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MATERNAL-FETAL MEDICINE

Placental diabesity: placental VEGF and CD31 expression according to pregestational BMI and gestational weight gain in women with gestational diabetes

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Abstract

Purpose The aim of this study is to investigate the placental expression of VEGF and CD31 in pregnancies complicated by gestational diabetes (GDM) and the influence of pregestational BMI and gestational weight gain (GWG) on this expression.

Methods We prospectively enrolled pregnant women with diagnosis of GDM and healthy controls who delivered in our Center between December 2016 and May 2017. Patients were grouped according to the presence of GDM and we compared pregnancy characteristics, placental VEGF and CD31 expression between the cases and controls. Immunochemistry analysis was performed to assess biomarkers positivity. Positivity of biomarkers was assessed in a dichotomic fashion with positivity set at 5% for VEGF and 1% for CD31.

Results 39 patients matched inclusion criteria, 29 (74.3%) women with GDM and 10 (25.7%) healthy controls. Immunochemistry analysis showed that VEGF was more expressed in placentas from women with GDM compared to controls (21/29, 72.4% vs 2/10, 20%; $p=0.007$), and CD31 was more expressed in placentas from women with GDM compared to controls (6/29, 20.7% vs 0/10, 0%; risk difference 0.2). VEGF positivity was associated with the presence of GDM (aOR 22.02, 95% CI 1.13–428.08, $p=0.04$), pregestational BMI (aOR 1.53, 1.00–2.34, $p=0.05$) and GWG (aOR 1.47, 95% CI 1.03–2.11, $p=0.03$). CD31 positivity was associated with the pregestational BMI (aOR 1.47, 95% CI 1.00–2.17, $p=0.05$) and with the gestational weight gain (aOR 1.32, 95% CI 1.01–1.72, $p=0.04$).

Conclusion Pregnancies complicated by GDM are characterized by increased placental expression of VEGF and CD31, and the expression of these markers is also independently associated to maternal increased pregestational BMI and GWG, defining the concept of "placental diabesity".

Keywords Gestational diabetes · VEGF · Placenta · Obesity · Pregnancy · Gestational weight gain

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What does this study add to the clinical work

Placental VEGF and CD31 expression is higher in placentas with GDM vs healthy controls.

CD31 expression is associated with pregestational BMI and gestational weight gain.

Placental VEGF expression is associated with GDM, pregestational BMI and gestational weight gain.

Introduction

Hyperglycemia in pregnancy (HIP) is one of the most relevant pathological conditions that affect pregnant women, complicating up to one in six pregnancies [1]. Apart from type 1 and type 2 pregestational diabetes, a growing rate of women in the last decades are developing gestational diabetes (GDM), a condition characterized by impaired insulin resistance and hyperglycemia which develops typically during pregnancy in non-diabetic women and resolves weeks after delivery [2]. Several associated risk factors for the development of GDM have been described so far; in particular, many authors have highlighted the role of pregestational BMI and the gestational weight gain (GWG) as key conditions acting on the maternal metabolic status, leading to an imbalanced insulin resistance and to impaired glycemic control [3–5].

Angiogenesis is a key step following trophoblast invasion during embryo implantation. Normal angiogenesis is crucial for successful implantation and placentation. Inadequate angiogenesis is associated with recurrent implantation failure and recurrent miscarriage, and some authors have demonstrated that VEGF is the most important angiogenic factor during embryo implantation [6–8]. Peculiar aspects have been described in placentas from pregnancies with GDM; in particular, placentas from GDM pregnancy are bigger and heavier than placentas from normal pregnancies, the thickness of the central region of the placenta and the number of placenta cotyledons are also higher in GDM compared with placentas from normal pregnancies [9, 10]. These changes are explained considering that maternal hyperglycemia is responsible for increased levels of glycated hemoglobin (Hba1c), which have a ten-fold affinity for oxygen compared to non-glycated hemoglobin, and increased tissue oxygen consumption due to maternal and fetal hyperinsulinemia. These changes lead to a chronic hypoxemic status and oxidative stress in placentas from women with GDM and the subsequent upregulation of hormones (EPO, FGF2, leptin, IGF2) and inflammatory cytokines (IL-6, TNFa) that may lead to placental neoangiogenesis and hypervascularization [11]. In this context, a higher number of branches per capillary in terminal villi occurs, known as chorangiosis, which is considered a structural adaptation to maintain normal placental efficiency. GDM is also responsible for the reduced apoptosis and increased inflammation pathways in human trophoblast, through the disruption of the TNFα and NFkB signalling. Li HP et al. demonstrated in a murine model that GDM and obesity are associated with placental hypoxic status, increased VEGF expression and increased pro-inflammatory factors TNF-α and IL-6 [12, 13].

Platelet endothelial cell adhesion molecule-1 (PECAM-1, CD31) is a cell adhesion and signalling receptor that is expressed on hematopoietic and endothelial cells. PECAM-1 is vital to the regulation of inflammatory responses, as it has been shown to serve a variety of pro-inflammatory and anti-inflammatory functions. In particular, CD31 helps to promote leukocyte chemokine-directed transendothelial migration and leukocyte accumulation at sites of inflammation [14].

Some authors have investigated the role of proangiogenic VEGF and pro-inflammatory CD31 in placentas from women with GDM, but conflicting results were published so far [15–17]. Furthermore, no data are available evaluating the role of pregestational BMI and GWG in the expression of placental proangiogenic and pro-inflammatory factors in these pregnancies. Thus, the aim of this study is to investigate the placental expression of VEGF and CD31 in pregnancies complicated by GDM and influence of pregestational BMI and GWG on this expression.

Methods

We prospectively enrolled pregnant women with diagnosis of GDM who delivered in our Center between December 2016 and May 2017. We included in our analysis consecutive singleton non-diabetic pregnant women who were diagnosed with GDM after a 75 g OGTT at 24–28 weeks according to the WHO criteria and healthy controls matched for maternal age with ratio 3:1 [18]. Exclusion criteria were presence of pregestational diabetes, overt diabetes in early pregnancy, early diagnosis of GDM in women with risk factors for GDM (obesity, previous GDM) who tested positive after a 75 g OGTT at 16–18 weeks, chromosomal or congenital anomalies, abnormal first or second-trimester ultrasound screening, hypertension, preeclampsia, autoimmune diseases, and other pregnancy complications apart from GDM. Gestational age was assessed by last menstrual period, if in agreement of 7 days with CRL dating using Robinson formula, or by CRL if there was more than 7 days

discrepancy. For each patient, we collected the following data: maternal age, pregestational weight and BMI, parity, maternal smoking, type of therapy for GDM (diet or insulin), GWG at delivery, gestational age (GA) at delivery, type of delivery, neonatal sex, neonatal birthweight, birthweight percentile according to the GA, placental weight, Apgar score at 1 and 5 min, large for gestational (LGA) newborn, diagnosed as neonatal birthweight percentile $\geq 90°$ centile.

After delivery, placentas were submitted to the histopathology laboratory and immediately fixed in 10% buffered formalin. After a fixation period of 24 h, the tissues underwent macroscopic examination for the definition of its shape, size, weight and any external evidence and the analysis of umbilical cord. All surgical specimens were fixed in 10% buffered formaldehyde, embedded in paraffin and then subsequent 5 micron-thick sections were stained with hematoxylin–eosin (H&E).

3 mm paraffin-embedded sections were placed on specific slides for the immunohistochemical evaluation. Tissue sections were treated to be adequate for the antigen using a 0.01 M of a NaH solution with a pH = 6 for 15 min in a microwave oven and then incubated with the primary antibody (VEGF, diluted 1:50; and CD31, diluted 1:50) for 1 h. Slides were then incubated with biotinylated goat anti-rabbit antibody (diluted 1:250; Vector Laboratories) for 45 min, washed and incubated with streptavidin-peroxidase (diluted 1:400; Immunotech, Beckman-Coulter). Antibodies were then visualized with the incubation in diaminobenzidine/H_2O_2. The VEGF e CD31 expression was evaluated by a pathologist blinded in regard of the placental conditions. The initial cut-off for the expression of VEGF in our study was defined at 1% staining of positive cells; however, in our specific patient study group, after analysing our data, we found that almost all the included cases showed VEGF expression that was greater than 1%. Hence, for VEGF, we used the 5% value as the minimal number of positive cells required for staining to be interpreted as positive, as previously reported in immunochemistry (ICH) semiquantitative scoring systems for VEGF [19]. For CD31, we used the 1% value as the minimal number of positive cells required for staining to be interpreted as positive. The manufacturer positive and negative cell-line control served as positive controls.

Statistical analysis

Patients were grouped according to the presence of GDM, and we compared pregnancy characteristics, placental VEGF and CD31 expression between the cases and controls. The Shapiro–Wilk test was performed to test for normality. When normally distributed, continuous variables were compared by Student's *t* test, otherwise by Kruskal–Wallis test; categorical variables were compared by Pearson Chi-square test or, when variables had frequency count less than 5, with Fisher's Exact test. Continuous data were expressed as mean and standard deviation when normally distributed or otherwise as median and interquartile range (IQR). Categorical variables were expressed as number (*n*) and percentage (%) of the group.

We performed a logistic regression analysis to test whether placental VEGF and CD31 expression was associated with maternal or neonatal variables in both cases and controls. Crude Odds Ratio (cOR) was calculated for univariate logistic regression analysis, evaluating the relationship between each pregnancy variable and the ICH positivity for VEGF or CD31. Furthermore, after the selection of adequate variables for the logistic regression model, we performed a multivariate logistic regression analysis to determine the independence and contribution of included variables associated with ICH positivity for VEGF or CD31 (maternal age, GDM, maternal BMI and GWG, smoke, insulin therapy, GA at delivery, neonatal birthweight, neonatal sex, VEGF and CD31 expression), and we calculated adjusted Odds Ratio (aOR) for each included variable adjusted for the other ones [20]. A p value < 0.10 was considered as significant. Statistical analysis was carried out using the Statistical Package for Social Sciences (SPSS) Statistics v. 19 (IBM Inc., Armonk, New York, USA).

Ethical approval

All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. This study obtained the approval from local ethical committee.

Results

We included in our analysis 39 patients matching the inclusion criteria, 29 (74.3%) women with GDM and 10 (25.7%) healthy controls. All included women were Caucasian. Maternal and ultrasound characteristics are summarized in Table 1. In 19 cases (65.5%), glycemic control was achieved by insulin therapy (type A2 GDM), while in 10 cases (34.5%), glycemic control was achieved with nutritional intervention (type A1 GDM). We found no difference in maternal age, pregestational BMI, GWG, parity, maternal smoking, GA at delivery, placental weight, neonatal birthweight, birthweight centiles, fetoplacental ratio, neonatal sex and incidence of LGA between cases and controls. None among included pregnancies had an Apgar score ≤ 7

at 5 min, while 19/29 (65.5%) of women with GDM was on insulin therapy.

Immunochemistry analysis showed that CD31 expression was higher in placentas from women with GDM compared to controls (6/29, 20.7% vs 0/10, 0%; risk difference 0.2) and VEGF expression was higher in placentas from women with GDM compared to controls (21/29, 72.4% Standard Error 0.08 vs 2/10, 20% Standard Error 0.13; $p=0.007$) (Fig. 1).

Univariate logistic regression analysis showed that VEGF positivity was associated with the presence of GDM (cOR 10.5, 95% CI 1.82–60.45, $p=0.01$) and insulin therapy (cOR 3.42, 95% CI 0.88–13.18, $p=0.07$). Multivariate logistic regression analysis showed that VEGF positivity was associated with the presence of GDM (aOR 22.02, 95% CI 1.13–428.08, $p=0.04$), pregestational BMI (aOR 1.53, 1.00–2.34, $p=0.05$) and gestational weight gain (aOR 1.47, 95% CI 1.03–2.11, $p=0.03$) Table 2.

Univariate logistic regression analysis showed that none of considered variables was associated with CD31 positivity. It was not statistically possible to assess the contribution of GDM for the positivity of CD31, since all CD31-positive pregnancies were in the GDM group. Multivariate logistic regression analysis showed that CD31 positivity was associated with the pregestational BMI (aOR 1.47, 95% CI 1.00–2.17, $p=0.05$) and with the gestational weight gain (aOR 1.32, 95% CI 1.01–1.72, $p=0.04$) (Table 3).

Discussion

Our study demonstrates that pregnancies complicated by GDM are characterized by increased placental expression of VEGF and CD31, and the expression of these markers is also independently associated to maternal increased pregestational BMI and GWG.

Pregnancy is considered a diabetogenic condition, since placental secretion of diabetogenic hormones, such as growth hormone, leptin, corticotropin releasing hormone, placental lactogen, and progesterone, leads to increasing insulin resistance [21, 22]. In most of pregnancies, pancreatic β-cells hyperplasia compensates the increased insulin resistance by increasing insulin secretion throughout the pregnancy [23]. In some women, prolonged insulin production in response to a chronic fuel load is responsible for β-cell dysfunction, characterized by inability to adequately sense blood glucose levels or insufficient insulin release in response to a glucose load [24].

The cause of insulin resistance in GDM is multifactorial; some authors have pointed out the role of inflammation in the pathophysiology of GDM [25]. Inflammation pathways, through c-JUN N-terminal kinase (JNK) and nuclear factor-kappa B (NF-kB), are responsible for phosphorylation of the serine residues in the insulin receptor substrate-1 (IRS-1) preventing insulin signalling through the tyrosine kinase cascade, the production of pro-inflammatory cytokines, and the recruitment leucocytes and monocytes [26, 27]. Furthermore, pro-inflammatory cytokine tumor-necrosis factor-α (TNF-α) reduces insulin sensitivity by disrupting the translocation of glucose transporter GLUT-4 and insulin signal transduction [28, 29]. A systematic review and meta-analysis showed that TNF-α levels are higher in GDM cases than in controls, and multivariate linear regression analysis reported that pregestational BMI was the most predictive indicator of TNF-α levels in women with GDM [30].

Hyperglycemia in pregnancy is also associated to abnormal placental vessels features. A recent systematic review and meta-analysis showed that, compared to

Table 1 Characteristics of included women

	GDM ($n=29$)	Controls ($n=10$)	p
Age (years)	34.31 ± 4.82	34.10 ± 4.82	0.906
Pregestational BMI (kg/m^2)	28.4 [25.26–30.98]	22.87 [21.37–23.66]	0.332
GWG (kg)	8.59 ± 6.66	9.33 ± 3.08	0.750
Insulin therapy	19 (65.5%)	–	
Parity	0.72 ± 0.80	0.50 ± 0.70	0.436
Smoking	2 (6.9%)	1 (10%)	0.751
GA at delivery (weeks)	39.48 [39.11–39.80]	39.4 [38.84–40.1]	0.795
Placental weight (g)	561.6 [487.65–613.81]	623.30 [593.42–683.51]	0.165
Neonatal weight (g)	3194.14 [2930.08–3337.15]	3380 [2957.5–3458.43]	0.248
Fetoplacental ratio	5.8 ± 0.94	5.5 ± 1.22	0.470
Neonatal weight percentile	39.05 [29.78–52.27]	56.60 [44.36–75.51]	0.128
Neonatal LGA	2 (6.9%)	1 (10%)	0.751
Neonatal sex (male)	19 (65.5%)	5 (50%)	0.384

Data given as mean ± standard deviation, median [interquartile range] (IQR) or n (%)

GDM gestational diabetes, *BMI* body mass index, *GWG* gestational weight gain, *GA* gestational age, *LGA* large for gestational age

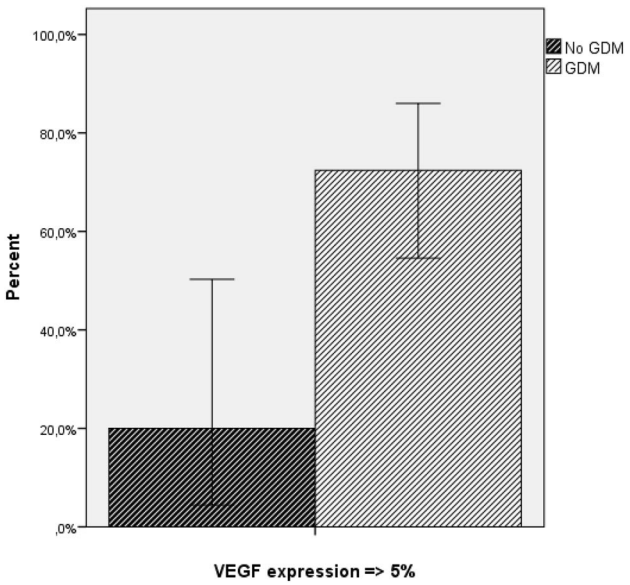

Fig. 1 Placental VEGF expression according to the presence of maternal GDM. Values are expressed as percentage and 95% CI error bars

healthy controls, placentas from GDM are characterized by increased placental weight, increased frequency of immature villi, increased mean number of redundant connections per terminal villi, increased incidence of fibrinoid necrosis and chorangiosis [31]. The mechanisms underlying the impairment of angiogenesis and vasculogenesis by hyperglycemia are still not completely understood [32, 33].

Hyperglycemia has been associated with an impaired response to hypoxia, via defects in the functional activity of hypoxia-inducible factor 1α (HIF-1α) and the accumulation of glycation end products such as methylglyoxal [34], and to the increase of oxidative stress products occurring during reoxygenation/reperfusion, leading to lower NO levels and insufficient vasodilatory response [35]. Hypoxia up-regulates VEGF-A, a potent mitogen for endothelial cells and pericytes. Glycation end products are toxic to pericytes, and it has been shown that glycated albumin induces angiogenesis, but the nascent vessels are deficient in pericytes [36, 37]. This deficiency can stimulate endothelial proliferation and lead to production of vessels which are more plastic (less mature) and more permeable, leading to an incompetent vasculogenesis [38]. Furthermore, hypoxia can also increase the production of erythropoietin, which itself has angiogenic properties [39]. Villous trophoblast secretes erythropoietin, and its receptor is found on trophoblast and the endothelium of fetal vessels [40].

Our findings show that placentas from GDM pregnancies are characterized by enhanced expression of VEGF and CD31, in line with increased neoangiogenesis and inflammation in these placentas compared to healthy controls. Up-to-date literature shows limited data with conflicting results in the evaluation of placental VEGF and CD31 expression in GDM pregnancies. In 2016, Meng et al. demonstrated that

Table 2 Logistic regression analysis: crude and adjusted odds ratios of variables associated with VEGF positivity

	cOR	*p*	95% CI	aOR	*p*	95% CI
Maternal age (years)	0.99	0.95	0.87–1.14	1.15	0.32	0.87–1.50
GDM	10.50	**0.01**	1.82–60.45	22.02	**0.04**	1.13–428.08
BMI (kg/m^2)	1.11	0.19	0.94–1.31	1.53	**0.05**	1.00–2.34
GWG (kg)	1.03	0.57	0.92–1.16	1.47	**0.03**	1.03–2.11
Smoke No (RC) Yes	3.14	0.36	0.26–37.99	172.98	0.17	0.10–293,304.19
Insulin therapy No (RC) Yes	3.42	**0.07**	0.88–13.18	0.35	0.45	0.02–5.13
GA at delivery (weeks)	0.79	0.53	0.37–1.68	0.40	0.19	0.10–1.56
Neonatal birthweight (g)	1.00	0.66	0.99–1.00	1.00	0.30	0.99–1.00
Fetoplacental ratio	1.32	0.41	0.67–2.60	1.34	0.59	0.45–3.96
Neonatal sex Female (RC) Male	1.46	0.57	0.39–5.39	1.08	0.94	0.11–10.15
CD31 No (RC) Yes	1.47	0.67	0.24–9.20	0.29	0.47	0.01–8.68

$p < 0.10$ shown in bold. Multivariate logistic analysis was performed to calculate aOR for each included variable adjusted for the other ones included in the table

cOR crude odds ratio, *aOR* adjusted odds ratio, *CI* confidence interval, *RC* reference category, *GDM* gestational diabetes, *BMI* body mass index, *GWG* gestational weight gain, *GA* gestational age

Table 3 Logistic regression analysis: crude and adjusted odds ratios of variables associated with CD31 positivity

	cOR	*p*	95% CI	aOR	*p*	95% CI
GDM	–	0.99	–	–	0.99	–
BMI (kg/m$^{2)}$	1.09	0.23	0.94–1.27	1.47	**0.05**	1.00–2.17
GWG (kg)	1.10	0.31	0.91–1.32	1.32	**0.04**	1.01–1.72
Insulin therapy No (RC) Yes	1.06	0.95	0.18–6.05	0.05	0.13	0.01–2.49
GA at delivery (weeks)	1.06	0.90	0.38–2.95	0.87	0.86	0.14–5.27
Neonatal birthweight (g)	1.00	0.91	0.99–1.00	1.00	0.41	0.99–1.00
Neonatal sex Female (RC) Male	3.68	0.26	0.39–35.14	3.42	0.41	0.18–66.69
VEGF No (RC) Yes	1.47	0.68	0.24–9.21	0.12	0.20	0.01–3.02

$p < 0.10$ shown in bold. Multivariate logistic analysis was performed to calculate aOR for each included variable adjusted for the other ones included in the table

cOR crude odds ratio, *aOR* adjusted odds ratio, *CI* confidence interval, *RC* reference category, *GDM* gestational diabetes, *BMI* body mass index, *GWG* gestational weight gain, *GA* gestational age

VEGFA and VEGFR2 were significantly reduced in GDM placentas compared to controls [15]. Troncoso et al. showed that, compared to controls, no increased VEGF expression in human umbilical vein endothelial cells (HUVEC) was found, even after stratifying for BMI, but increased CD31 expression in GDM HUVEC [16]. In contrast, Zhou et al. found no difference in CD31 expression in HUVEC between 7 GDM pregnancies and 7 controls [17]. The conflicting results from these studies, compared to our findings, may be due to the limited samples included in the analyses and to the different tissues included in the previous analysis. In fact, expression of neoangiogenesis and inflammatory markers in HUVEC may differ from the expression of the same markers in the placental layers due to the different grade of exposure to the maternal metabolic milieu. Furthermore, our study is the first to validate the association between placental expression of VEGF and CD31 and the maternal GDM with a multivariate logistic regression analysis, including the possible confounding factors.

Obesity is a strong independent risk factor for GDM, since the risk of developing GDM is increased 1.3–3.8 times in obese women compared to women of normal BMI [41]. A recent study by Grieger et al. showed that serum triglycerides level and waist circumference are among the most important contributors to the development of GDM, while Xi et al. demonstrated that second-trimester and third-trimester maternal triglycerides levels are correlated to the risk of neonatal LGA and macrosomia [42, 43]. Furthermore, Yoles et al. found that maternal obesity and fetal macrosomia in the first pregnancy are among the main risk factors for developing GDM in the subsequent pregnancy [44]. Adipose tissue is considered an endocrine organ that secretes adipokines including adiponectin and leptin and cytokines (TNF-α, IL-6, IL-1) [45, 46]. In addition to adipocytes, also macrophages from adipose tissue secrete pro-inflammatory adipokines [47]. Therefore, obesity is associated with chronic low-grade inflammation, termed "metainflammation", or metabolically induced inflammation, which impairs insulin signalling [48]. Furthermore, adipocytes synthetize macrophage chemotactic protein-1 (MCP-1), vascular and intracellular adhesion molecules (VCMA, ICAM), which recruit lymphocytes and macrophages, perpetrating the inflammation cycles, with effects including insulin resistance and endothelial dysfunction [49].

We found only one study evaluating the placental expression of VEGF according to the presence of obesity. This study from Dubova et al. confirmed that VEGF and VEGFR-2 expression was increased in placentas from obese women compared to normal weight pregnancies [50].

The most important strength of this study is that this is the first study to describe the possible correlation between pregestational BMI and gestational weight gain and the placental expression of neoangiogenesis and inflammation markers in pregnancies with GDM. Our data analysis not only confirms that placental VEGF and CD31 expression is increased in placentas from GDM pregnancies compared to healthy controls, but it also assesses that, apart from GDM, increased pregestational BMI and GWG are independent risk factors for the expression of placental neoangiogenesis and inflammation markers in these pregnancies.

Interestingly, even if univariate logistic analysis showed a correlation between the need of insulin therapy and the placental expression of VEGF, when we analysed all the cofactors in the multivariate logistic regression, we found that

the main contributors to placental VEGF expression were maternal BMI and GWG instead of insulin requirement.

Therefore, based on our preliminary data, we hypothesize that maternal hyperglycemia and increased maternal adipose tissue contributes in synergy to the imbalance of the placental function through the same biochemical pathways, contributing to define the concept of "placental diabesity".

Interestingly, multivariate logistic regression data show that placental VEGF and CD31 expression in GDM pregnancies is not dependent on the type of therapy (insulin therapy vs nutritional intervention).

Other strengths of our study are the prospective design, the strict inclusion criteria for both cases and controls, the availability of delivery outcome data of included pregnancies, and the rigorous evaluation of possible risk biases through a multivariate logistic regression analysis. Limitations of the study include limited number of included pregnancies, the lack of glucose control evaluation through Hba1c dosage in included GDM pregnancies, as well as other diabetogenic hormones (leptin, CRH, hPL), neoangiogenesis and inflammation markers.

This is the first study to describe how GDM and obesity may independently impact placental development and the expression of pro-angiogenetic and pro-inflammatory makers. Our data point out how preconception intervention in obese women and weight gain control in pregnant women could ameliorate the impact of metabolic disturbances on placental development in women at risk or diagnosed with GDM, reducing the expression of placental pro-inflammatory and neoangiogenesis markers. Further research on a larger cohort is needed to confirm our findings and to establish whether the increased placental expression of VEGF and CD31 is correlated to the incidence of adverse pregnancy and neonatal outcomes in GDM pregnancies.

Recent studies on murine models of diabetic retinopathy, which is also characterized by increased neoangiogenesis and inflammation, showed that metformin treatment inhibited VEGF signalling and attenuated diabetic retinopathy progression [51, 52]. Furthermore, cinnamaldehyde, one of the active components derived from Cinnamon, has been shown to reduce VEGF expression via suppressing HIF-1α gene expression in a murine model of cancer cells [53] and, recently, Hosni et al. demonstrated that cinnamaldehyde mitigates GDM-induced placental vascular dysfunction and protected from fetal hypoxia in a murine model [54]. In particular, cinnamaldehyde showed to reduce placental expression of VEGFA. It would be of interest to evaluate the effect of metformin and/or cinnamaldehyde on the placental expression of VEGF and CD31 in a human model, and whether these effects would impact perinatal outcomes of pregnancy.

Author contributions Conceptualization: AS and DP; methodology: VAD; validation: VA, AL and DP; formal analysis: EDR; investigation: AS; resources: AR and MDL; data curation: LT; writing—original draft preparation: AS; writing—review and editing: DP; visualization: VA; supervision: AL; project administration: AS. All authors have read and agreed to the published version of the manuscript.

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Declarations

Conflict of interest The authors declare no conflict of interest.

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