



Omentin-1 and its relationship with inflammatory factors in maternal plasma and visceral adipose tissue of women with gestational diabetes mellitus

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Abstract

Purpose To investigate the association of omentin-1 and inflammatory factors in serum and visceral adipose tissue (VAT) of women with gestational diabetes mellitus (GDM) compared to normal pregnant (NP) subjects. Furthermore, to examine their correlation with maternal clinical characteristics.

Methods We compared 116 GDM women to 115 NP women, at the time of cesarean section. Circulating omentin-1 and pro-inflammatory (IL-1 β , IL-6, TNF- α), and anti-inflammatory cytokines (IL-1RA, IL-10) were examined. Moreover, their mRNA expression in VAT, along with inflammatory factors involved in the NF- κ B pathway (TLR2, TLR4, NF- κ B, IK κ B), were examined.

Results Circulating omentin-1 ($p=0.022$) was lower and circulating IL-1- β , IL-1RA, as well as IL-10 ($p=0.005$, $p=0.007$, and $p=0.015$, respectively), were higher in GDM compared to NP women. Omentin-1 correlated negatively with pre-pregnancy and gestational BMI, and HOMA-IR in all women, but was not associated with cytokines. TLR2, TLR4, IL-1 β , IL-1RA, IL-6, IL-10 mRNA expression in VAT was lower in GDM compared with controls ($p<0.05$ all). In multivariate analysis, BMI at delivery was significantly correlated to omentin-1 concentrations in all and NP subjects. In addition, omentin-1 expression was correlated to inflammatory gene expression in all, GDM and NP, women ($p<0.05$ all).

Conclusion Serum levels and VAT gene expression of omentin-1 are not independently linked to GDM; notwithstanding, GDM women have a VAT-altered inflammatory status. In addition, no systemic association between omentin-1 and inflammatory factors was found, whereas associations between their expression in all women were observed, indicating that expression of these adipokines is linked between them regardless of GDM.

Keywords Gestational diabetes mellitus · Omentin-1 · Cytokines · Visceral adipose tissue · mRNA expression

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Introduction

Gestational diabetes mellitus (GDM) is the most common medical complication of pregnancy, affecting from 1 to 30% of pregnant women worldwide. Maternal overweight and obesity are major GDM risk factors [1]. In recent years, various adipokines (adipocyte-secreted proteins) have been involved in the pathophysiology of GDM through their role in the regulation of insulin resistance, β -cell dysfunction, and bodyweight gain [2].

Omentin-1, also known as intelectin-1, is a recently-identified adipokine mainly expressed in visceral fat as well as vascular cells, intestine, and placenta [3]. Circulating omentin-1 levels are decreased in obesity and in patients with type 2 diabetes (T2DM) [4, 5]. Evidence has shown

that omentin-1 stimulates glucose uptake via AMP-activated protein kinase/Akt in cultured adipocytes [3]. Furthermore, omentin-1 has anti-inflammatory properties, in cultured endothelial cells suppresses TNF- α -induced vascular inflammation and in smooth-muscle cells attenuates TNF- α -induced monocyte adhesion [6, 7].

Several cross-sectional studies have described decreased circulating concentrations of omentin-1 in women with GDM compared to controls [8–12]. Furthermore, a prospective study found that omentin-1 less than 38.4 ng/mL at 12–15 weeks of gestation was predictive of increased risk of GDM [13]. However, other studies have found no difference in omentin-1 circulating levels in women with GDM [14, 15], and recently, a few other studies have not shown GDM effect on omentin-1 gene expression in visceral adipose tissue (VAT) [8, 9].

GDM is characterized by elevated circulating free fatty acids and advanced glycation end products which activate toll-like receptors 2 and 4 (TLR2, TLR4) to promote the production of pro-inflammatory cytokines from macrophages and adipose tissue through the up-regulation of the nuclear transcription factor- κ B (NF- κ B) [16]. These cytokines can inhibit insulin action in the muscle and adipose tissue [17, 18]. Collectively, omentin-1 and inflammatory factors are associated with GDM [19, 20]; however, interactions among them in terms of their expression in VAT and how this correlates with their blood levels, and with related clinical parameters, have not yet been determined in a single setting.

Therefore, we aimed to investigate the association of circulating and mRNA expression in VAT of omentin-1 and pro-inflammatory (IL-1 β , IL-6, TNF- α), and anti-inflammatory cytokines (IL-1RA, IL-10) along with inflammatory factors involved in the nuclear factor- κ B (NF- κ B) pathway (TLR2, TLR4, NF- κ B, IK κ B), in GDM compared to normal pregnant (NP) subjects. The secondary objective was to explore the correlation between omentin-1, inflammatory cytokines and clinical maternal characteristics.

Materials and methods

The cross-sectional study was approved by the Institutional Review Board of the Instituto Mexicano del Seguro Social (IMSS) in Mexico City (R-2018-785-026), and all participants gave written informed consent. Women who were scheduled for an elective Caesarean section at term (37–41 weeks of gestation) were recruited for participation in this study (indications for Caesarean section were breech presentation, previous Caesarean section, and/or when macrosomia was suspected by ultrasonography at 38 weeks of gestation) were consecutively recruited, attended in Hospital of Gynecology and Obstetrics 3, Medical Center La Raza, IMSS (Mexico City), and the Hospital of Gynecology and

Obstetrics 221, IMSS (Toluca, State of Mexico). Women with pre-gestational diabetes or hypertension, and immunosuppressive, autoimmune, thyroid, renal, liver, infectious diseases or smoking and alcohol habits, as well as pregnancies complicated by fetal anomalies, were excluded from the study. This group of women has been previously analyzed for various other adipokines [21].

All women were screened for GDM at 24–28 weeks of gestation, and women with a negative screen were classified in the NP group. Women with GDM were diagnosed according to the International Association of Diabetes and Pregnancy Study Groups criteria (one or more abnormal glucose value during a 75 g-oral glucose tolerance test (OGTT), with fasting levels \geq 5.1 mmol/L, 1 h \geq 10 mmol/L or 2 h \geq 8.5 mmol/L). treatment for GDM began with nutrition therapy (1600–1800 kcal/day, restricting carbohydrates to 35–40%), and moderate physical activity (30 min of moderate-intensity aerobic exercise at least 5 days a week), with assessments of glycemic control with fasting glucose and 2-h postprandial blood glucose at 2–4 week intervals. Women who did not achieve glycemic control with diet (fasting glucose levels $>$ 5.27 mmol/L, and postprandial blood glucose values $>$ 6.66 mmol/L at 2 h) began pharmacological therapy. Of the GDM cases, 33% ($n = 38$) were treated by diet and exercise only, and 14% ($n = 16$) received insulin, 41% ($n = 48$) metformin, or 12% ($n = 14$) a combination of both to reach glycemic goals.

A baseline questionnaire included information about current and previous pregnancies from medical records, demographic characteristics such as maternal age, past history of GDM and family history of T2DM in a first-degree relative. Maternal pre-pregnancy body mass index (BMI) was calculated from self-reported pre-pregnancy weight and height measured during the first visit of gestation. BMI was calculated using the subject's weight in kilograms divided by the square of her height in meters, and was recalculated at the time of delivery. Maternal gestational weight gain (GWG) was obtained by the difference between the last recorded pre-delivery weight reported pre-pregnancy weight.

Biochemical analysis

On the day programmed for cesarean section, maternal fasting blood samples for biochemical analysis were obtained by venipuncture, along with the samples for routine laboratory tests. The samples were allowed to clot for at least 30 min before 15 min of centrifugation at 1000 g. Serum aliquots were frozen at -70 °C until assayed. Levels of glucose, total cholesterol, high-density lipoprotein (HDL) cholesterol and triglycerides were measured in fresh samples on an ARCHITECT Plus c4000 Clinical Chemistry Analyzer (Abbot Diagnostics, Abbott Park, IL, USA). Levels of low-density lipoprotein (LDL)

cholesterol were estimated with the use of the Friedewald formula. Omentin-1 determination was performed with an enzyme-linked immunoassay kit from BioVendor R&D Products (Brno, Czech Republic). IL-1 β , IL-6, TNF- α , IL-1RA, IL-10, and insulin were measured through multiplex immunoassay using Magpix technology (Milliplex Map, Billerica, MA, USA). The calculated inter-assay and intra-assay coefficients of variation were all < 10%. Insulin resistance was calculated using the homeostasis model assessment for insulin resistance (HOMA-IR) method, where $\text{HOMA-IR} = \text{fasting insulin concentration } [\mu\text{U/mL}] \times \text{fasting glucose concentration } [\text{mmol/L}] / 22.5$ [22].

VAT collection

Omental adipose tissue was obtained from 50 NP women matched with 50 GDM patients according to age. Tissue was obtained within ten minutes of delivery and was washed in water treated with diethylpyrocarbonate to remove any blood, and then dissected into fragments that were placed in TRIzol® Reagent (In-vitrogen™, Carlsbad, CA, USA) and stored at $-70\text{ }^{\circ}\text{C}$ until RNA extraction.

RNA extraction, cDNA synthesis and qPCR

Total RNA was isolated from VAT using Direct-zol™ RNA MiniPrep (Zymo Research Corp, CA, USA) according to the manufacturer's protocol. RNA concentrations were quantified using a spectrophotometer (NanoDrop 2000, Thermo Fisher Scientific, DE, USA), and RNA quality was determined via the A_{260}/A_{280} ratio. One μg of RNA was converted to cDNA using the SuperScript®III First-Strand kit (Invitrogen™, Carlsbad, CA, USA) according to the manufacturer's recommendations. The cDNA expression level was quantified by a real-time PCR using Taqman® Gene Expression Assays and Taqman® Universal PCR Master Mix (Applied Biosystems™, Foster City, CA, USA) according to the manufacturer's protocol. Real-time PCR was performed with the StepOnePlus™ Real-Time PCR System (Applied Biosystems™, Foster City, CA, USA). The $2^{-\Delta\text{CT}}$ method of relative quantification was used to determine the fold change in the mRNA expressions with the GAPDH transcript as endogenous control. All the primers and probes were acquired from Applied Biosystems™: omentin-1 (Hs00914745_m1), IL-1 β (Hs01555410_m1), IL-6 (Hs00985639_m1), TNF- α (Hs01113624_g1), IL-1RA (Hs00893626_m1), IL-10 (Hs00961622_m1), TLR2 (Hs02621280_s1), TLR4 (Hs00152939_m1), NF- κB (Hs00765730_m1), IK κB (Hs01559460_m1), and GAPDH (PN 4326317E).

Statistical analysis

Data distribution was assessed using Kolmogorov–Smirnov test. Results are presented as the medians with interquartile range (IQR). Differences between groups were analyzed by Mann–Whitney U test and correlations calculated using Spearman's test. Correction for potential confounders was performed with analysis of covariance. Multiple regression analysis was used to determine which variables were independently associated with maternal levels and mRNA expression of omentin-1. We used IBM SPSS Statistics 23.0 (IBM SPSS Inc., Chicago, IL) for statistical analysis, and a $p < 0.05$ was considered statistically significant.

Results

One hundred sixteen women with GDM and 115 healthy NP women were enrolled. The clinical and metabolic maternal characteristics of the NP and GDM women are summarized in Table 1. Women diagnosed with gestational diabetes had a higher age, pre-pregnancy BMI, gestational BMI, parity, family history of T2DM, previous GDM, fasting glucose at delivery, and triglycerides compared with the NP individuals. In contrast, gestational weight gain, gestational age at delivery and HDL levels were significantly lower in GDM subjects than NP subjects.

Maternal circulating plasma levels

Circulating omentin-1 ($p = 0.022$) was lower and circulating IL-1 β , IL-1RA, as well as IL-10 ($p = 0.005$, $p = 0.007$, and $p = 0.015$, respectively) were higher in GDM compared to NP women (Fig. 1). These significances did not continue after adjusting for age, pre-pregnancy BMI, BMI at term, and gestational age at delivery. No differences were found in circulating levels of IL-6 and TNF- α between GDM and non-GDM women (Table 2). We assessed the effect of pre-existing maternal obesity on omentin-1 and inflammatory cytokine levels in GDM participants. There was no difference in omentin-1, IL-1 β , IL-6, IL-10, and TNF- α levels between obese and non-obese women. Circulating IL-1RA levels were significantly higher in women with obesity compared to non-obese women [8.1 (5.1–14.5 pg/mL) vs. 5.57 (3.1–8.7 pg/mL), respectively, $p = 0.020$]. In addition, no associations of maternal plasma omentin-1 levels with inflammatory factors were observed.

Omentin-1 and inflammatory markers gene expression

Median TLR2, TLR4, IL-1 β , IL-1RA, IL-6, IL-10 expression levels in visceral adipose tissue were lower in GDM

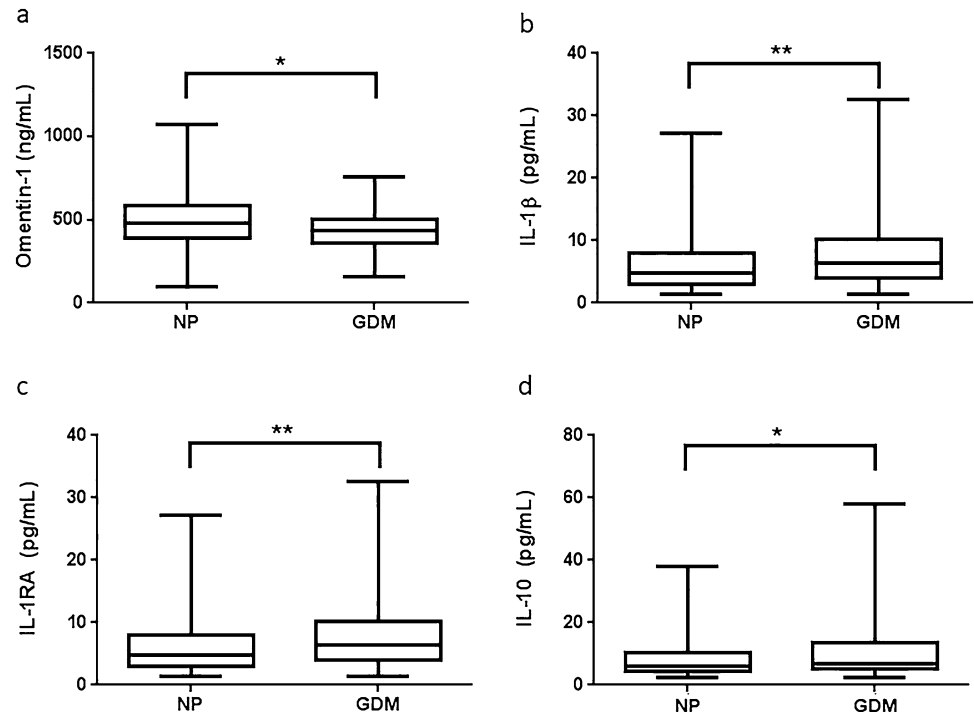
Table 1 Maternal and metabolic characteristics in the study population

	NP (n=115)	GDM (n=116)	p value
Maternal age (years)	26.0 (22.0–32.0)	33.0 (29.0–37.0)	<0.001
Pre-pregnancy BMI (Kg/m ²)	25.3 (22.3–27.3)	29.4 (26.6–33.9)	<0.001
BMI at term (Kg/m ²)	29.5 (27.1–31.6)	33.3 (30.1–36.3)	<0.001
Gestational weight gain (Kg)	10.0 (7.1–12.4)	8.2 (5.5–11.0)	0.002
Gestational age at delivery (weeks)	39.0 (38.4–39.8)	38.0 (37.3–38.6)	<0.001
Parity n (%)			0.016
Nulliparous	39 (33.9)	23 (19.8)	
One or greater	76 (66.1)	93 (80.2)	
Family history of T2DM n (%)	14 (12.2)	61 (52.6)	<0.001
Previous GDM n (%)	1 (0.87)	7 (6.0)	0.03
Fasting glucose at delivery (mg/dL)	74.0 (64.3–83.4)	84.5 (74.5–92.7)	<0.001
Fasting insulin at delivery (pg/mL)	324.3 (213.7–497.2)	311.8 (198.6–419.6)	0.477
HOMA-IR at delivery	1.4 (0.9–2.3)	1.6 (1.2–2.4)	0.467
Triglycerides at delivery (mg/dL)	253.2 (200.5–310.3)	276.2 (218.7–350.5)	0.015
HDL at delivery (mg/dL)	95.0 (77.7–116.4)	87.0 (62.5–104.5)	0.003
LDL at delivery (mg/dL)	76.6 (57.8–99)	76.0 (44.3–122.1)	0.708
Total cholesterol at delivery (mg/dL)	224.3 (195.4–254.7)	214.7 (195–257.5)	0.443

Data are presented as medians (interquartile range) as well as counts and percentages

NP normal pregnancy; GDM gestational diabetes mellitus; BMI body mass index; T2DM type 2 diabetes mellitus; HOMA-IR homeostasis model assessment-insulin resistance; HDL high-density lipoprotein cholesterol; LDL low-density lipoprotein cholesterol

Fig. 1 Box and whisker plots of circulating omentin-1, IL-1 β , IL-1RA, and IL-10 in GDM compared to NP. GDM group showed decreased omentin-1 levels (a), and increased IL-1 β (b), IL-1RA (c), as well as IL-10 (d) levels compared with controls. NP, n = 115; GDM, n = 116. * $p < 0.05$, ** $p < 0.01$. NP normal pregnancy; GDM gestational diabetes mellitus; IL-1 β interleukin 1 β ; IL-1RA IL-1 receptor antagonist; IL-10 interleukin 10



compared with controls (Fig. 2). There was no difference in VAT omentin-1, IK κ B, NF- κ B, TNF- α expression levels between GDM and controls (Table 2). As it has been suggested that an impaired balance between pro- and anti-inflammatory cytokines may play a role in the pathogenesis

of GDM, we decided to investigate the pro-inflammatory cytokines/anti-inflammatory cytokines balance and we observed that VAT TNF- α /IL-10 expression ratio was higher in GDM compared with controls ($p = 0.002$). However, after controlling for gestational age, pre-pregnancy BMI and BMI

Table 2 Omentin-1 and inflammatory cytokines in the study population

	NP	GDM	<i>p</i> value
Serum levels			
Omentin-1 (ng/mL)	477.2 (391.2–582.4)	436.0 (360.1–501.7)	0.022
IL-1 β (pg/mL)	30.8 (19.8–38.4)	35.3 (29.7–40.5)	0.005
IL-6 (pg/mL)	3.5 (2.3–9.9)	4.9 (2.2–14.9)	0.381
TNF- α (pg/mL)	15.2 (11.3–19.8)	17.2 (12.0–24.2)	0.083
IL-1RA (pg/mL)	4.7 (3.1–7.9)	6.6 (3.9–10.7)	0.007
IL-10 (pg/mL)	6.0 (4.2–10.3)	7.2 (5.1–18.7)	0.015
mRNA expression in VAT			
Omentin-1	59.9 (42.7–106.9)	53.09 (27.4–100.9)	0.190
IL-1 β	0.42 (0.22–0.86)	0.23 (0.10–0.54)	0.012
IL-6	0.35 (0.14–0.92)	0.12 (0.04–0.41)	0.002
TNF- α	0.02 (0.01–0.03)	0.02 (0.009–0.033)	0.767
IL-1RA	0.04 (0.02–0.08)	0.03 (0.01–0.07)	0.05
IL-10	0.10 (0.06–0.22)	0.04 (0.02–0.13)	0.001

Data are presented as medians (interquartile range)

NP normal pregnancy; GDM gestational diabetes mellitus; VAT visceral adipose tissue; IL-1 β interleukin 1 β ; IL-6 interleukin 6; TNF- α tumor necrosis factor alpha; IL-1RA IL-1 receptor antagonist; IL-10 interleukin 10

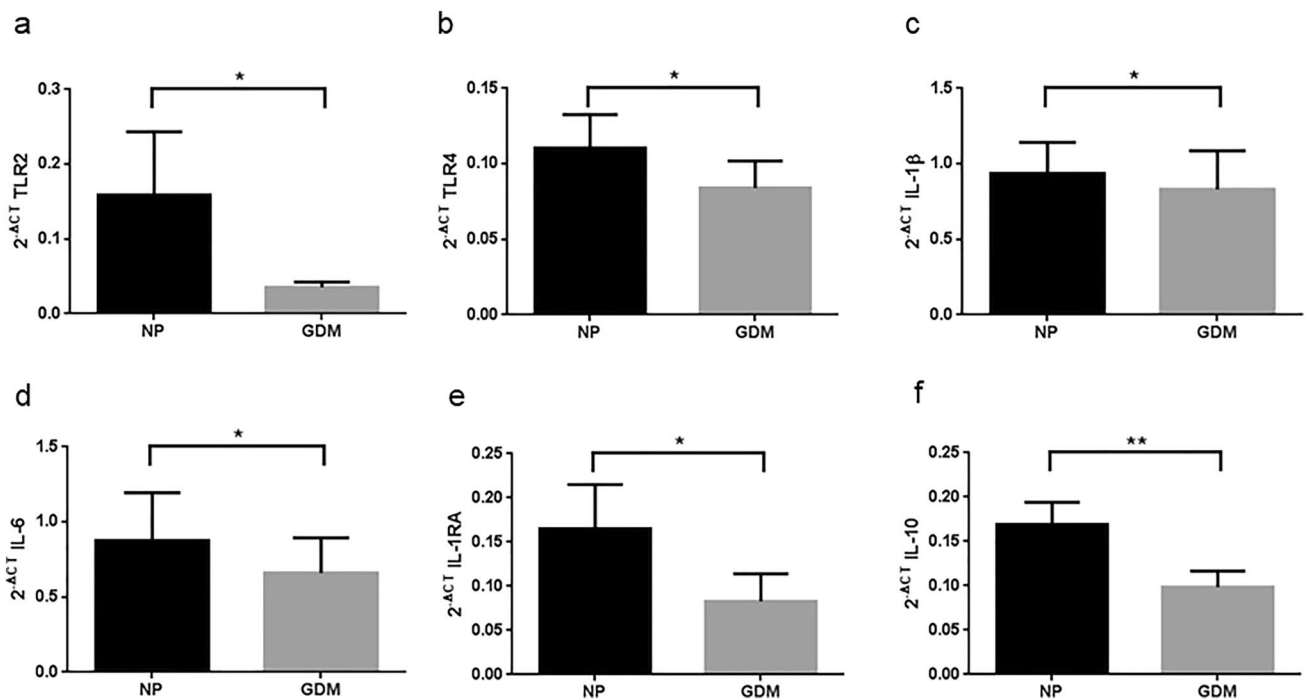


Fig. 2 Inflammatory markers gene expression in visceral adipose tissue in GDM compared to NP. TLR2 (a), TLR4 (b), IL-1 β (c), IL-6 (d), IL-1RA (e), and IL-10 (f) expression levels were lower in GDM compared with controls. NP, *n* = 50; GDM, *n* = 50. **p* < 0.05,

***p* < 0.001. Each bar represents the mean \pm SEM. NP normal pregnancy; GDM gestational diabetes mellitus; TLR2 toll-like receptor 2; TLR4 toll-like receptor 4; IL-1 β interleukin 1 β ; IL-6 interleukin 6; IL-1RA IL-1 receptor antagonist; IL-10 interleukin 10

at term, only IL-10 expression remained significantly lower in participants with GDM. Moreover, there was no significant difference in omentin-1 and cytokines expression and serum levels between women with GDM who were managed

by dietary modification alone compared with women who were treated with insulin or metformin.

Omentin-1 and inflammatory cytokines VAT gene expression were not associated with maternal blood levels.

Additionally, positive associations between omentin-1 and inflammatory markers expression in VAT in all, NP and GDM women, were observed (Table 3). The significance of the above correlations continued after adjustment for gestational age, pre-pregnancy BMI and BMI at term.

Correlations between omentin-1 and inflammatory markers serum levels as well as VAT gene expressions with maternal clinical parameters

In all women, serum omentin-1 levels correlated negatively with pre-pregnancy BMI, gestational BMI, and HOMA-IR ($r = -0.196$, $p = 0.003$, $r = -0.289$, $p = 0.001$, $r = -0.244$, $p = 0.048$, respectively), and positively with HDL ($r = 0.146$, $p = 0.035$). IL-1 β concentration was positively correlated with age ($r = 0.175$, $p = 0.031$), pre-pregnancy BMI ($r = 0.201$, $p = 0.014$), BMI at term ($r = 0.165$, $p = 0.044$), insulin ($r = 0.344$, $p = 0.007$), and HOMA-IR ($r = 0.275$, $p = 0.042$). The TNF- α level was positively correlated with BMI at term ($r = 0.191$, $p = 0.020$). IL-1RA had a positive correlation with BMI at term ($r = 0.175$, $p = 0.033$), and HOMA-IR ($r = 0.326$, $p = 0.015$). Furthermore, IL-10 was significantly negatively correlated with GWG ($r = -0.245$, $p = 0.002$). In GDM subjects, serum IL-1 β levels were positively correlated with insulin ($r = 0.626$, $p = 0.022$). IL1RA had positive correlations with BMI at term ($r = 0.249$, $p = 0.034$), and HOMA-IR ($r = 0.553$, $p = 0.021$). IL-10 levels were positively correlated with HOMA-IR ($r = 0.660$, $p = 0.004$), and were negatively associated with GWG ($r = -0.278$, $p = 0.016$). In NP women, circulating omentin-1 correlated negatively with pre-pregnancy BMI and at term BMI ($r = -0.298$, $p = 0.001$, $r = -0.379$, $p = 0.001$, respectively), and correlated positively with triglycerides ($r = 0.253$, $p = 0.006$), and VLDL ($r = 0.224$, $p = 0.016$).

In all women, omentin-1 mRNA expression was positively related to the patient's age ($r = 0.277$, $p = 0.005$), and IL-6 expression was positively associated with GWG ($r = 0.249$, $p = 0.013$). In GDM subjects, omentin-1, TLR2, TLR4, TNF- α , and NF- κ B expression were positively correlated with maternal age ($r = 0.287$, $p = 0.044$, $r = 0.441$, $p = 0.001$, $r = 0.341$, $p = 0.015$, $r = 0.312$, $p = 0.028$, $r = 0.399$, $p = 0.004$, respectively), and NF- κ B expression was positively associated with pre-pregnancy BMI ($r = 0.317$, $p = 0.028$). In NP women, omentin-1 expression had a positive correlation with age ($r = 0.320$, $p = 0.023$). TLR2 expression was positively correlated with GWG ($r = 0.391$, $p = 0.005$). Finally, IL-1 β and IL-10 expression had positive correlations with fasting plasma glucose levels ($r = 0.290$, $p = 0.041$, $r = 0.300$, $p = 0.034$).

Multiple linear regression analysis was performed to study the variables independently associated with maternal levels and expression of omentin-1 (Table 4). BMI at delivery was significantly correlated to omentin-1 concentrations in all and NP subjects. In addition, omentin-1 expression was significantly correlated to inflammatory markers mRNA expression.

Discussion

As far as we know, this is the first study that simultaneously determined adipose tissue expressions and serum levels of omentin-1 and inflammatory factors in GDM women at the time of delivery. Moreover, this study determined correlations between them and maternal parameters. We found that omentin-1 serum levels were lower in GDM compared with NP, and correlated negatively with BMI, and HOMA-IR in all women; however, there was no difference after

Table 3 Correlations between omentin-1 and inflammatory markers mRNA expression in VAT in all, NP and GDM subjects

	All <i>n</i> = 100		NP <i>n</i> = 50		GDM <i>n</i> = 50	
	<i>r</i>	<i>p</i>	<i>r</i>	<i>p</i>	<i>r</i>	<i>p</i>
TLR2	0.330	<0.001	0.210	0.143	0.413	0.003
TLR4	0.377	<0.001	0.326	0.021	0.405	0.004
IK κ B	0.412	<0.001	0.343	0.015	0.406	0.003
NF- κ B	0.403	<0.001	0.444	0.001	0.350	0.013
IL-1 β	0.507	<0.001	0.507	<0.001	0.450	0.001
IL-6	0.362	<0.001	0.373	0.008	0.305	0.031
TNF- α	0.373	<0.001	0.229	0.110	0.471	<0.001
IL-1RA	0.405	<0.001	0.502	<0.001	0.291	0.041
IL-10	0.405	<0.001	0.488	<0.001	0.286	0.044

VAT visceral adipose tissue; NP normal pregnancy; GDM gestational diabetes mellitus; TLR2 toll-like receptor 2; TLR4 toll-like receptor 4; IK κ B I- κ B-Kinase β ; NF- κ B nuclear factor- κ B; IL-1 β interleukin 1 β ; IL-6 interleukin 6; TNF- α tumor necrosis factor alpha; IL-1RA IL-1 receptor antagonist; IL-10 interleukin 10

Table 4 Multivariate regression analysis for serum levels and adipose tissue expression of omentin-1 in all, NP and GDM subjects

	Coefficient β	95% CI	<i>p</i> value
Serum omentin-1 levels			
For all subjects			
Maternal BMI	− 0.702	− 31.786 to − 1.945	0.027
For NP subjects			
Maternal BMI	− 0.286	− 16.592 to − 1.525	0.019
Omentin-1 expression			
For all subjects			
TLR2	0.375	106.271 to 418.312	0.001
TLR4	− 0.754	− 2480.554 to − 707.434	0.001
NF- κ B	0.912	254.29 to 420.71	<0.001
IL-1 β	0.349	26.164 to 76.781	<0.001
IL-6	− 0.549	− 0.106.329 to − 61.752	<0.001
IL-10	0.602	796.821 to 1446.966	<0.001
For NP subjects			
IL-1 β	− 0.476	− 218.533 to − 45.351	0.004
IL-6	− 0.662	− 149.162 to − 87.459	<0.001
IL1-RA	0.409	146.487 to 781.652	0.005
For GDM subjects			
TLR4	− 0.525	− 1093.757 to − 60.772	0.029
IL-1 β	1.446	46.249 to 115.047	<0.001
TNF- α	0.202	17.911 to 759.658	0.040
IL-1RA	− 0.701	− 868.177 to − 22.287	0.040

NP normal pregnancy; GDM gestational diabetes mellitus; BMI body mass index; TLR2 toll-like receptor 2; TLR4 toll-like receptor 4; NF- κ B nuclear factor- κ B; IL-1 β interleukin 1 β ; IL-6 interleukin 6; TNF- α tumor necrosis factor alpha; IL-1RA IL-1 receptor antagonist; IL-10 interleukin 10

adjustment for maternal factors such as age and BMI, and the result of the multiple regression analysis showed that BMI affects omentin-1 levels. In agreement with this, it has been shown that in conditions of increased adiposity, increased visceral adipocyte parameters including area, width, height, and perimeters are related to decreased serum levels of omentin-1 [23].

Case–control studies related to omentin-1 levels between GDM and NP women have had variable results. Barker et al. and others found that maternal omentin-1 was significantly lower in women with GDM compared with controls [8–12]. It is noteworthy that in the studies in which women were classified according to their BMI, omentin-1 was significantly decreased only in the non-obese subgroup of GDM women. On the other hand, Abell et al., in a longitudinal study, found that omentin-1 < 38.66 ng/mL at 12–15 weeks of gestation was associated with a fourfold increased risk of GDM [13]. However, Lewandowski et al., and Franz et al. did not find any difference in omentin-1 in women with GDM [14, 15]. The disagreement between different studies could be due to the definition of GDM, maternal BMI, study design, the different gestational ages at the time of the maternal blood collection, the methodological heterogeneity selected for the analysis, and the different treatments used

for GDM. In this study, pharmacological treatment did not significantly affect adipokine levels.

Interestingly, in this study, omentin-1 mRNA synthesis was not significantly different in VAT of women with GDM compared with pregnant controls. Similarly, Barker et al. and Tsiotra et al. reported VAT omentin-1 mRNA expression being comparable between the NP and GDM groups [8, 9]. This is evidence that omentin-1 does not have a role in the modulation of adipocyte dysfunction in GDM. Of note, Barker et al. demonstrated that pre-existing maternal obesity was associated with lower expression and release of omentin-1 in VAT. Several studies have shown that serum levels of this adipokine reflect its expression in VAT, which decreases in parallel with the increase in VAT [23–25]. However, in this study, omentin-1 mRNA expression was not correlated with its serum levels, indicating that serum levels of omentin-1 might primarily be correlated to its secretion from other tissue, such as placental. It has recently been shown that placenta is the major source of omentin-1 levels, and its expression in this tissue is decreased in GDM compared to non-obese NP [8].

Omentin-1 is a recently-discovered adipokine originally identified as a soluble galactofuranose-binding lectin, and later identified from a human omental adipose tissue cDNA

library [3]. Omentin-1 is abundantly expressed in the stromal vascular fraction of VAT, as well as vascular cells, intestine, and placenta. It has been shown that omentin-1 stimulates glucose uptake in cultured adipocytes *in vitro* in response to insulin, suggesting that may improve insulin sensitivity. Circulating omentin-1 levels are decreased in obese individuals, in patients with T2DM, with metabolic syndrome and in pregnancy [4, 5]. Additionally, it has been reported that omentin-1 has anti-inflammatory properties. In human endothelial cells inhibits TNF- α induced vascular inflammation, and in vascular smooth muscle cells it inhibits TNF- α -induced superoxide production, suggesting a protective role in vascular disease [6, 7]. In addition, in high fat diet-induced obese mice, omentin-1 attenuates adipose tissue inflammation, and in macrophages it attenuates its lipopolysaccharide-induced activation by inhibiting the TLR4/MyD88/NF- κ B signaling [26, 27].

Increasing evidence suggests that GDM is a systemic pro-inflammatory state. It has been shown that pro-inflammatory cytokines such as IL-1 β , TNF- α , and IL-6 increase in GDM conferring insulin resistance in liver, skeletal muscle, and vascular endothelium [16–18]. In this study, circulating IL-1 β , IL-1RA, as well as IL-10, were higher in GDM compared to NP women. However, these significances did not continue after adjusting for maternal characteristics. We also observed that obese GDM women had higher IL-1RA levels than non-obese women, and that maternal BMI was in correlation with the serum IL-1 β , IL-1RA and TNF- α levels, suggesting that one of the main sources of cytokine production is the adipose tissue, although it has also been documented that the feto-placental unit may also be an important source of TNF- α [2, 28]. Additionally, serum omentin-1 levels were not associated with circulating inflammatory factors.

Adipose tissue from GDM pregnancies displays an altered physiology; adipocytes display increased cell size, and adipose tissue displays reduced capillary density and also increased expression of markers of endothelial dysfunction and inflammatory cytokines when compared to NP [29]. However, it still remains to be determined whether the expression of these factors precedes the development of GDM or is rather secondary to the metabolic disturbances in GDM patients. In this study, we found that TLR2, TLR4, IL-1 β , IL-1RA, IL-6, IL-10 mRNA expression in VAT was lower, and TNF- α /IL-10 ratio was higher, in GDM compared with controls. However, after controlling for maternal characteristics, only IL-10 expression remained significantly lower in participants with GDM, which further indicates altered anti-inflammatory status in patients with GDM. In agreement with this, several studies have shown decreased levels of IL-10 in GDM women [30].

Both age and BMI before pregnancy increase the risk of GDM. We showed that TLR2, TLR4, TNF- α , and NF- κ B VAT expression were positively correlated with maternal

age, and NF- κ B expression was positively associated with pre-pregnancy BMI in GDM women. NF- κ B is a primary regulator of inflammatory response. This nuclear factor exists in an inactive dimeric form in the cytoplasm bound by the inhibitory proteins I κ Bs. After activation by stimuli such as pathogens, T-cell activation signals, and pro-inflammatory cytokines, I κ Bs become phosphorylated by I- κ B-Kinase β (IKK β), thus allowing the NF- κ B to translocate to the nucleus and activate gene transcription of several genes, including pro-inflammatory cytokines, chemokines and adhesion molecules. Recent studies implicate a role of NF- κ B and TLR in ageing, obesity and insulin resistance [31–33]. In GDM, several studies have reported increased expression of TLR4 mRNA and NF- κ B mRNA in the placenta [34]. On the other hand, it has been shown that an elevated TLR2 expression in peripheral blood mononuclear cells of women with GDM [35], and high glucose and saturated fat increase TLR expression in these cells [33, 36].

In this study, we also reported associations between omentin-1 and inflammatory marker mRNA expression in VAT in all, NP, and GDM women, and the significance of correlations continued after adjustment for maternal characteristics, indicating that expression of these adipokines is linked between each other regardless of GDM. Interestingly, in NP, but not in GDM women, expression of omentin-1 was positively correlated with the expression of anti-inflammatory cytokine IL-1RA and was negatively correlated with pro-inflammatory cytokines IL1 β , and IL-6, suggesting an anti-inflammatory property. One explanation of different associations between omentin-1 and cytokines expressions between GDM and non-GDM subjects might be because a hypertrophy of adipocytes is seen in individuals with GDM, which could contribute to a failure in expression levels of adipokines [29].

Our study has several limitations. First, the cross-sectional data analyses cannot make causal inferences regarding the relationships between omentin-1 and inflammatory factors in GDM. Second, there were differences in age and BMI between groups, although adjustments for these variables were made. Third, we did not measure the secretion of adipokines from adipose tissue obtained from studied women. It has been suggested that IL-1 β gene expression on adipose tissue is independent of gene transcription. In addition, maternal adipokines were measured at the time of Caesarean section in only one population, there was a lack of HbA1c, and all the GDM patients received intervention once diagnosed, including diet, exercise and pharmacological treatment. The influence of these treatments on the cytokine levels is unclear and should be considered in future studies. Thus, large prospective cohort studies will be necessary to provide evidence concerning the association of VAT expression and release of the examined adipokines with GDM.

Conclusion

Serum levels and VAT gene expression of omentin-1 are not independently linked to GDM; notwithstanding, GDM women have a VAT-altered inflammatory status. In addition, no systemic association between omentin-1 and inflammatory factors was found, whereas associations between their expression in all women were observed, indicating that expression of these adipokines is linked between each other regardless of GDM.

Author contribution MIPC, JVO, and RS planned and designed the study. MIPC, and JVO performed laboratory biomarker analysis. EMA, MFDV, RGD, and RS drafted the manuscript. MIPC, and RS performed the data analysis. All authors provided significant intellectual contribution in interpreting data and critical review of the manuscript.

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Availability of data and material The data used to support the findings of this study are available from the corresponding author by request.

Code availability Not applicable for that section.

Declarations

Conflict of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

Ethics approval The study protocol was approved by the Institutional Review Board of the Instituto Mexicano del Seguro Social (IMSS) in Mexico City (R-2018-785-026).

Consent to participate All participants gave written informed consent.

Consent for publication All authors approved the final version of the manuscript.

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