Endokrynologia Polska
DOI: 10.5603/EP.2014.0023
Tom/Volume 65; Numer/Number 3/2014
ISSN 0423–104X

The IL-6/IL-6R/sgp130 system and Th17 associated cytokines in patients with gestational diabetes

Stężenie interleukiny-6, receptora dla interleukiny-6 i glikoproteiny 130 oraz cytokin zależnych od limfocytów Th17 u pacjentek z cukrzycą ciążową

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Abstract

Introduction: Interleukin-6 (IL-6) is a pleiotropic cytokine which signals through a cell surface receptor complex consisting of a cognate receptor subunit (IL-6R) and glycoprotein 130 (gp130), which is considered an antagonist to the IL-6R/IL-6 pathway. The aim of the present study was to assess IL-6/IL-6R/gp130 system and Th17 associated cytokines in different time points during and after pregnancy in women with gestational diabetes mellitus (GDM) and normal glucose tolerance (NGT).

Material and methods: Serum levels of IL-6, sIL6R, sgp130, IL-17 and IL-23 were measured in 91 women divided into three groups: GDM in the 24th–28th week of gestation (visit 1), NGT at the 1st visit and GDM in the 29th-32nd week, and NGT at both visits.

Results: The patients with GDM recognised at the 1st visit had significantly higher IL-6 ($p = 0.02$) and sgp130 ($p = 0.03$) concentrations than had the women with NGT, whereas the women with GDM diagnosed at the 2nd visit had elevated sIL-6R concentrations ($p = 0.03$). The patients with low sIL-6R but high sgp130 concentration had significantly higher glucose levels ($p = 0.04$) and lower IL-6 values ($p = 0.04$) than had the patients with low sIL-6R and sgp130 concentrations. IL-17 and IL-23 were detected in approximately one-third of the population studied. A trend towards higher IL-17 levels was observed in the subjects with GDM, but the differences were not significant.

Conclusions: Our results suggest that an increased serum sgp130 concentration in the patients with GDM might represent a compensatory mechanism, controlling intracellular IL-6 signalling and preventing the activation of the IL-6/IL-6R pathway. **(Endokrynol Pol 2014; 65 (3): 169–175)**

Key words: *IL-6; IL-6R; gp130; IL-17; IL-23; gestational diabetes*

Streszczenie

Wstęp: Interleukina-6 (IL-6) jest plejotropową cytokiną, która przekazuje sygnał do wnętrza komórki za pośrednictwem receptora błonowego złożonego z właściwej podjednostki receptorowej (Il-6R) i glikoproteiny 130 (gp130), uważanej za antagonistę kompleksu IL-6R/IL-6. Celem pracy była analiza szlaku IL-6/IL-6R/gp130 i cytokin zależnych od limfocytów Th17 w II i III trymestrze ciąży oraz po porodzie u pacjentek z cukrzycą ciążową i prawidłową tolerancją glukozy.

Materiał i metody: Dokonano pomiaru stężenia IL-6, IL6R, gp130, IL-17 i IL-23 w surowicy 91 kobiet podzielonych na 3 podgrupy: pacjentki z cukrzycą ciążową zdiagnozowaną w 24.–28. tygodniu ciąży (wizyta 1), pacjentki z prawidłową tolerancją glukozy w trakcie 1 wizyty i cukrzycą ciążową rozpoznaną w 29.–32. tygodniu ciąży oraz pacjentki z prawidłową tolerancją glukozy w trakcie obu wizyt.

Wyniki: U pacjentek z cukrzycą ciążową rozpoznaną w trakcie 1 wizyty wykazano istotnie wyższe stężenia IL-6 ($p = 0,02$) i gp130 ($p = 0,03$) w porównaniu z kobietami z prawidłową tolerancją glukozy, podczas gdy pacjentki z cukrzycą ciążową rozpoznaną w trakcie 2 wizyty charakteryzowały się wyższym stężeniem IL-6R ($p = 0,03$). U pacjentek z niskim stężeniem IL-6R i wysokim stężeniem gp130 obserwowano znamiennie wyższe wartości glikemii ($p = 0,04$) i niższe stężenia IL-6 ($p = 0,04$) w porównaniu z kobietami z niskimi stężeniami IL-6R i sgp130. Obecność krążących IL-17 i IL-23 stwierdzono u około 1/3 spośród badanych kobiet. Wykazano również tendencję do wyższych stężeń IL-17 u pacjentek z cukrzycą ciążową, różnice nie były jednak istotne statystycznie.

Wnioski: Wyniki sugerują, że podwyższone stężenie gp130, obserwowane u kobiet z cukrzycą ciążową, może stanowić mechanizm kompensacyjny, zapobiegający nadmiernej aktywacji szlaku IL-6/IL-6R. **(Endokrynol Pol 2014; 65 (3): 169–175)**

Słowa kluczowe: *IL-6; IL-6R; gp130; IL-17; IL-23; cukrzyca ciążowa*

This study was supported by the State Committee for Scientific Research (grant No. N N407 141937).

Introduction

Interleukin-6 (IL-6) is a pleiotropic cytokine that regulates various physiological processes including inflammation, haematopoiesis, immune responses, and host defence mechanisms [1]. It signals through a cell surface type I cytokine receptor complex consisting of two subunits: a cognate receptor subunit (IL-6R), which

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specifically recognises IL-6, and a signal-transducing element, the glycoprotein 130 (gp130) [2–4]. Soluble forms of IL-6R (sIL-6R) and gp130 (sgp130), which can be detected in the blood, are biologically active, but exert opposite functions. In the extracellular compartment, sIL-6R acts as an agonist to IL-6 by forming sIL-6R/IL-6 complex, which can bind to membrane gp130 and initiate the signal transduction that accounts for most of the biological activity of IL-6 [5]. In contrast, sgp130 is considered an antagonist to the sIL-6R/IL-6 complex, preventing sIL-6R/IL-6 from binding to the membrane receptor [4, 6]. While IL-6 plasma levels change remarkably over time, sIL-6R and sgp130 concentrations fluctuate much less, and give more precise information about the activation of IL-6 pathway [2, 4, 5]. Though characterised as both a pro- [7–9] and anti-inflammatory [10, 11] cytokine, IL-6 has been identified as a potential mediator linking chronic low-grade inflammation with insulin resistance [12]. Moreover, recent studies have shown that increased secretion of IL-6 can promote the differentiation of Th17 lymphocytes, resulting in elevated levels of IL-17 [13], which in turn promotes inflammation, triggering downstream nuclear factor-κB and cytokine production [14–16]. Another main inducer of IL-17 seems to be monocyte and dendritic cell-derived IL-23 [16–18]. Conversely, adipocytes express significant levels of the IL-17 receptors and respond to IL-17 by secreting IL-6 [19].

In the last decade, elevated IL-6 levels have been repeatedly found in patients with gestational diabetes mellitus (GDM) compared to healthy pregnant women [20-23], strongly suggesting that IL-6 may contribute to the development of glucose intolerance during pregnancy. However, the IL-6 trans-signalling pathway, and in particular sgp130, has never been studied in patients with GDM. Therefore in the present study we analysed the IL-6/IL-6R/sgp130 system at different time points during and after pregnancy in relation to (i) the disturbances of glucose tolerance, (ii) the indices of insulin sensitivity and insulin secretion, and (iii) the levels of Th17 associated cytokines such as IL-17 and IL-23.

Material and methods

Study population

The group studied consisted of 91 pregnant women attending the gynaecological out-patient clinic of the Medical University of Bialystok and tested for GDM with a 75 g 2-h oral glucose tolerance test (OGTT) between the 24th and 28th week of gestation (visit 1). GDM was diagnosed according to the criteria of the Polish Diabetological Association, with the following threshold glucose levels: fasting ≥ 100 mg/dL (5.5 mmol/L), 1 h ≥ 180 mg/dL (10.0 mmol/L) and 2 h ≥ 140 mg/dL (7.8 mmol/L). In order to evaluate whether women who developed GDM in the 2nd trimester showed the same disturbances of insulin sensitivity and pro-inflammatory cytokines secretion as those who developed GDM in a late phase of pregnancy, the patients with NGT at the 1st visit were tested again four weeks later (visit 2). All tests were repeated three months after childbirth. Patients with multiple pregnancy, abnormal glucose readings before recruitment, or complications such as pregnancy-induced hypertension or pre-eclampsia were excluded. Written informed consent was obtained from all participants, and the protocol was approved by the local ethics committee (Medical University of Bialystok).

Analytical methods

The OGTT was performed after an overnight fast and blood samples were collected at 0, 30, 60 and 120 min after glucose load. Plasma glucose concentration was measured using the oxidase method (CORMAY, Poland), serum insulin was assayed by the immunoradiometric method (Biosource Europe SA, Belgium) and glycated haemoglobin (HbA1c) was evaluated by a high performance liquid chromatography technique (BIO-RAD Laboratories, Germany). Total cholesterol, HDL-cholesterol, LDL-cholesterol and triglyceride concentrations were measured by enzymatic methods (ANALCO-GBG, Poland). Serum IL-6, sIL-6R and sgp130 concentrations were measured using commercial immunoassays (Quantikine, R&D Systems, USA). Serum IL-17 and IL-23 levels were also assayed by ELISA (Biosource Europe SA, Belgium and Invitrogen, USA, respectively).

Insulin secretion and sensitivity calculation

The following indices of insulin sensitivity and secretion were calculated:

— the Matsuda and de Fronzo index (IS_{OGTT}), defined as $10{,}000/\sqrt{[(FPG \times FPI) \times (G \times I)]}$, where FPG — fasting plasma glucose, FPI — fasting plasma insulin, G — mean glucose during the OGTT, and I — mean insulin (both calculated from glucose and insulin levels at 0, 30, and 120 min of the OGTT) [24];

— two disposition indices as the products of insulin sensitivity x insulin secretion ($DI_{30} = IS_{OGTT} \times AUC_{Ins30}/AUC_{Glu30}$ and $DI_{120} = IS_{OGTT} \times AUC_{Ins120}/AUC_{Glu120}$, where AUC_{Ins30}/AUC_{Glu30} — the ratio of the area under the insulin curve (AUC) to the area under the glucose curve during 0–30 min of the OGTT and $AUC_{Ins120}/AUC_{Glu120}$ — the ratio of insulin AUC to glucose AUC during 0–120 min of the OGTT) [25, 26]. The trapezoidal method was used to calculate glucose and insulin AUC during the OGTT;

— the homeostasis model assessment of insulin resistance (HOMA-IR) calculated as FPG × FPI/22.5 [27].

Statistical analysis

The data was analysed by the STATISTICA 10.0 for Windows Software (StatSoft.Inc, Tulsa, OK, USA). Before analysis, data was tested for normality of distribution using the Shapiro-Wilk test. Differences between the groups were compared by Mann-Whitney U test, and relationships between variables were tested by Spearman's rank correlations. One-way ANOVA with Bonferroni correction was used to compare the differences in interleukin concentrations during the course of pregnancy, after log-transformation of non-normally distributed variables. A p value of less than 0.05 was regarded as statistically significant.

Results

Clinical characteristics of the groups studied

The clinical characteristics of the groups studied are summarised in Table I. On the basis of the OGTT, the patients were divided into three groups: GDM at the first visit (GDM1, n = 24), normal glucose tolerance at the 1st visit and GDM at the 2nd visit (GDM2, n = 22), and the patients with normal glucose tolerance at the first visit who remained so four weeks later (NGT, n = 45).

The patients from the GDM1 group had significantly higher glucose and insulin at 120 min of the OGTT ($p < 0.00001$ and $p = 0.002$, respectively), and higher triglyceride concentrations ($p = 0.03$), as well as lower IS_{OGTT} ($p = 0.03$), DI_{30} ($p = 0.0005$) and DI_{120} ($p = 0.0005$) than had the women with NGT. At the 1st visit the women classified as having NGT after the first OGTT but who later developed GDM (GDM2), had markedly higher glucose at 120 min of the OGTT ($p = 0.02$), fasting and post-load insulin ($p = 0.006$ and $p = 0.04$, respectively), higher HbA1c ($p = 0.04$), HOMA-IR ($p = 0.01$) and triglyceride concentration ($p = 0.006$), as well as lower DI_{30} ($p = 0.02$) and DI_{120} ($p = 0.02$) compared to the subjects with NGT throughout pregnancy. The patients with GDM recognised at the 2nd visit had significantly higher fasting ($p = 0.02$) and 120 min. OGTT glucose levels ($p < 0.00001$), higher fasting and post load insulin ($p = 0.03$ and $p = 0.004$, respectively), higher HOMA-IR ($p = 0.001$), as well as markedly lower IS_{OGTT} ($p = 0.003$), DI_{30} ($p = 0.0009$) and DI_{120} ($p = 0.0002$) than had the women with NGT. Three months postpartum, there were no significant differences in anthropometric and metabolic variables between the patients with and without GDM in pregnancy.

The levels of IL-6/sIL-6R/sgp130 and Th17 associated cytokines in patients with and without GDM

At the first visit, the patients from the GDM1 group had significantly higher IL-6 ($p = 0.02$) and sgp130 ($p = 0.03$) concentrations than had the women with NGT (Table II). The women with low sIL-6R (below median value) and high sgp130 concentration had significantly higher fasting and 120′ postload glucose levels ($p = 0.04$), as well as lower DI_{30} ($p = 0.02$) and IL-6 values ($p = 0.04$) than had the patients with low sIL-6R and sgp130 concentrations.

At the 1st visit, IL-17 was detected in 35.5% of the women with NGT, 42% of the patients with GDM and 23% of the women with normal result of the 1st OGTT who later developed GDM (GDM2). The highest level of IL-17 was found in the GDM1 group, but the difference was not significant. IL-23 was detected in 31% of the subjects with NGT, 17% of the patients with GDM and 41% of the subjects with normal glucose tolerance after the 1st OGTT who later developed GDM. At the 1st visit, the lowest levels of IL-23 were observed in the GDM1 group (Table II).

At the 2nd visit, the women with GDM2 had markedly higher sIL-6R levels ($p = 0.03$) compared to the NGT subjects. There was also a trend towards higher IL-6 levels in patients with GDM2, but the difference was not significant. The patients with comparatively high sgp130 and low sIL-6R levels had significantly higher HbA1c value ($p = 0.04$) and lower IL-6 ($p = 0.04$) concentration compared to the subjects with high sIL-6R and low sgp130 values.

At the 2nd visit, IL-17 was detected in 51% of the women with NGT and 64% of the women with GDM, whereas IL-23 was detected in 20% of the subjects with NGT and 35.5% of the patients with GDM. Median concentrations of both interleukins did not differ significantly between the groups studied.

Three months postpartum, the women with previous GDM had significantly higher sgp130 concentrations than had the women with NGT during pregnancy ($p = 0.01$), whereas other interleukins levels did not differ between the two groups (Table II). IL-17 and IL-23 were detected in 33% and 37% of the women with previous GDM, as well as in 22% and 35% of women with NGT in pregnancy, respectively.

Serum sIL-6R and sgp130 levels increased significantly between the 1st and the 2nd visit in the GDM2 group ($p = 0.02$ and $p = 0.04$, respectively), whereas in the subjects with NGT, sgp130 concentrations increased in the same period with a borderline significance ($p = 0.057$). In both groups there was also a trend towards higher IL-6 levels at the 2nd visit, but the differences were not significant. Three months postpartum, sgp130 concentrations increased markedly in the women with previous GDM compared to the values observed in the 3rd trimester ($p = 0.04$). There was also an increase in IL-23 concentrations in both groups — with and without GDM – but the difference was significant only when all the patients were analysed together ($p = 0.039$).

Table I. *Characteristics of the groups studied*
Tabela I. *Charakterystyka badanych grup*

	1st visit			2nd visit		3 months postpartum	
	NGT	GDM2	GDM1	NGT	GDM2	NGT	GDM1 + GDM2
n	45	22	24	45	22	45	46
Age (years)	32 (28–35)	32 (28–34)	33 (31–34)	32 (28–35)	32 (28–34)	32 (28–35)	33 (29–35)
Gestational age (weeks)	25 (24–27)	25 (24–28)	25 (24–27)	30 (29–32)	30 (29–32)	N[A]	N[A]
Pre-pregnancy BMI [kg/m^2]	23.0 (20.4–26.2)	25.0 (24.2–28.0)	24.0 (22.1–27.0)	23.0 (20.4–26.2)	25.0 (24.2–28.0)	23.0 (20.4–26.2)	24.4 (22.4–28.0)
Current BMI [kg/m^2]	26.7 (22.9–29.4)	28.0 (27.0–31.1)	27.3 (25.2–31.1)	28.0 (25.5–30.8)	28.8 (27.1–31.4)	24.1 (21.5–27.3)	24.9 (22.9–29.8)
Birth weight [g]						3,400 (3,120–3,600)	3,440 (2,950–3,900)
Fasting glucose [mmol/L]	4.4 (4.1–4.6)	4.6 (4.4–4.8)	4.8 (4.0–5.2)	4.3 (4.0–4.8)	4.7 (4.4–4.8)[d]	4.8 (4.4–4.9)	4.9 (4.4–5.5)
Glucose 120 min [mmol/L]	6.1 (5.2–6.7)	6.6 (6.4–7.2)[d]	8.8 (8.2–9.2)[a]	6.2 (5.7–7.0)	8.4 (8.3–8.6)[a]	5.1 (4.6–6.4)	5.6 (4.6–6.5)
Fasting insulin [pmol/L]	82.0 (41.7–111.9)	112.4 (96.2–130.6)[d]	85.0 (70.1–149.9)	76.0 (42.4–117.0)	131.0 (99.0–146.7)[d]	78.0 (36.0–114.6)	89.3 (46.7–115.2)
Insulin 120 min [pmol/L]	385 (337–684)	462 (336–629)[d]	766 (610–881)[c]	502 (312–768)	834 (517–1,130)[c]	210 (140–352)	205 (161–371)
HbA1c (%)	4.7 (4.3–5.0)	5.0 (4.8–5.1)[d]	4.8 (4.4–4.9)	5.1 (4.8–5.5)	5.1 (4.9–5.4)	5.1 (4.9–5.3)	5.2 (4.8–5.5)
ISOGTT [mU/L, mg/dL]	4.71 (3.29–6.20)	3.91 (2.83–4.55)	3.31 (2.10–4.62)d	3.86 (3.21–4.75)	2.60 (2.00–3.51)[c]	6.70 (4.59–9.57)	5.35 (4.17–8.00)
DI30	218.6 (185.3–265.9)	186.8 (151.7–216.9)[d]	130.1 (97.0–210.2)[c]	214.0 (170.0–310.2)	131.6 (129.1–158.8)[a]	193.0 (149.0–251.1)	160.1 (125.0–221.6)
DI120	321.4 (261.3–409.0)	241.1 (210.5–271.9)[d]	201.5 (142.7–253.4)[c]	361.6 (274.1–426.4)	237.1 (186.8–264.2)[a]	50.0 (28.0–75.1)	53.0 (31.3–67.8)
HOMA-IR [mU/L, mmol/L]	2.37 (1.08–3.14)	3.14 (2.51–3.75)[d]	2.78 (2.00–4.99)	2.1 (0.98–3.39)	3.81 (2.69–4.29)[c]	2.30 (1.17–3.50)	2.66 (1.60–3.69)
Total cholesterol [mmol/L]	5.8 (5.4–6.5)	6.2 (6.0–7.0)	6.6 (5.5–7.0)	6.1 (5.8–7.0)	7.2 (6.4–8.1)	4.1 (3.7–4.7)	4.5 (4.3–5.5)
HDL-cholesterol [mmol/L]	1.8 (1.4–2.2)	1.8 (1.7–2.1)	1.8 (1.7–2.2)	1.8 (1.7–1.9)	1.8 (1.6–2.1)	1.7 (1.3–1.9)	1.7 (1.3–1.9)
LDL-cholesterol [mmol/L]	3.2 (2.5–3.8)	3.3 (3.0–4.5)	3.5 (2.5–3.8)	3.7 (2.6–4.9)	4.0 (3.6–5.0)	2.3 (1.9–2.9)	2.4 (2.2–3.1)
Triglycerides [mmol/L]	1.6 (1.4–2.2)	2.6 (2.1–2.9)[c]	2.3 (1.8–3.0)[d]	2.1 (1.7–2.5)	2.4 (2.1–3.0)	0.94 (0.70–1.00)	0.93 (0.69–1.06)

Data is shown as medians (interquartile range); NGT — normal glucose tolerance; GDM1 — gestational diabetes mellitus at the 1st visit; GDM2 — NGT at the 1st visit and GDM at the 2nd visit; IS_{OGTT} — OGTT insulin sensitivity index; DI — disposition index; HOMA-IR — homeostasis model assessment of insulin resistance; differences between NGT and GDM were significant at [a]$p < 0.0001$, [b]$p < 0.001$, [c]$p < 0.01$ and [d]$p < 0.05$ by Mann-Whitney U test

Table II. *Serum levels of interleukins studied in patients with and without GDM*

Tabela II. *Stężenia badanych interleukin u pacjentek z cukrzycą ciążową i prawidłową tolerancją glukozy.*

	1st visit			2nd visit		3 months postpartum	
	NGT	GDM2	GDM1	NGT	GDM2	NGT	GDM1 + GDM2
	45	22	24	45	22	45	46
IL-6 [pg/mL]	0.89 (0.75–1.07)	0.94 (0.77–1.30)	1.04 (0.96–1.24)[b]	1.02 (0.83–1.22)	1.13 (0.98–1.57)	0.96 (0.76–1.10)	0.87 (0.76–1.47)
sIL-6R [ng/mL]	49.34 (38.18–56.26)	46.32 (38.42–56.79)	52.95 (38.82–62.37)	37.71 (34.30–54.42)	51.49 (40.78–59.32)[b]	41.07 (31.80–54.52)	47.16 (40.70–54.07)
sgp130 [ng/mL]	307.0 (265.8–334.0)	304.1 (283.6–347.0)	336.9 (297.8–370.3)[b]	333.7 (295.4–389.1)	333.4 (294.7–357.9)	318.8 (302.7–332.2)	358.4 (327.7–393.8)[a]
IL-17 [pg/mL]	5.14 (2.73–25.69) n = 16	4.66 (3.90–7.81) n = 5	13.74 (10.00–16.92) n = 10	3.41 (2.04–23.60) n = 23	3.60 (2.01–4.72) n = 14	16.20 (13.41–66.06) n = 10	10.04 (3.18–18.00) n = 15
IL-23 [pg/mL]	20.03 (7.87–44.93) n = 14	11.41 (7.87–21.73) n = 9	3.7 (0.36–6.19) n = 4	7.87 (4.23–11.41) n = 9	11.41 (4.23–18.32) n = 10	16.61 (6.05–23.41) n = 16	21.73 (11.41–31.78) n = 17

Data is shown as medians (interquartile range); NGT — normal glucose tolerance; GDM1 — gestational diabetes mellitus at the 1st visit; GDM2 — NGT at the 1st visit and GDM at the 2nd visit; IL-6 — interleukin-6; sIL-6R — soluble IL-6 receptor; sgp130 — soluble glycoprotein 130; differences between NGT and GDM were significant at ap < 0.0001 and bp < 0.05 by Mann-Whitney U test

Correlations between serum levels of interleukins studied and other variables

At the 1st visit in the GDM1 group, there was a negative correlation between IL-6 concentration and DI_{30} (R = –0.63, p = 0.03), as well as a positive correlation between sgp130 and IL-17 concentration (R = 0.79, p = 0.03). In the women with NGT, IL-6 and IL-23 levels correlated positively with HbA1c (R = 0.34, p = 0.04 and R = 0.58, p = 0.01, respectively).

At the 2nd visit in the patients with GDM2, there was a negative correlation between IL-6 concentration and IS_{OGTT} (R = –0.53, p = 0.01), whereas in the healthy pregnant women IL-6 concentration correlated positively with HOMA-IR (R = 0.54, p = 0.038), as well as negatively with DI_{30} (R = –0.54, p = 0.04) and DI_{120} (R = –0.68, p = 0.005). In the same group, sgp130 level correlated positively with HBA1c (R = 0.65 p = 0.006).

Three months after childbirth in the patients with previous GDM, there was a positive correlation between IL-6 and IL-17 concentrations (R = 0.70, p = 0.03), whereas in the group with NGT during pregnancy IL-6 concentration correlated positively with fasting insulin (R = 0.72, p = 0.005) and HOMA-IR (R = 0.57, p = 0.04), as well as negatively with IS_{OGTT} (R = –0.59, p = 0.03) and DI_{120} (R = –0.67, p = 0.01). In the same group sgp130 level correlated positively with HbA1c (R = 0.56, p = 0.04).

Discussion

In the last few years, it has been suggested that the presence of GDM is connected with decreased oestrogen receptors expression [28] and abnormal adipokines production by adipose tissue [29], elevated expression of adenosine receptors in leukocytes [30], and an imbalance between circulating pro- and anti-inflammatory cytokines [20–23], but their contribution to the pathogenesis of GDM remains unclear.

Our study showed that the patients with GDM recognised between the 24th–28th week of gestation had significantly higher IL-6 and sgp130 concentrations than had the women with NGT, whereas the women with GDM diagnosed in the 29th–32nd week of pregnancy had elevated sIL-6R and an insignificant trend towards higher IL-6 levels. During the four weeks between the 1st and the 2nd visits, IL-6 and sgp130 concentrations rose in both groups studied, whereas sIL-6R level increased only in the patients who developed GDM. Three months postpartum, sgp130 concentrations increased markedly in the women with previous GDM compared to the values observed in the 3rd trimester and were significantly higher than in the patients with NGT.

These results are in agreement with our previous findings [21, 22, 31], as well as with other studies [20, 23] consistently showing higher IL-6 levels in patients with GDM compared to healthy pregnant women. However, there is still some controversy regarding the source(s) of elevated IL-6 in GDM subjects. In our previous studies, there were no significant differences in IL-6 mRNA expression in fat and placental tissue obtained from the patients with and without GDM [31, 32]. Similarly, Lappas et al. [33] did not find any marked differences in IL-6 release from the placenta, adipose tissue and skeletal muscle of the women with GDM compared to the NGT subjects. In contrast, Kleiblova et al. [28] demonstrated higher IL-6 gene expression in subcutaneous adipose tissue from the patients with GDM. Furthermore, the causal relationship between IL-6, insulin resistance and GDM seems far from clear. It has been shown that chronic and acute IL-6 exposure causes impaired insulin signalling in adipocytes [7, 8] and liver cells [9] but anti-inflammatory and insulin-sensitising effects of this cytokine have also been described [10, 11]. Although the cross-sectional design of our study limits any speculation about a causal relationship between IL-6 and insulin resistance, it is worth noting that in both groups studied, IL-6 concentration correlated negatively with the indices of insulin sensitivity and beta-cells response to pregnancy-induced insulin resistance. We should also mention that some authors have found elevated IL-6 levels several months after childbirth in patients with previous GDM [23, 34], but the present study did not confirm this observation.

In contrast to IL-6, which has been extensively studied in pregnant women, the other elements of the IL-6 signalling pathway have never been assessed in GDM patients. This seems rather surprising since gp130 trans-signalling is considered to be critical for the regulation of IL-6 induced cellular response [2–4]. The present study showed elevated sgp130 serum levels in the patients with GDM both between the 24th–28th week of gestation and three months postpartum. Additionally, in the group with NGT, sgp130 concentration correlated positively with HbA1c values both during and after pregnancy. Moreover, the women with comparatively low sIL-6R and high sgp130 concentration had significantly lower IL-6 levels, as well as higher fasting and post-load glucose values and lower $DI_{30,}$ which is considered to be a measure of beta cell response to the certain level of insulin resistance [26]. Although there is no other data concerning sgp130 in GDM subjects, elevated sgp130 levels have been observed in various diseases connected with insulin resistance, such as metabolic syndrome [35] and polycystic ovary syndrome [36], as well as in pre-eclampsia [37]. On the basis of these findings, it can be hypothesised that in the presence of IL-6 mediated chronic low-grade inflammation, increased sgp130 levels might represent a compensatory mechanism controlling intracellular IL-6 signalling and preventing the activation of the IL-6/Il-6R pathway. However, since there is good evidence that gp130 activators might be useful in the treatment of obesity and insulin resistance [38], the hypothesis that the inhibition of gp130 trans-signalling could promote insulin resistance cannot be excluded. Since in the present study all measurements were performed at two time points during pregnancy, we had an unique opportunity to assess the IL-6/IL-6R/gp130 system in the patients who were normoglycaemic at the time of sampling but later developed GDM; however, neither IL-6 nor sIL-6R and sgp130 levels were elevated in this group before the diagnosis of GDM.

The next hypothesis tested in the present study was the assumption that increased secretion of IL-6 in GDM subjects may lead to a pro-Th17 skewing milieu [13], which in turn results in elevated levels of so-called Th17 cytokines. Other studies have shown that the IL-17/IL-23 axis is elevated in obese patients [39] and that IL-17 is an important inhibitor of adipocyte differentiation and insulin-induced glucose uptake in adipose tissue [40]. In the present study, IL-17 was detected in 42% of the GDM subjects and in 35% of the women with NGT in the 2nd trimester, whereas four weeks later this percentage increased to 64% in the group with GDM and to 51% in the group with NGT. IL-23 was detected in approximately one third of the patients at both visits. There was also a trend towards higher IL-17 and — rather surprisingly — low IL-23 levels in the subjects with GDM in the 24th-28th week of gestation but the differences were not significant, probably due to a relatively small number of samples and large variations among the patients studied. Three months postpartum, IL-23 concentrations increased markedly, whereas IL-17 levels were comparable with the values observed during pregnancy. It is also worth noting that in the patients with previous GDM, IL-17 correlated positively with IL-6 concentration, suggesting a functional relationship between these two cytokines.

In conclusion, our results suggest that an increased sgp130 concentration observed in the patients with GDM might represent a compensatory mechanism controlling intracellular IL-6 signalling and preventing the activation of the IL-6/IL-6R pathway.

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