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ORIGINAL ARTICLE

Circulating IL-8 levels are increased in patients with type 2 diabetes and associated with worse inflammatory and cardiometabolic profile

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Abstract
Aims Interleukin-8 (IL-8) is a chemokine involved in systemic immunity, macrophages infiltration and activation in adipose tissue and may play a significant role in the pathogenesis of type 2 diabetes (T2D) and atherosclerosis. Aims of this study were to evaluate circulating IL-8 levels in adult patients with T2D in comparison with non-diabetic subjects and to describe clinical and biochemical correlates of IL-8 concentration.
Methods For this cross-sectional study, we enrolled 79 consecutive T2D individuals referring to our diabetes outpatient clinics at Sapienza University of Rome, and 37 sex, age and BMI comparable non-diabetic subjects as a control group. Clinical parameters and medical history were recorded; fasting blood sampling was performed for biochemistry and for measuring serum IL-8, IL-6, TNF-α, CRP, adiponectin and 25(OH)vitamin D [25(OH)D] levels.
Results Patients with T2D exhibited significantly higher serum IL-8 levels than non-diabetic subjects (69.27 ± 112.83 vs. 16.03 ± 24.27 pg/mL, $p < 0.001$). In diabetic patients, increased IL-8 concentration correlated with higher IL-6 ($p < 0.001$), TNF-α ($p = 0.02$), FBG ($p = 0.035$), HbA1c ($p = 0.04$) and LDL-C ($p = 0.04$) and with lower adiponectin ($p = 0.02$) and 25(OH)D ($p = 0.003$) concentrations.
Conclusions Patients with T2D display a marked elevation of circulating IL-8 levels which identify subjects with worse inflammatory, glycometabolic and lipid profile and lower vitamin D levels. Further studies are warranted for evaluating a possible role of IL-8 as a novel marker for risk stratification in T2D patients.

Keywords Type 2 diabetes · Interleukin-8 · Adipose tissue dysfunction · Cytokines · Clinical science

Introduction

Type 2 diabetes (T2D) is characterized by a condition of systemic low-grade inflammation which represents a key factor for the development of insulin resistance and associated comorbidities, such as non-alcoholic fatty liver disease (NAFLD) and atherosclerosis [1–7]. In this scenario, adipose tissue (AT) acts as an endocrine and immune organ by secreting many bioactive peptides and, thus, influencing insulin action. Advances in obesity research have led to the recognition that insulin resistance is tightly connected with immunity. In condition of obesity, AT undergoes infiltration and activation of immune cells and, mostly, macrophages, which represent a major source of inflammatory mediators [8] and trigger, in turn, the development of AT-insulin resistance [9, 10].

Indeed, the coexistence of systemic and AT-associated chronic inflammation and impaired insulin sensitivity lay the basis for the development of T2D [11].

Several cross-sectional studies showed that insulin resistance and T2D are associated with higher circulating levels of C-reactive protein (CRP), IL-6 and TNF-α

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[12–17]; in addition, the chemokine system, in particular interleukin-8 (IL-8), came more recently into the focus of metabolic inflammation research [18–20].

IL-8 is a pro-inflammatory polypeptide belonging to the CXC chemokine superfamily, characterized by the presence of two cysteine residues separated by an intervening aminoacid in the first three positions [21], and is secreted by several cell types, including adipocytes, monocytes/macrophages, T-lymphocytes, endothelial and epidermal cells [22, 23]. As a multifunctional chemokine, it has chemoattractant and mitogenic effects on neutrophils [24], as well as on T-cells, vascular smooth muscle cells, vascular endothelial cells and monocytes [25–28].

Several studies have described an association between IL-8 and hepatic metabolic pathways in humans. In particular, IL-8 was shown to be produced by hepatocytes under the stimulus of free fatty acids via the activation of NF-kB and JNK pathways [29]. Moreover, IL-8 secretion significantly increased in condition of chronic liver diseases, suggesting a role for this cytokine in the recruitment and activation of hepatic macrophages [30]. Indeed, several studies demonstrated a role of IL-8 in pathogenesis and progression of NAFLD [31–37].

Among its multiple actions, IL-8 also promotes macrophages infiltration in AT [38], inducing local and systemic inflammation and representing, in turn, a potential link between AT dysfunction and insulin resistance-related conditions [31–40].

Data showed that IL-8 enhanced the IL-8 mRNA expression in human adipocytes, and adipocytes expressed the main receptor for IL-8, playing, thus, an autocrine effect on these cells [41]. Furthermore, Kobashi et al. [41] demonstrated that the inflammatory stimulation creates a vicious circle of IL-8 production in human adipocytes via ERK pathway and/or p38 MAPK pathway. Taken together, these data suggest that IL-8 plays an important role in both the initiation and the maintenance of inflammation in the AT. Additionally, in vitro studies showed that IL-8 induced insulin resistance via the inhibition of insulin-induced Akt phosphorylation in human adipocytes and suggested that the attenuation of IL-8 production and/or action may represent a target for the prevention of diabetes and its complications. These results support the hypothesis of a central role of IL-8 in influencing adipocyte physiology beyond its traditional function in the recruitment of inflammatory cells, but despite the bulk of data on IL-8 involvement in cardiometabolic diseases, studies specifically designed for assessing the role of IL-8 in diabetes are lacking. Therefore, aims of this study were to evaluate circulating IL-8 levels in adult patients with T2D in comparison with non-diabetic subjects and to describe clinical and biochemical correlates of IL-8 concentration.

Methods

For our purposes, we enrolled 79 patients with T2D (M/F: 45/34, age: 58 ± 9 years, BMI: 33.65 ± 6.3 kg/m^2, T2D duration: 7.1 ± 6.3 years) among those referring to our diabetes outpatient clinics at Sapienza University of Rome, and 37 non-diabetic subjects comparable for sex, age and BMI (M/F: 20/17, age: 54 ± 17 years, BMI: 34.29 ± 4.4 kg/m^2, *p*-values not significant) not affected by any comorbidities and not treated with any medications at the time of study recruitment, as a control group.

Each subject underwent medical history collection, physical examination, with measurement of height, weight, waist circumference, systolic and diastolic blood pressure (SBP, DBP; mmHg), hepatic ultrasound (US) for the evaluation of NAFLD, and blood sampling for assessing biochemistry and immune-inflammatory profile. Fasting blood glucose (FBG, mg/dL), glycosylated hemoglobin (HbA1c, %–mmol/mol), total cholesterol (mg/dL), HDL cholesterol (mg/dL), triglycerides (mg/dL), blood ureic nitrogen (BUN, mg/dL), creatinine (mg/dL), aspartate aminotransferase (AST, IU/L), alanine aminotransferase (ALT, IU/L), alkaline phosphatase (ALP, IU/L) and gamma-glutamyl transpeptidase (γ-GT, IU/L) were measured by standard laboratory methods. LDL-cholesterol (mg/dL) was obtained using the Friedwald formula. Fasting plasma insulin levels were measured by radio-immuno-assay (μU/L, ADVIA Insulin Ready Pack 100, Bayer Diagnostics, Italy); indexes of insulin resistance (HOMA-IR) and secretion (HOMA-β%) have been calculated as reported elsewhere [42].

Serum IL-8, IL-6 and TNF-α levels (pg/mL) were measured by BioPlex Multiplex Immunoassays Biorad on sera frozen immediately after separation and stored at −25 °C for few days. Circulating adiponectin concentration was assessed by immunoenzymatic assay (Phoenix Pharmaceutical, Inc.) and CRP by high-sensitivity CRP assay (mg/dL, latex-enhanced immunoturbidimetric).

Among molecules with immunomodulation properties, we assessed systemic levels of vitamin D by dosing serum concentration of 25(OH)vitamin D [25(OH)D], the most stable form of this hormone, by colorimetric method (LIAISON, DiaSorin).

Statistics

SPSS version 17 statistical package was used to perform all the analyses. Student's *T* test for continuous variables and χ^2 test for categorical variables were performed to compare mean values between two independent groups. Skewed variables underwent logarithmic transformation before the analyses. Correlations between continuous variables were calculated by Pearson's coefficient, whereas Spearman's coefficient was used for dichotomic/ordinal parameters.

Correlation coefficients are reported as *r* values in the text. Bivariate and multivariate linear regression models were used to detect the association between serum IL-8, considered as a continuous variable, and all possible determinants. This is the first study specifically designed to investigate IL-8 profile in subjects with T2D in relation to the inflammatory and metabolic profile, therefore in order to calculate the study power we performed a post hoc sample size calculation showing that based on the two groups IL-8 mean and common standard deviation, a minimum of 23 subjects per group were needed for obtaining statistically significant results with α-error = 0.05 and power = 0.80.

Data are shown as mean ± standard deviation (SD) in both text and tables. A *p*-value <0.05 was considered statistically significant.

Results

Patients with T2D had significantly higher serum IL-8 levels than non-diabetic subjects (mean ± SD: 69.27 ± 112.83 pg/mL vs. 16.03 ± 24.27 pg/mL, $p < 0.001$); of note, diabetic patients also displayed higher CRP levels, total cholesterol, transaminases, SBP and lower adiponectin concentration than control group. Clinical and biochemical characteristics of study populations are summarized in Table 1.

In T2D patients, increased serum IL-8 levels correlated with higher IL-6 ($r = 0.33$, $p < 0.001$) and TNF-α ($r = 0.22$, $p = 0.02$). The positive association between IL-8 and TNF-α applied also to non-diabetic controls ($r = 0.46$, $p = 0.005$). Furthermore, in the diabetes cohort, higher IL-8 correlated with worse glycemic control (FBG, $r = 0.22$, $p = 0.035$; HbA1c, $r = 0.25$, $p = 0.04$) and lipid profile (LDL, $r = 0.23$, $p = 0.04$) and with lower serum concentration of adiponectin ($r = -0.29$, $p = 0.02$) and 25(OH) D ($r = -0.35$, $p = 0.003$). By contrast, no association was found between serum IL-8 and either parameters of total

Table 1 Clinical and biochemical characteristics of patients with T2D and controls

	T2D ($n = 79$)	Controls ($n = 37$)	*p*-value
Gender (M/F)	45/34	20/17	n.s.*
Age (years)	58 ± 9	54 ± 11	n.s.
BMI (kg/m^2)	33.65 ± 6.3	34.29 ± 4.4	n.s.
Waist circumferences (cm)	113.7 ± 12.8	98.7 ± 16.5	n.s
SBP (mmHg)	130.2 ± 16.4	124.8 ± 10.5	0.01
DBP (mmHg)	81.7 ± 9.7	79.3 ± 8	n.s.
FBG	132.1 ± 38.1	97.9 ± 16	0.001
HbA1c (%–mmol/mol)	6.8 ± 1.02–49 ± 5	–	–
Triglycerides (mg/dL)	134.2 ± 62.7	122.4 ± 70.9	n.s.
Total cholesterol (mg/dL)	178.6 ± 37	158 ± 24.7	0.02
HDL (mg/dL)	50.3 ± 14.7	54.7 ± 17.8	n.s.
LDL (mg/dL)	101.3 ± 33.2	117.9 ± 32.2	n.s.
AST (IU/L)	24.12 ± 12.44	18.35 ± 4.49	0.04
ALT (IU/L)	32.63 ± 21.38	24.65 ± 13.3	n.s.
GGT (IU/L)	31.87 ± 5.99	19.1 ± 8.26	–
Fasting plasma insulin (μU/L)	12.15 ± 5.52	16.3 ± 10.3	n.s.
HOMA-IR	3.84 ± 1.81	4.7 ± 2.5	n.s.
HOMA-β (%)	87.89 ± 63.8	200 ± 127.9	0.01
25(OH)D (ng/mL)	13.5 ± 7.5	17.9 ± 9.3	n.s.
IL-8 (pg/mL)	69.27 ± 112.8	16.03 ± 24.2	<0.001
IL-6 (pg/mL)	9.2 ± 52.1	2.9 ± 4.3	n.s.
TNF-α (pg/mL)	8.3 ± 49.9	2.7 ± 4.6	n.s.
CRP (mg/L)	3.05 ± 3.92	0.8 ± 1.63	<0.001
Adiponectin (ng/mL)	6.8 ± 3.2	13.4 ± 5.6	0.02
Statins (%)	52%	0	<0.001*
Antidiabetic therapy (%)	89%	0	<0.001*
Insulin therapy (%)	17%	0	<0.001*

Values are expressed by mean ± SD. *T* test for independent samples test applied. * χ test applied. *p*-values <0.05 are considered statistically significant

body adiposity, such as obesity, BMI and waist circumference, or systemic insulin resistance/secretion, as expressed by HOMA-IR and HOMA-β%. Moreover, no correlation was found between circulating IL-8 levels and the presence of US-detected NAFLD and serum transaminases. In total, 72% of T2D patients had systemic blood hypertension, 87% non-alcoholic fatty liver disease (NAFLD), 52% dyslipidemia, 56% obesity and 5% suffered from ischemic cardiopathy. No association was found between comorbidities and serum IL-8 levels in this population (hypertension: $r = -0.09$, $p = 0.43$; NAFLD: $r = 0.03$, $p = 0.23$; dyslipidemia: $r = -0.13$, $p = 0.24$; obesity: $r = -0.02$, $p = 0.89$; ischemic cardiopathy: $r = -0.15$, $p = 0.23$).

Within T2D subgroup, 85% of patients were treated with oral antidiabetic agents (OAD) and 17% with insulin, with or without ADO; 52% were also in treatment with statins. Thus, we performed bivariate correlation analyses showing no association between circulating IL-8 levels and therapies, both when considering just the presence of OAD treatment per se and when comparing different classes of OAD or insulin. Similarly, treatment with statins did not affect IL-8 in our population. Correlations among IL-8 concentration and clinical/biochemical parameters are shown in Table 2. For ascertaining the main determinant of increased IL-8 levels in the whole study population, we performed a multivariate linear regression analysis, showing that T2D was associated with higher IL-8 independently from the possible confounders (β: 0.52, $p = 0.0001$) (Table 3).

In addition, although higher CRP levels were observed in T2D patients compared to controls, CRP was not related to markers of increased risk profile, such as impaired glycemic control and lipid profile at the bivariate correlation analyses (data not shown). Furthermore, although higher CRP was associated with the presence of obesity ($r = 0.28$, $p = 0.009$), increased CRP levels did not identify a cytokine pattern suggestive of AT-related inflammation, showing no correlation with adiponectin ($r = 0.025$, $p = 0.85$), IL-6 ($r = 0.16$, $p = 0.15$) or TNF-α ($r = -0.07$, $p = 0.51$) concentration.

Table 2 Serum IL-8 levels in patients with type 2 diabetes—bivariate correlation analyses

Parameter	Correlation coefficient	*p*-value
Sex	0.07	0.4
Age	0.04	0.8
Diabetes' duration	0.05	0.68
BMI	−0.08	0.56
Waist circumference	−0.1	0.24
SBP	0.15	0.38
DBP	0.09	0.41
Total cholesterol	0.07	0.46
HDL	−0.1	0.9
LDL	0.23	0.04
Triglycerides	0.08	0.39
FBG	0.22	0.035
AST	0.02	0.87
ALT	0.04	0.73
GGT	0.05	0.63
NAFLD	0.03	0.23
HbA1c	0.25	0.04
HOMA-IR	0.14	0.3
HOMA-β (%)	−0.08	0.56
25(OH)D	−0.35	0.003
IL-6	0.33	<0.001
TNF-α	0.22	0.02
Adiponectin	−0.29	0.02
CRP	0.18	0.09
Obesity (yes/no)	−0.05	0.62

Discussion

Our study demonstrates that patients with T2D display significantly higher IL-8 levels than non-diabetic subjects which identify individuals with greater AT-associated inflammation, worse glycometabolic profile and low vitamin D levels.

Few previous studies investigated circulating IL-8 concentration in presence of diabetes, reporting inconclusive results. Zozulinska et al. [43] first found increased IL-8 levels in a small cohort of both type 1 and type 2 diabetic patients in poor glycemic control, in comparison with non-diabetic subjects. According to our results, they observed a linear correlation between higher IL-8 levels and HbA1c, but no data regarding systemic immune-inflammatory profile, clinical parameters such as adiposity and dysmetabolic conditions other than diabetes, were available in this study population [43].

Higher IL-8 levels in T2D, tightly associated with FBG, were also found in a report by Esposito et al. [44] examining a panel of inflammatory biomarkers in a cohort of 30 non-obese individuals with newly diagnosed T2D, naive for antidiabetic treatments. Contrarily, in a small intervention trial testing the effect of ticlopidine therapy on vascular complication in T2D, no difference was found in IL-8 levels between diabetic and non-diabetic individuals [45].

Interestingly, Samaras et al. [46] investigated SAT and VAT gene expression in individuals with or without T2D and demonstrated significantly higher IL-8 expression in VAT from T2D patients compared to VAT from non-diabetic controls. These data, along with our findings on increased circulating IL-8 levels in T2D subjects, support the hypothesis of a role for IL-8 as a marker of AT inflammation in presence of diabetes.

Table 3 Multivariate linear regression analysis

Parameter	Unstandardized coefficients		Standardized coefficients	*t*	*p*-value
	β	Standard deviation error	β		
Constant	1.439	0.403		3.567	0.001
DMT2	0.526	0.128	0.376	4.121	0.0001
Age	−0.007	0.006	−0.111	−1.193	0.236
Sex (M/F)	−0.135	0.124	−0.103	−1.087	0.279
BMI	−0.003	0.006	0.039	0.409	0.684
Waist circumference	−0.786	0.666	0.312	1.180	0.244

Circulating IL-8 concentration is the dependent variable. $R^2 = 0.41$

Of note, in our study population, IL-8 assessment showed high standard deviation and, as observed for other circulating molecules, several study participants reported undetectable serum IL-8 levels. However, unlike other cytokines, increased IL-8 concentration was able to identify a specific phenotype among patients with T2D, characterized by the presence of worse glycometabolic and inflammatory systemic profile.

This is the first study specifically designed to investigate IL-8 profile in subjects with T2D in relation to both systemic and AT-associated inflammation and clinical markers of metabolic diseases. Thus, beside showing the existence of a relationship between higher IL-8 and the presence of diabetes, our study demonstrated that, differently from what observed for a specific pro-inflammatory markers such as CRP, increased IL-8 levels are associated with unfavorable systemic inflammatory pattern—as expressed by higher IL-6 and TNF-α and lower adiponectin and active vitamin D levels—and worse glycemic and lipid profile.

In condition of chronic positive energy balance, AT is challenged to store large amounts of triglycerides and glucose-derived free fatty acids, leading to adipocytes expansion; however, once they are no longer able to increase in volume for safely storing nutrients, AT becomes dysfunctional, instigating FFAs spillover and releasing pro-inflammatory cytokines, thus contributing to AT-insulin resistance and chronic low-grade inflammation [47]. Indeed, in condition of visceral AT expansion, the main contributor to the development of AT inflammation is likely represented by the limited AT capacity to appropriately react to calories overload, as shown in non-obese subjects with T2D [48].

Notably, in this study no direct correlation was found between IL-8 and indicators of adiposity, such as BMI and waist circumference, likely showing that AT inflammation and impaired physiology, rather than obesity *per se*, may represent the key determinant of increased circulating IL-8 concentration in diabetes. This conclusion is reinforced by the evidence of an association between AT dysfunction and impaired IL-6, TNF-α and adiponectin concentrations [48–50] in dysmetabolic conditions. Thus, the above cytokines tightly associated with higher IL-8 levels in our study.

As to immune-inflammatory profile, we also observed an inverse association between IL-8 and circulating levels of vitamin D, known to display, beside its action on calcium and bone metabolism, a modulatory action on both innate and adaptive immunity [51, 52].

Since hypovitaminosis D is a risk factor for macrovascular complications in diabetes [53–55] and for all-cause and cardiovascular mortality in the general population [56], it is plausible to hypothesize that increased IL-8 levels and impaired vitamin D status may act "in synergy" determining worse metabolic risk profile in presence of diabetes. Thus, a preexisting condition of hypovitaminosis D and subsequent impaired immune regulation [51] could enhance the systemic low-grade inflammation underlying insulin resistance and diabetes with detrimental effects on cardiovascular outcomes.

Our study has some limitations. First, the cross-sectional design of this study does not allow to establish a certain causal relationship between increased IL-8 production and AT dysfunction, but we may speculate that, in T2D patients, higher circulating IL-8 levels represent and expression of AT inflammation, which, in turn, could lead to worse risk profile in diabetes. Second, the lack of AT histological data still considered the gold standard for identifying AT inflammation.

In conclusion, our study demonstrates that patients with T2D display a marked elevation of circulating IL-8 levels which identify subjects with worse inflammatory state and metabolic control independently from total adiposity and other possible confounders. Prospective studies will be needed to further evaluate the relevance of IL-8 in the disease process and to clarify whether it can be considered as a novel marker and a useful tool for risk stratification in T2D patients.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

Ethical standard All procedures performed in the study were in accordance with the ethical standards of the institutional (Sapienza University of Rome) and national research committee and with the 1964 Helsinki Declaration and its 2008 amendments.

Informed consent Informed consent was obtained from all individual participants included in the study.

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