

Research Article

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Evaluation of second trimester plasma lipoxin A4, VEGFR-1, IL-6, and TNF- α levels in pregnant women with gestational diabetes mellitus

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Abstract: In this study, our objective was to explore the association between gestational diabetes mellitus (GDM) and second trimester maternal plasma levels of lipoxin A4 (LXA4), along with proinflammatory markers such as interleukin-6 (IL-6) and tumour necrosis factor alpha (TNF- α), and the anti-angiogenic factor vascular endothelial growth factor receptor 1 (VEGFR-1) in pregnant women. The study included a cohort of 30 pregnant women with GDM and a control group of 30 normoglycaemic pregnant women matched for age, body mass index, and gestational age. Plasma samples were collected and analysed by enzyme-linked immunosorbent assay to assess specific biomarkers. The GDM group had significantly lower levels of LXA4 and higher levels of TNF- α and VEGFR-1 compared to the control group ($p = 0.038$, $p = 0.025$, and $p = 0.002$, respectively). A statistically significant decrease in

the LXA4/TNF- α ratio was observed in the GDM group ($p = 0.004$). The results suggest that each unit decrease in the LXA4/TNF- α ratio is associated with a 1.280-fold increase in the risk of GDM. These findings suggest a potential diagnostic role for the LXA4/TNF α ratio as a marker for women with GDM. This work provides new insights into the pathogenesis of GDM and highlights the important interplay between inflammation and metabolic dysregulation.

Keywords: gestational diabetes mellitus, lipoxin A4, pro-inflammatory cytokines, tumour necrosis factor-alpha, vascular endothelial growth factor receptor-1

1 Introduction

Gestational diabetes mellitus (GDM) is a pathological condition characterised by varying degrees of hyperglycaemia and glucose intolerance that is either initiated or first identified during pregnancy [1]. A physiologically normal second trimester of pregnancy typically exhibits a complex balance between insulin resistance, compensatory β -cell proliferation, and hyperinsulinemia, all induced by increased maternal adiposity and placenta-secreted diabetogenic hormones. Non-diabetic pregnant women can successfully mitigate this increased insulin resistance by upregulating insulin production. Conversely, GDM manifests in those who are unable to increase insulin secretion sufficiently to compensate for the metabolic stress induced by insulin resistance during pregnancy. The estimated prevalence of GDM in pregnancies is approximately 7%, although this rate can range from 1 to 14% based on demographic factors and variations in screening and diagnostic approaches [2].

GDM shares similar pathophysiological mechanisms and risk factors with type 2 diabetes mellitus (T2DM), including genetic predisposition, sedentary lifestyle, and obesity. Obesity, a major risk factor for T2DM, has been shown to correlate with subacute inflammation and elevated levels of pro-inflammatory

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molecules that exacerbate insulin resistance [3]. However, the presence of a chronic inflammatory state in GDM remains controversial. The hypothesis that modulation or suppression of inflammatory dysfunction may play a critical role in the pathogenesis of GDM has been raised in recent scientific discourse. Lipoxins are recently identified endogenous anti-inflammatory autacoids of lipid origin that are synthesised from arachidonic acid via a lipoxygenase-mediated transcellular biosynthetic pathway. Lipoxin A4 (LXA4) and its analogs are often referred to as “brake signals” of inflammation, modulating the onset and acting as agonists during the resolution phase of inflammatory responses. LXA4 mediates its effects at inflammatory sites by binding to the high-affinity G protein-coupled receptor N-formyl peptide receptor 2 (FPR2/ALX), which has been documented as the primary receptor responsible for the anti-inflammatory effects of LXA4 *in vivo*. Its modulatory role includes inhibition of neutrophil and eosinophil chemotaxis and antagonism to peptidoleukotrienes, while stimulating macrophage-mediated phagocytosis of apoptotic cells and reducing inflammatory infiltrates and oedema *in vivo* [4].

Endogenous LXA4 plays a critical role in maintaining a physiologically normal pregnancy by modulating inflammation-related factors, mast cells, and other cellular components. However, comprehensive data on the association between serum LXA4 levels and GDM are lacking in the current scientific discourse. Proinflammatory cytokines can potentially trigger the onset of insulin resistance and impaired glucose metabolism by interfering with insulin signalling pathways [5]. Previous studies have shown down-regulation of anti-inflammatory markers such as interleukin (IL)-4 and -10, and increased levels of pro-inflammatory cytokines such as IL-6 and tumour necrosis factor- α (TNF- α) in patients with GDM [6,7]. However, the role of vascular endothelial growth factor (VEGF) and its associated receptors, VEGF receptor (VEGFR)-1 and VEGFR-2, which are essential for normal placental angiogenesis, have been postulated as important modulators in the regulation of GDM [8]. While some studies have reported increased levels of these pro-inflammatory and angiogenic factors in GDM, others have failed to confirm these findings [9].

The primary objective of this study was to accurately quantify plasma levels of LXA4 in relation to pro-inflammatory factors, specifically VEGFR-1, IL-6, and TNF- α , in the context of GDM. The importance of this objective lies in its potential to elucidate the complex pathophysiological processes underlying GDM, thereby providing a clearer understanding of its development and progression.

2 Methods

Prior to commencement of the study, approval was obtained from the Inonu University Clinical Research Ethics Committee (approval number: 2021/115). In accordance with the tenets of the Declaration of Helsinki (as revised in 2013), all participating women were provided with both written and verbal information about the study, and informed consent was subsequently obtained. The study population comprised pregnant women who were diagnosed with GDM and presented to the Prenatal Diagnosis and Treatment Unit, Faculty of Medicine, Inonu University, during the period from 1 May 2021 to 1 May 2022. Among these patients, a subset of 30 women diagnosed with GDM formed the study group, while age, gestational age, and body mass index (BMI) matched cohort of 30 normoglycaemic women formed the control group. The gestational age of the participating women was validated by first trimester ultrasound measurements. Eligibility criteria for participation in the study were defined as follows:

Inclusion criteria:

- Age between 18 and 45 years
- Carrying a single viable pregnancy
- BMI below 35 kg/m²
- Normal obstetric and medical history

Exclusion criteria:

- Multiple gestation
- Presence of hypertensive disorders of pregnancy, including pre-eclampsia, eclampsia and HELLP syndrome, or concomitant maternal systemic disease such as dyslipidaemia, chronic hypertension, chronic renal failure, malignancy, asthma, or pulmonary or cardiac disease.
- Presence of abnormal karyotypes or faetal malformations
- Use of alcohol or tobacco

All pregnant women without pre-existing risk factors were screened for GDM between 24 and 28 weeks of pregnancy. The GDM screening protocol was a one-step procedure using a 75 g oral glucose tolerance test. The initial blood sample was obtained after 8–10 h overnight fast, between 08:00 and 09:00 AM, by drawing approximately 2 mL of blood into a standard biochemistry tube. Following this initial sample, patients were given a solution containing 75 g of anhydrous glucose in 300 mL of water to be consumed within 5 min. Further blood samples were taken at intervals of 1 and 2 h after glucose ingestion. A diagnosis of GDM was made if one or more of the thresholds

defined by the International Diabetes in Pregnancy Working Group were met or exceeded. These thresholds were set at a fasting glucose level of 92 mg/dL, a 1 h postprandial level of 180 mg/dL, and a 2 h postprandial level of 153 mg/dL.

Standard serum analyses were performed using the Abbott Architect c8000 system (Abbott Diagnostics, USA) at the Biochemistry Laboratory, Inonu University School of Medicine. For GDM screening, a 2 mL peripheral blood sample was collected in EDTA-anticoagulated tubes from all participants at baseline. Plasma was isolated by centrifugation at 3,000 g for 15 min at room temperature and stored at -80°C until analysis. When the required sample size was reached, the plasma samples were thawed and the quantitative levels of LXA4, VEGFR-1, IL-6, and TNF- α were determined by enzyme-linked immunosorbent assay (ELISA). ELISA kits for LXA4, IL-6, TNF- α (Cat. No: E3155Hu, E2063Hu, and E0796Hu, respectively; Sunredbio Corp, China) and VEGFR-1 (Cat. No: E3155Hu; Cloud-Clone Corp, China) were used according to the manufacturer's protocols. The assays for LXA4, IL-6, TNF- α , and VEGFR-1 provided measuring ranges of 0.1–38.0 nmol/L, 1–400, 0.5–150, and 0.3–90.0 ng/mL, respectively. The coefficients of variation for both inter- and intra-assay precision were less than 10 and 8%, respectively, for all ELISA kits used. Results were calculated using a standard curve, and the measurements were expressed in picograms per millilitre (pg/mL) for all four ELISA assays. To ensure the precision and accuracy of the measurements, the ELISA assays were performed in duplicate.

Demographic and clinical variables were meticulously recorded, including the participant's age, parity, number of caesarean sections, BMI measured in kg/m^2 , and any history of GDM in previous pregnancies. Fasting blood glucose (mg/dL), first hour postprandial blood glucose (mg/dL) and second hour postprandial blood glucose (mg/dL) were also recorded. Gestational age at GDM screening was noted. In addition, plasma levels of LXA4 (pg/mL), VEGFR-1 (ng/dL), IL-6 (pg/mL), and TNF- α (ng/dL) were recorded. Finally, the mode of delivery, the birth weight of the newborn, the newborn's gender, and cord blood parameters of neonate were likewise documented.

2.1 Sample size calculation

A power analysis was performed to determine the sample size required for this study. The analysis was based on the assumption of a statistically significant decrease in the LXA4 ratio of 1.0 pg/mL, corresponding to a standard deviation of 1.7, in pregnant women with complications of GDM. Calculations showed that at least 30 participants in each

group were needed to detect this expected difference with 80% power at the 5% two-sided significance level.

2.2 Statistical analysis

Statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) software, version 22.0 (SPSS Inc, New York, USA). Baseline characteristics of the study and control groups were presented as median ranges and/or interquartile ranges, while continuous variables were expressed as mean values, standard deviations, and minimum and maximum values. In the initial stages of statistical comparison, the Shapiro–Wilk test was used to assess the normality of the distribution of the data. Once normality was confirmed, two-sample *t*-tests were used to compare the main differences between the study and control groups for normally distributed data. In cases where the data did not conform to a normal distribution, the Mann–Whitney *U* test was used for comparative analyses. Categorical variables were summarised as frequencies and percentages, with comparisons using Pearson's exact chi-squared test and continuity-corrected chi-squared tests. Binary logistic regression models were constructed with group classification as the dependent variable and LXA4, TNF- α , VEGFR-1, and LXA4/TNF- α ratio as the independent variables. The goodness of fit of the binary logistic regression model was assessed using the Hosmer–Lemeshow test, and a two-tailed *p*-value less than or equal to 0.05 ($\alpha = 0.05$) was considered statistically significant.

3 Results

3.1 Clinical characteristics of the study population

Compared with the normoglycaemic control group, no statistically significant differences were found in the variables of age, gravidity, parity, BMI, gestational age at screening for GDM, and gestational age at delivery in pregnant women diagnosed with GDM ($p = 0.151$, $p = 0.368$, $p = 0.367$, $p = 0.095$, $p = 0.148$, and $p = 0.501$, respectively). When analysing birth outcomes between the study and control groups, no statistically significant differences were observed in the gestational age at delivery, mode of delivery, cord blood pH, and cord blood base deficit scores ($p = 0.501$, $p = 1.000$, $p = 0.994$, and $p = 0.600$, respectively). However, a significant

increase in birth weight was observed in pregnancies complicated by GDM compared to the control group ($p = 0.027$). The detailed maternal characteristics and birth outcomes for both the study and control groups are comprehensively summarised in Table 1.

3.2 Pro- and anti-inflammatory mediators in GDM patients

The concentrations of pro- and anti-inflammatory mediators in the peripheral blood plasma of the study and control groups were analysed. A significantly lower concentration of LXA4 was observed in the plasma of pregnant women diagnosed with GDM compared to the control group ($p = 0.038$). IL-6 levels did not show a statistically significant difference between the two groups ($p = 0.518$). In contrast, plasma levels of TNF- α and VEGFR1 were found to be statistically significantly elevated in the GDM group compared to the control group ($p = 0.025$ and $p = 0.002$, respectively). The plasma concentrations of LXA4, IL-6, TNF- α , and VEGFR1 in the study and control groups are shown in Table 2.

Table 2: Plasma LXA4, IL-6, TNF- α , and VEGFR1 values of the study and control groups

Variable	GDM ($n = 30$)	Control ($n = 30$)	p value
LXA4 (ng/mL)*	31.58 (11.42–74.63)	39.38 (1.01–71.34)	0.038
IL-6 (ng/L)*	15.66 (1.06–199.65)	9.93 (1.91–255.97)	0.518
TNF- α (ng/L)*	200.08 (63.57–1121.25)	111.5 (56.31–1243.6)	0.025
VEGFR1 (pg/mL)*	1476.81 (500.66–5455.19)	944.9 (82.4–1660.65)	0.002

*median (minimum–maximum).

LXA4: Lipoxin A4; IL: Interleukin; TNF: Tumour necrosis factor; VEGFR-1: Vascular endothelial growth factor receptor 1; DM: Diabetes mellitus.

Bold values denote statistical significance at the $p < 0.05$ level.

The ratios of LXA4/TNF- α and LXA4/IL-6 were also evaluated in both the GDM and control groups. A statistically significant decrease in the LXA4/TNF- α ratio ($p = 0.004$) was observed in the pregnant women diagnosed with GDM compared to the control group (Table 3 and Figure 1a). On the other hand, no statistically significant difference was observed when comparing the LXA4/IL-6

Table 1: Clinical characteristics and birth outcomes of the GDM and control groups

Variable	GDM ($n = 30$)	Control ($n = 30$)	p value
Age (years)*	33 (28–42)	31 (19–35)	0.151
Gravidity*	3 (1–5)	2 (0–4)	0.368
Parity*	1 (0–3)	1 (0–4)	0.367
Weight (kg)**	83.3 \pm 17.87	77.38 \pm 9.38	0.301
Height (cm)**	160.63 \pm 4.44	161.77 \pm 5.62	0.462
BMI (kg/m ²)**	32.32 \pm 7.09	29.73 \pm 3.23	0.095
GDM history in previous pregnancy	3 (10.0)	0 (0.0)	0.236
Gestational age at screening (weeks)*	26 (24–28)	25 (24–28)	0.148
Fasting glucose level (mg/dL)**	98.77 \pm 15.32	84.97 \pm 4.26	<0.001
1 h glucose level (mg/dL)**	187.37 \pm 24.67	146.43 \pm 13.67	<0.001
2 h glucose level (mg/dL)**	176.97 \pm 37.95	124.3 \pm 14.79	<0.001
Gestational age at birth (weeks)*	37 (31–40)	38 (28–40)	0.501
Mode of delivery***			
	Vaginal delivery	3 (10.0)	1.000
	Caesarean section	27 (90.0)	
Birthweight (g)*	3,065 (1,140–4,800)	2,740 (960–3,727)	0.027
Gender***			
	Female	14 (46.7)	0.300
	Male	16 (53.3)	
Cord blood pH*	7.33 \pm 0.14	7.31 \pm 0.15	0.994
Cord blood base excess*	–4.95 \pm 3.35	–5.55 \pm 4.58	0.600

*mean value \pm standard deviation; **median (minimum–maximum); *** n (%).

BMI: Body mass index, GDM: Gestational diabetes mellitus.

Bold values denote statistical significance at the $p < 0.05$ level.

Table 3: Comparison of groups according to LXA4/TNF- α and LXA4/IL-6 ratios

Variable	GDM ($n = 30$)	Control ($n = 30$)	p value
LXA4/TNF- α *	0.21 \pm 0.07	0.28 \pm 0.11	0.004
LXA4/IL-6*	3.75 \pm 1.66	3.32 \pm 1.52	0.243

*mean value \pm standard deviation.

LXA4: Lipoxin A4; IL: Interleukin; TNF: Tumour necrosis factor; DM: Diabetes mellitus.

Bold values denote statistical significance at the $p < 0.05$ level.

ratio between the GDM group and the control group ($p = 0.243$) (Table 3 and Figure 1b).

3.3 Predictive value of inflammatory mediators for GDM

To assess the predictive ability of pro-inflammatory molecules in relation to GDM, we used a binary logistic regression model. The independent variables in the model were LXA4, TNF- α , VEGFR-1, and LXA4/TNF- α ratio, while the

dependent variable was the binary variable representing the GDM and control groups. We used the Hosmer–Lemeshow statistic to assess the goodness of fit of the model, which confirmed its statistical validity for estimating differences between the data groups (GDM vs control) ($\chi^2 = 11.329$, $df = 8$, $p = 0.184 > 0.05$).

Our findings revealed an inverse relationship, where each one-unit increase in LXA4 was associated with a 0.918-fold decrease in the risk of GDM. In addition, our analysis showed that each unit increase in TNF- α was associated with a 1.023-fold increase in the risk of GDM. Similarly, a one-unit increase in VEGFR-1 was associated with a 1.031-fold increase in the odds of GDM. Moreover, our results indicated that a unit increase in the LXA4/TNF- α ratio was associated with an 0.826-fold decrease in the risk of GDM. Detailed information on parameter estimates (β), standard errors (SE), Wald statistics (W), degrees of freedom (df), odds ratios [$\text{Exp}(\beta)$], and 95% confidence intervals (CI) are shown in Table 4.

4 Discussion

Emerging evidence suggests that the aetiology of GDM may be intricately linked to inflammatory responses and endothelial dysfunction; however, a comprehensive understanding of these factors remains incomplete. The current study provides insight into the role of pro-inflammatory cytokines, anti-angiogenic factors and, in particular, LXA4 – an endogenous anti-inflammatory mediator – in the pathogenesis of GDM. The complex mechanisms regulating inflammation during pregnancy require a delicate balance between pro- and anti-inflammatory mediators. Disruption of this balance, as indicated by changes in monocyte patterns, may contribute to the development of GDM. Prior to our study, the role of LXA4 in the regulation of inflammation in the context of GDM was unexplored. We hypothesised that LXA4 may be involved in the pathogenesis of GDM because of its known role in preventing the onset of inflammation and facilitating the resolution of inflammatory processes. Our results support this hypothesis, showing significantly lower plasma levels of LXA4 in women diagnosed with GDM compared to normoglycaemic pregnant women. This suggests a possible deficiency of this anti-inflammatory mediator in the setting of GDM, potentially disrupting the balance of inflammatory regulation.

Lipoxins, including LXA4, are derivatives of arachidonic acid that have significant anti-inflammatory activity both *in vivo* and *in vitro* [10]. Pregnancy represents a unique physiological state that transitions from an anti-inflammatory

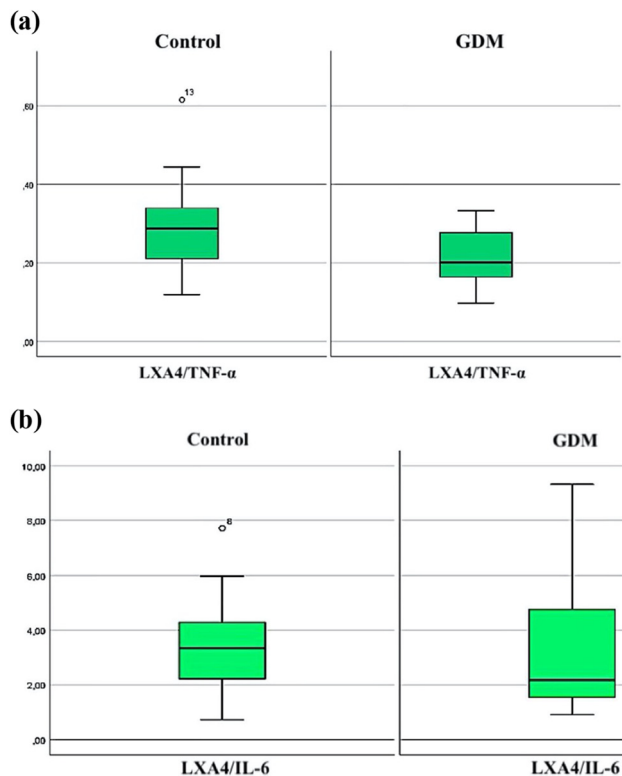


Figure 1: LXA4/TNF- α ratio in the second trimester (a) and LXA4/IL-6 ratio (b) in pregnant women with GDM and in the control group.

Table 4: Estimated values of the parameters in the model

Variables	β	SE	W	df	p value (sig)	Exp(β)	95% CI for Exp(β)	
							Lower limit	Upper limit
LXA4 (ng/mL)	-0.011	0.005	3.978	1.000	0.046	0.918	0.850	0.981
TNF- α (ng/L)	0.003	0.001	5.030	1.000	0.025	1.023	1.112	1.129
VEGFR1 (pg/mL)	0.001	0.000	7.437	1.000	0.016	1.031	1.215	1.517
LXA4/TNF- α (ng/mL)	-9.919	3.703	7.177	1.000	0.007	0.826	0.785	0.870
Constant	2.408	0.953	6.387	1.000	0.011	11.114		

β : parameter estimation; SE: standard error; W: Wald statistic, df: degrees of freedom; Exp(β): odds ratio; 95% CI: confidence interval; LXA4: Lipoxin A4; TNF: Tumour necrosis factor; VEGFR-1: Vascular endothelial growth factor receptor 1.

milieu in early pregnancy to a more pro-inflammatory environment in the second half of pregnancy and just prior to delivery. LXA4 has been proposed to modulate these fluctuations, with lower levels being associated with blastocyst implantation and delivery, and higher levels being beneficial for maintaining the mid-gestational period and the normal course of pregnancy [11]. In the context of pregnancy complications, LXA4 has been reported to suppress the overproduction of pro-inflammatory cytokines such as TNF- α and IL-1 β in monocytes from individuals with severe pre-eclampsia, thereby contributing to the amelioration of inflammatory symptoms and promoting a healthy pregnancy outcome [12]. Furthermore, LXA4 competes with oestrogen and progesterone receptors, critical hormonal regulators during pregnancy, highlighting its integral role in pregnancy physiology. Remarkably, a reduction in plasma LXA4 levels and its corresponding receptor, FPR2/ALX, has been identified in pre-eclamptic pregnancies. This finding suggests an underlying imbalance between LXA4 and pro-inflammatory cytokines like IL-1 β and TNF- α [13]. Early pregnancy has also been associated with increased LXA4 levels, possibly due to the secretion of human chorionic gonadotropin [14]. The results of this study showed a significant decrease in LXA4 levels in the GDM patient group compared to the control group. These findings are consistent with the notion that changes in LXA4 levels may be associated with the development or progression of GDM. To further investigate the role of LXA4 in GDM, a study by Gázquez *et al.* focused on pregnant mice with diabetes. In contrast, their results showed no significant difference in urinary LXA4 levels between the GDM group and the control group [15]. This discrepancy in results between the two studies may be due to differences in study design, sample size or even species differences between humans and mice. However, further research is needed to fully elucidate the reasons for this discrepancy. In addition, the researchers in our study assessed the ratio of LXA4 to proinflammatory cytokines, specifically IL-6 and TNF- α , in pregnant women with GDM, and the control group. Notably,

there was no statistically significant difference in the LXA4/IL-6 ratio between the groups. However, the LXA4/TNF- α ratio was significantly lower in pregnant women with GDM compared to the control group. These findings suggest that the imbalance between LXA4 and TNF- α may play a crucial role in the pathogenesis of GDM. Furthermore, utilising a binary logistic regression model, the researchers demonstrated that a unit increase in the LXA4/TNF- α ratio was associated with an 0.826-fold reduction in the risk of GDM. This observation highlights the potential utility of the LXA4-TNF- α ratio as a predictive marker for GDM. Notably, this study is the first to demonstrate a clear association between changes in the LXA4/TNF- α ratio and the risk of developing GDM. These findings provide valuable insights into the pathophysiological mechanisms underlying GDM and offer potential avenues for further research and the development of therapeutic interventions targeting the LXA4-TNF- α axis.

TNF- α and IL-6 are cytokines known to play a critical role in maintaining a balanced inflammatory milieu at the maternal-faetal interface during pregnancy [16]. The current study demonstrates the inhibitory effects of LXA4 on LPS-induced pro-inflammatory factors, including TNF- α , as well as on anti-angiogenic factors such as VEGFR-1. In line with these findings, a recent study was conducted in pregnant women with pre-eclampsia to investigate the effect of LXA4 on pro-inflammatory cytokines. In this study, reduced levels of LXA4 were observed in pre-eclamptic individuals, together with a significant increase in TNF- α , IL-6, and interferon gamma levels [17]. These collective findings suggest that endogenous LXA4 plays a pivotal role in maintaining normal pregnancies by modulating inflammation-related factors and preventing inflammation-associated obstetric complications such as GDM.

The development of peripheral insulin resistance in GDM has been attributed to increased levels of proinflammatory cytokines [18]. In addition, the human placenta itself expresses several cytokines, including TNF- α and

IL-6, which originate from adipocytes, as well as adipokines such as resistin and leptin, which are involved in insulin regulation [19]. However, conflicting results have been reported regarding the levels of pro-inflammatory cytokines in GDM. Some studies have shown elevated levels of IL-6, IL-10, and TNF- α in patients with GDM compared to controls [20], while others have found no significant differences [21,22]. In our study, no significant difference in serum IL-6 levels was observed between the GDM patient group and the control group, whereas TNF- α levels were significantly higher in pregnant women with complicated GDM. It is important to note that cytokine levels fluctuate throughout pregnancy, from a pro-inflammatory state in the first and third trimesters to an anti-inflammatory state in the second trimester. The discrepancies in inflammatory markers observed between GDM and non-GDM pregnancies are most pronounced during the first half of pregnancy [23]. These conflicting findings highlight the complex and dynamic nature of inflammatory processes during pregnancy and suggest that GDM-related inflammatory responses may exhibit different patterns compared to non-GDM pregnancies. Further investigation is warranted to elucidate the precise mechanisms underlying these differences and to comprehensively assess the role of proinflammatory cytokines in the pathogenesis of GDM.

Soluble VEGFR-1 (sVEGFR-1), a splice variant of VEGFR-1 lacking transmembrane and cytoplasmic domains, circulates in the bloodstream and acts as a potent antagonist of the angiogenic factors VEGF and placental growth factor (PLGF), effectively inhibiting their interaction with cellular receptors [24]. The formation and continuity of the placenta depends on the synchronised processes of angiogenesis and vasculogenesis involving key ligand-receptor systems such as VEGF, PLGF, and VEGF receptors (Flt-1). Activation of placental angiogenesis and vasculogenesis is mediated by Flt-1 interactions with VEGF and PLGF, whereas inactivation is achieved by sFlt-1 interactions with VEGF and PLGF. Thus, sFlt-1 plays a crucial role in the development of endothelial dysfunction. In the current study, we found that plasma Flt-1 levels were elevated in second trimester GDM patients compared to healthy pregnant individuals. Existing literature supports a strong association between elevated Flt-1 levels and the risk of GDM. In healthy pregnancies, Flt-1 levels remain stable throughout the first and second trimesters, but show a sharp increase between 33 and 36 weeks' gestation. Elevated levels of sFlt-1 have been associated with reduced circulation of VEGF and PLGF in pre-eclampsia. In addition, increased circulation of sVEGFR-1 levels have been implicated in the development of pre-eclampsia-related hypertension, proteinuria, and glomerular endotheliosis

in a rat model [25,26]. Meng et al. reported a significant decrease in VEGF-A and VEGFR-2 expression in placental tissue of GDM patients compared to controls [27]. Under hypoxic conditions, VEGF expression is oxygen dependent and upregulated. In normal pregnancy, placental VEGF is elevated during the first trimester when oxygen levels are low and then decreases [28]. In GDM, high glucose levels, inhibition of VEGF/VEGFR2 binding, hypercapillary reduction, and hypoxia/ischemia differentially regulate VEGFR levels under hyperglycaemic conditions. A separate study reported significantly higher VEGFR-1 release from adipose tissue in patients with GDM compared to women with normal glycaemic tolerance [29]. The increased VEGFR-1 levels found in this study may be due to increased adipose tissue and placental inflammation, leading to increased production of angiogenic molecules in the adipose tissue of GDM patients.

Although the present study provides valuable insights into the role of LXA4 and inflammatory cytokines in the context of GDM, it is important to acknowledge some limitations. The study was conducted in a single centre, limiting the diversity of the study population and potentially limiting the generalisability of the findings. In addition, the sample size, although adequate for an initial analysis of plasma LXA4 levels and inflammatory cytokines, was relatively small, which may reduce statistical power and potentially mask the presence of certain effects. In addition, the assessment of plasma LXA4 levels was limited to the second trimester, without the inclusion of serial LXA4 assays at different stages of pregnancy, which might have provided a more comprehensive understanding of LXA4 dynamics throughout pregnancy.

Nevertheless, the study has notable strengths. The main strength of the study was the first assessment of LXA4 levels in pregnant women complicated by GDM and controls. It represents a pioneering effort to assess LXA4 levels in pregnant women with complicated GDM and to compare these levels with those in normoglycaemic control pregnant women. This approach has revealed important patterns of difference, thereby enhancing our understanding of the underlying pathophysiological mechanisms of GDM. Another major strength of the study is its prospective cohort design, which allowed real-time tracking of patient progression and the establishment of temporal relationships between variables. As a result, the findings of this study not only contribute to the current body of knowledge in this field, but also lay the groundwork for future research aimed at further delineating the etiopathogenesis of GDM and potentially identifying novel diagnostic and therapeutic targets.

In this study, pro- and anti-inflammatory mediators were examined together for the first time, with the aim

of shedding light on the etiopathogenesis in pregnant women with complicated GDM. The results of the study suggest that the LXA4/TNF α ratio may serve as a potential diagnostic marker and a prognostic tool for monitoring women diagnosed with GDM. This study also provides new insights into the pathogenesis of GDM, highlighting the significant interplay between inflammation and metabolic dysregulation, with a focus on the balance between pro- and anti-inflammatory responses. Importantly, the differential pattern of LXA4 and TNF α in GDM revealed by this study may elucidate a potential pathophysiological mechanism whereby altered anti-inflammatory responses lead to an inflammatory predominance, contributing to the development and progression of GDM. In addition, these findings may pave the way for the identification of new biomarkers for the early detection of GDM. By enabling earlier diagnosis and intervention in high-risk populations, such an approach may potentially reduce the maternal and neonatal complications associated with GDM, thereby improving overall pregnancy outcomes. While the current study provides promising results, it also highlights the need for further research to confirm the findings and explore the potential of the LXA4/TNF α ratio as a therapeutic target. In addition, future studies should consider a larger, more diverse sample size to validate these findings in different populations and clinical contexts.

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Informed consent: All participating women were provided with both written and verbal information about the study, and informed consent was subsequently obtained.

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Data availability statement: The datasets generated during the current study are available from the corresponding author on reasonable request.

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