $p=0.013$). These factors entered in a stepwise regression model to determine their independent roles in predicting TAC. The results of stepwise regression analyses showed that uric acid (β = 0.453, $p<0.001$), albumin (β = 0.316, $p=0.003$), and HOMA-IR (β = −0.251, $p=0.045$) were independently predictive of TAC in patients with T2DM. Similar results were obtained in the control group, where TAC showed a positive association with serum albumin as well as uric acid and negative correlation with HOMA-IR (Table 3). The findings of stepwise regression analyses revealed independent role of uric acid (β = 0.462, $p<0.001$), albumin (β = 0.323, $p=0.001$), and HOMA-IR (β = −0.324, $p=0.002$) in predicting TAC values in the control individuals. No significant association was observed between TAC and serum Kyn, Trp, and KTR.

Biochemical characteristics of T2DM patients based on glycemic control status

Table 4 reveals the results of biochemical characteristics in the T2DM patients with poor glycemic control (G2 group; A1c ≥ 8.0), the patients with good glycemic control (G1 group; A1c < 8.0), and healthy control individuals. As to the age and BMI of the studied groups, there was no significant difference. The values of systolic and diastolic blood pressures in the T2DM patients with poor and good glycemic control were significantly higher compared to control subjects while no significant difference was observed between the T2DM patients with poor and good glycemic control. As shown in the Table 4, we found no significant difference in the serum Kyn levels between the control and T2DM patients. However**,** in the T2DM patients' lower serum Trp levels were observed in those with poor glycemic control (G2 group; A1c ≥ 8.0) compared to the diabetic patients with good glycemic control and as well as control group. The level of KTR was significantly higher in the T2DM patients with poor glycemic control (G2 group; A1c ≥ 8.0) compared to the patients with good glycemic control and as well as control group. No significant difference was observed in KTR between the T2DM patients with good glycemic control and healthy control group.

Discussion

Clinical and experimental studies have revealed a critical role of chronic low-grade inflammation in the pathogenesis of T2DM and its complications [30]. Thus, identifying molecular pathways that link inflammation to T2DM may help to develop new preventive and therapeutic strategies for T2DM. IDO is an immunomodulatory enzyme that is involved in chronic low grade inflammation [31]. It is proposed that IDO might be involved in the pathogenesis of T2DM. In this regard, the association of dysregulated IDO activity with IR and impaired beta cell function, hall marks of T2DM, has been revealed in a previous study [18]. Furthermore, Laurans et al. have reported that inhibition or deletion of IDO gene in mice improved insulin sensitivity and decreased blood sugar [32]. The results of the present study demonstrated a significantly higher value of KTR, an index of IDO activity, in patients with T2DM compared to healthy control individuals. This finding is in agreement with the study of Hussain et al., who showed a higher serum Kyn level in patients with T2DM compared to the control group, suggesting a higher IDO activity in T2DM patients [33]. However, they estimated IDO activity by a colorimetric measurement of serum Kyn, without measuring serum Trp, which is not an accurate method for IDO assay. In the present study, we used HPLC method for measuring Kyn

Table 4 Biochemical characteristics based on glycemic control status

Variables	Control	Type 2 diabetes		P value		
	C: A1c < 6.0 n = 74	G1:A1c < 8.0 n = 36	G2:A1c ≥ 8.0 n = 38	C &G1	C& G2	G1 & G2
HbA1c (%)	4.6 ± 0.7	6.2 ± 1.0	10.2 ± 1.6	<0.001	<0.001	<0.001
Age(years)	46.1 ± 6.1	48.1 ± 6.7	48.2 ± 6.1	0.105	0.187	0.943
BMI (kg/m^2)	27.9 ± 4.0	29.4 ± 5.0	28.9 ± 5.5	0.084	0.363	0.702
Systolic (mmHg)	119 ± 14	125 ± 15	127 ± 13	0.036	0.027	0.562
Diastolic (mmHg)	75 ± 13	85 ± 10	79 ± 9	0.002	<0.001	0.182
FBS (mg/dl)	92.3 ± 12.2	125.9 ± 25.4	242.2 ± 88.8	<0.001	<0.001	<0.001
Insulin (μIU/dl)	7.2 ± 5.1	7.9 ± 7.8	7.4 ± 7.1	0.506	0.856	0.764
HOMA-IR	1.7 ± 1.3	2.5 ± 2.4	5.2 ± 4.8	0.151	<0.001	<0.001
HOMA-B (%)	94.7 ± 66.8	56.4 ± 58.1	16.3 ± 13.6	<0.001	<0.001	0.008
QUICKI	0.373 ± 0.047	0.351 ± 0.034	0.332 ± 0.045	0.005	<0.001	0.081
TAC (nmole/lit)	1139 ± 258	999 ± 248	1013 ± 272	0.002	0.034	0.824
Trp (mmole/lit)	69.5 ± 17.8	75.9 ± 32.3	52.5 ± 28.1	0.427	0.019	0.012
Kyn (mmole/lit)	0.88 ± 0.56	1.19 ± 0.55	1.12 ± 0.65	0.106	0.146	0.768
KTR	0.013 ± 0.006	0.018 ± 0.015	0.025 ± 0.015	0.208	0.002	0.173

FBS fasting blood sugar, *HOMA-IR* Homeostatic model assessment of insulin resistance, *QUICKI* quantitative insulin sensitivity check, *HOMA-B* homeostatic model assessment to quantify beta-cell function, *TAC* total antioxidant capacity, *Trp* Tryptophan, *Kyn* Kynurenine, *C* control group, *G1* T2DM patients with HbA1c < 8.0, *G2* T2DM patients with A1c ≥ 8.0. The presented data are mean ± SD. One-way ANOVA followed by LSD post hoc tests or Kruskal-Wallis test were used to analyze the data. $p < 0.05$ was considered as significant difference between groups

and Trp that is the most sensitive and accurate method for measuring IDO activity [34]. The association between low grade chronic inflammation and poor glycemic control in patients with T2DM has been shown in previous studies [35, 36]. Our data revealed a significant lower amount of serum Trp in the T2DM patients with poor glycemic control (A1c ≥ 8.0) compared to those with moderate glycemic control as well as the control group. In addition, our results exhibited a significant higher IDO activity in the T2DM patients with poor glycemic control (HbA1c ≥ 8.0) [37] compared to control subjects, while we could find no significant difference between IDO activity of control individuals and T2DM patients with moderate glycemic control, suggesting the inducing effects of hyperglycemia on IDO activity. In line with these findings, Kartika et al. [38] have recently shown IDO upregulation in PBMC cells of T2DM patients both in basal state and following stimulation with phytohemagglutinin, a stimulant of IF-γ secretion, suggesting that the low-grade chronic inflammation present in patients with T2DM causes dendritic cells and macrophages to increase IDO expression. IDO then ameliorates chronic inflammation through suppression of effector T cells activity.

IR and impaired beta cell function are two main hallmarks of T2DM [39]. In this study, we used HOMA-IR and HOMA-B to evaluate IR and beta cell dysfunction, respectively. Our results showed higher level of HOMA-IR in patients with T2DM compared to the control group, suggesting insulin resistance in our patients. Spearman correlation analyses revealed that HOMA-IR was positively correlated with HbA1c and inversely associated with TAC. Furthermore, in the regression analyses models, both HbA1c and TAC remained as predictive factors of HOMA-IR. These findings are consistent with those of previous studies and suggest independent roles of both hyperglycemia and oxidative stress in the development of IR in T2DM patients [40, 41]. The mean of HOMA-B, an index of beta cell function, was significantly lower in patients with T2DM compared to the control group, suggesting the presence of impaired beta cell function in T2DM patients. Spearman correlation analyses revealed a significant positive correlation between HOMA-B and serum Trp levels, while HbA1c, TAC, and age of the patients were inversely correlated with HOMA-B. Bozkurt et al. showed that in women with gestational diabetes, increased HbA1c is associated with beta cell dysfunction [42]. The association between oxidative stress and dysfunction of beta cells has also been shown in several studies [43]. Chen et al. [44] have also revealed a direct association between circulating Trp concentration and function of beta cells, which is consistent with our findings.

It has been reported that exercise could alter Kyn and Trp circulating levels through changes in the expression of enzymes involved in the Kyn metabolism [45]. In addition, plasma Trp and Kyn are also influenced by diet [45, 46]. A limitation of our study is that we did not consider the effects of these confounding factors. Furthermore, our study is limited in sample size. Finally, our data revealed that IDO

activity in the patients with T2D is positively correlated with the level of hyperglycemia, however the mechanism did not investigate in the present study. Therefore, further studies with a larger sample size and considering confounding variables are necessary.

All in all, these results suggest that T2DM patients with poor glycemic control have higher IDO activity. Due to the relationship between increased activity of this enzyme and diabetes complications such as nephropathy and retinopathy, control of the activity of this enzyme can be considered to overcome the complications of diabetes.

Acknowledgements This study has been extracted from the MSc thesis of Sara Abedi from the Department of molecular biology-Biochemistry, Islamic Azad university, Shiraz branch, Shiraz, Iran. We are thankful to all the staff members of the Diagnostic Laboratory Sciences and Technology Research Center for their technical assistance.

Declarations

Conflict of interest No conflict of interest is declared by the authors.

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