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SHORT COMMUNICATION

Neutrophil gelatinase-associated lipocalin (NGAL) levels in twin pregnancy and association with gestational diabetes

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Introduction

Gestational diabetes mellitus (GDM) is the most common metabolic complication of pregnancy, affecting up to 4–8% of pregnancies. The incidence of GDM in twin pregnancies is higher compared to singleton pregnancies [1]. The pathogenesis of GDM remains largely unknown, but it is associated with changes in metabolism during pregnancy such as maternal body weight gain, hyperglycemia, hyperinsulinemia, and insulin resistance. Identifying novel biomarkers for GDM to be used in clinical practice could improve the diagnosis and the management of the disease.

Neutrophil gelatinase-associated lipocalin (NGAL), also known as lipocalin-2 (LCN-2), is a 25-kDa glycoprotein, first identified as a matrix protein of specific granules of human neutrophils. NGAL is also expressed in many other human tissues and has a pleiotropic effect on immune function, cellular metabolism, homeostasis, and hormone function. Previous studies have shown the association between NGAL and cardiovascular disease [2]. The strict association between NGAL and kidney tubular damage has facilitated its current use in clinical practice, where it is measured in serum and urine to corroborate the diagnosis of acute kidney injury.

Some clinical studies have demonstrated that the serum NGAL levels of patients with type 2 diabetes mellitus were significantly correlated with insulin resistance [3]. A recent meta-analysis has demonstrated that serum NGAL levels were higher in pregnancies subsequently complicated by pre-eclampsia when compared to a control group [4].

However, we could not find any study that associates NGAL in twin pregnancies with GDM.

Therefore, this study aims to evaluate the serum NGAL levels in twin pregnancies during each trimester of gestation and its association with GDM and other clinical maternal characteristics.

A prospective, observational study was conducted between January 2018 and February 2022 at Careggi University Hospital in Florence, Italy. Thirty-nine women with twin pregnancies were enrolled and blood samples were collected in each trimester of gestation. All women gave written informed consent before inclusion. Of the 39 women, 15 developed GDM during pregnancy (cases) and 24 did not (controls). We included diet-controlled GDM cases as well as those requiring insulin treatment. GDM was diagnosed based on fasting serum glucose $\geq$ 5.1 mmol/L, and/or 1-h serum glucose $\geq$ 10.0 mmol/L, and/or 2-h serum glucose $\geq$ 8.6 mmol/L in the 75 g oral glucose tolerance test.

Both pregnancies conceived spontaneously and through assisted reproductive technologies (ART), either dichorionic or monochorionic, were included. Exclusion criteria were previous preterm birth, pregestational diabetes, chronic hypertension, renal disease, monochorionic–monoamniotic twin pregnancy, first or second trimester pregnancy loss, termination of pregnancy, major fetal anomalies, and loss to follow-up during prenatal care.

Blood samples were collected, and centrifuged at 750 × g/10 min. The serum was stored at − 80 °C. Then

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Table 1 Demographic, maternal and neonatal characteristics of the pregnant women with GDM compared to the control group

Clinical characteristics	All pregnant women ($n=39$)	Pregnant women without GDM (Control, $n=24$)	Pregnant women with GDM (Cases, $n=15$)	p-value
Ethnicity (n, %)				*0.085
Caucasian	32 (82.1%)	22 (91.7%)	10 (66.7%)	
Asian	7 (17.9%)	2 (8.3%)	5 (33.3%)	
Parity (n, %)				*0.500
0	26 (66.6%)	17 (70.8%)	9 (60%)	
≥ 1	13 (33.33%)	7 (29.2%)	6 (40%)	
Maternal age at sampling (years) (median and IQR)	36.0 (33.0–39.0)	34.0 (33.0–37.0)	38.0 (35.0–40.0)	#0.220
Pregestational BMI (median and IQR)	21.75 (20.50–23.52)	21.10 (19.80–22.80)	21.90 (21.30–24.00)	#0.260
Assisted reproductive technology (n, %)	12 (30.8%)	4 (16.7%)	8 (53.3%)	*0.030
Monochorionic-diamniotic pregnancy (n, %)	10 (25.6%)	9 (37.5%)	1 (6.7%)	*0.060
Dichorionic-diamniotic pregnancy (n, %)	29 (75.4%)	15 (62.5%)	14 (93.3%)	
Intrahepatic cholestasis of pregnancy (n, %)	8 (20.5%)	5 (20.9%)	3 (20.0%)	*0.950
Gestational age at sampling in the 1st trimester (weeks) (median and IQR)	12.40 (11.30–13.40)	12.50 (11.62–13.35)	12.40 (10.30–13.60)	#0.762
Gestational age at sampling in 2nd trimester (weeks) (median and IQR)	21.20 (20.00–23.00)	21.10 (20.10–23.00)	22.00 (18.40–22.40)	#0.919
Gestational age at sampling in 3rd trimester (weeks) (median and IQR)	29.35 (28.32–31.00)	29.40 (28.70–31.15)	29.20 (28.00–31.00)	#0.409
Gestational age at delivery (weeks) (median and IQR)	36.40 (35.10–37.10)	36.10 (34.52–36.90)	37.00 (35.60–37.30)	#0.120
Spontaneous preterm birth	6 (20.5%)	6 (25%)	0	*0.065
1st Baby sex (n, %)				*0.100
Male	20 (51.3%)	15 (62.5%)	5 (33.3%)	
Female	19 (48.7%)	9 (37.5%)	10 (66.7%)	
2nd Baby sex (n, %)				*1.000
Male	26 (66.6%)	16 (66.7%)	10 (66.7%)	
Female	13 (33.3%)	8 (33.3%)	5 (33.3%)	
1st Baby birth-weight (g) (Mean ± SD)	2320.64 ± 381.5	2288.0 ± 396.0	2373.0 ± 363.0	+0.500
2nd Baby birth-weight (g) (Mean ± SD)	2377.0 ± 343.2	2330.80 ± 388.0	2452.0 ± 250.6	+0.290

Normality of data was tested by the Kolmogorov–Smirnov test

Data are n (%), median IQR, Mean ± SD, * χ^2 test for categorical data, #Mann–Whitney U test for non-parametric variables, +t-student test

NGAL levels (ng/ml) were evaluated by using DuoSet ELISA KIT (Biotechne, USA). Optical density was determined at 450 nm, using a Victor Nivo microplate reader (PerkiElmer, USA).

Table 2 Statistical comparison of the level of serum NGAL between control group and patient group

Serum NGAL level (ng/ml)	Control group	Cases group	#p-value
1st trimester	14.51 (12.00–13.50)	16.00 (12.20–15.33)	0.363
2nd trimester	14.65 (16.60–15.70)	17.22 (17.00–19.35)	0.004
3rd trimester	15.90 (13.20–23.00)	17.13 (16.70–19.00)	0.262

Normality is tested by the Kolmogorov–Smirnov test

NGAL: Neutrophil gelatinase-associated lipocalin

Data are median IQR, #Mann–Whitney U test

The statistical analysis was performed using the IBM SPSS Statistics, version 28 A. The normality of the distribution was tested by the Kolmogorov–Smirnov test. Normally distributed data were analyzed with an independent t-student test. Non-parametric variables were compared using the Mann–Whitney U test for continuous data. The χ^2 test was used for categorical data. A multivariable regression analysis was used to assess if serum level of NGAL are independent predictors of GDM. Spearman correlation analysis was performed to assess the relationship between continuous variables. Results were statistically significant when the p value was < 0.05.

Demographic, clinical information, maternal and neonatal outcomes of the enrolled pregnant women are reported in Table 1. No significant differences were observed in ethnicity, maternal age at the time of sampling and in pregestational body mass index (BMI) between cases and controls.

Table 3 Multinomial logistic regression model for the prediction of gestational diabetes

All pregnant women (n = 39)	OR	95% Confidence Interval		*p*
		Lower Bound	Upper Bound	
Diabetes				
Intercept		0.043		
Age	1.267	0.996	1.611	0.054
Pregestational Body mass index	1.075	0.784	1.473	0.655
Gestational age at sampling in 2nd trimester	1.031	0.718	1.482	0.867
NGAL level (ng/ml) 2nd trimester	1.382	1.022	1.868	0.036

In addition, there was no significant difference in the median gestational age at serum collection in each trimester and at delivery. The percentage of pregnancies conceived using ART was significantly higher in the case group compared to controls.

The results of the comparison of the serum NGAL level between cases and controls at the univariate analysis are presented in Table 2. Second trimester NGAL levels were significantly higher among cases compared to controls ($p = 0.004$), with no difference noted in the first- and third-trimester between the groups. Multinomial regression analysis (Table 3) identified second trimester NGAL level as an independent predictor of gestational diabetes (OR 1.38, 95% CI 1.02–1.87), after adjusting for potential confounders. Second trimester NGAL levels were not correlated with BMI nor with maternal age ($p = 0.078$ and $p = 0.20$, respectively).

Our results showed that in twin pregnancies serum NGAL levels in the second trimester may be predictive of the onset of diabetes in pregnancy. Interestingly, the correlation analysis did not show any significant association between the serum levels of NGAL and maternal characteristics such as maternal age and pregestational BMI. These results are partially in line with the study of Wang et al. [3] which failed to demonstrate a positive correlation between NGAL and women's BMI [3].

Lu et al. [5] demonstrated a positive association between serum NGAL levels in the first trimester of pregnancy and the risk of developing GDM later in pregnancy, suggesting that NGAL is an early predictive biomarker for GDM, which could help guide the clinical practice of antenatal care. However, the authors did not measure the changes in NGAL levels during the second and third trimesters [5].

In conclusion, this is the first study to measure NGAL in each trimester of gestation in women with twin pregnancies and to compare serum levels of this protein between women with or without GDM. The level of NGAL in the second trimester was significantly increased compared to the control group. Further in-depth research studies involving larger sample sizes within each group, and incorporating additional biomarkers are necessary to confirm this preliminary data and clarify the role of NGAL in gestational diabetes.

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Declarations

Conflict of interest The authors declare that they have no conflicts of interest.

Ethical standard statement The study was approved by the Ethical Committee of Azienda Ospedaliero-Universitaria Careggi (Ref. No.10255/2017). All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2008.

Informed consent An informed consent was obtained from all patients for being included in the study.

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