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Original Research

Differential changes in maternal proinflammatory IL6 plasma levels as a putatively surrogate marker of candidacy and clinical utility during mid- and late pregnancy hyperglycemia: interventional impact of clinical pharmacist on maternal and neonatal outcomes in a randomized clinical trial

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Abstract

Background/methods: The impact of clinical pharmacist on undiagnosed pregnancy hyperglycemia (PHG) in mid- and late- pregnancy as a major preventable cause of maternal and neonatal (M/N) complications is investigated. This longitudinal randomized controlled study of changes in plasma levels of predictive/prognostic/diagnostic biomarkers of oxytocin, thrombospondin, MCP1, IL6, MIF, insulin and LAR and undesirable M/N pregnancy outcomes in women with/out PHG (pregnancy normoglycemia; PNG) following the implementation of clinical pharmacist interventions were investigated. **Results:** A total of 68 PHG (36 intervention vs. 32 non-intervention) vs. 21 PNG participants were enrolled at 20–28 weeks and followed up till delivery. BMI of intervention PHG (unlike non-intervention) was greater (p=0.036) compared to PNG's. LAR and insulin, oxytocin, thrombospondin1, adiponectin and MCP1 plasma levels and their differences between 2nd and 3rd pregnancy trimesters lacked discrepancies in participants. Both PHG groups in mid pregnancy had substantially greater HbA1c %, FPG and IL6 levels vs. PNG, while PHG non-intervention' leptin was greater than PNG's. In late pregnancy, greater SBP, IL6 and MIF levels between either PHG groups vs. PNG's were observed. Unlike PHG non-intervention and PNG; IL6 level in PHG intervention group decreased (-2.54±6.61; vs. non-intervention PHG's 4.26±5.28; p<0.001 and vs. PNG's 2.30±4.27; p=0.023). None of the assessed M/N outcomes was found of differential significance between any of the three study groups. **Conclusions:** Proinflammatory IL6 as a robust and generalizable cardiometabolic risk-based and related pharmacotherapy biomarker in mid and late hyperglycemic pregnancy with likely implications of novel therapeutic targets was delineated by clinical pharmacist interventions.

Keywords: clinical pharmacist intervention; gestational diabetes cardio-metabolic risk-based and related pharmacotherapy; interleukin 6; insulin, leptin/adiponectin ratio; monocyte chemoattractant protein 1; macrophage migration inhibitory factor; oxytocin; thrombospondin 1

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INTODUCTION

Gestational diabetes mellitus (GDM), defined as any degree of glucose intolerance with onset or first recognition during pregnancy, is characterized by underlying maternal defects in the β-cell response to insulin during pregnancy. Impressively, the high prevalence of glucose intolerance in the early postpartum period in women with previous GDM has been described; with polycystic ovarian syndrome (PCOS) emerging as a new strong antepartum predictor of prediabetes.[1] The prevalence of GDM in pregnant women with a body mass index (BMI) ≥29.0 kg/m^2 is substantial, and posses a significant health burden to these pregnancies and to the future health

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of the mother.[2] Furthermore, women with a previous history of GDM have a greater than 7-fold higher risk of developing postpartum diabetes compared with women without GDM. Various risk factors for postpartum diabetes have been identified, including maternal age, glucose levels in pregnancy, family history of diabetes, pre-pregnancy and postpartum body mass index, dietary patterns, physical activity, and breastfeeding.[2-3] Genetic studies revealed that GDM shares common genetic variants with type 2 diabetes mellitus (T2D). The prevalence of GDM has been rising steadily over the past few decades, coinciding with the ongoing epidemic of obesity and T2D. Infants born to mothers with GDM also have a higher risk of developing T2D in their teens or early adulthood. Women who develop GDM generally have higher BMI when compared with healthy pregnant women, and obesity-related insulin insensitivity, progressive β-cell demise or dysfunction can induce low-grade inflammation. Chronic low-grade inflammation induces the synthesis of xanthurenic acid, known to be associated with the GDM, T2D and pre-diabetes development.[4] Hyperglycemia accelerates purine nucleotide synthesis, which in turn stimulates nucleotide breakdown and increases the concentration of nucleotide degradation products, including superoxide molecules and uric acid. Obviously, first trimester uric acid level and subsequent development of GDM correlated proportionally.[5] Reactive oxygen species (ROS) and excessive intracellular uric acid may also have direct effects on the development of the disease or further deterioration of the condition. Whereas leptin levels were higher, adiponectin levels in first or second trimester of pregnancy were lower among pregnant women who later develop GDM than non-GDM women.[6] Significantly lower maternal 25(OH)D concentrations in overweight/obese pregnant women at high-risk of GDM are associated with increased cardiometabolic risks during pregnancy and adverse pregnancy outcomes. These associations may be mediated by high molecular weight (HMW)-adiponectin but neither omentin nor visfatin.[7] Furthermore, maternal hormones such as leptin, insulin, ghrelin, adiponectin, resistin, obestatin and insulin-like growth factor-1 copeptin, apelin, and nesfatin, among others, have been identified in the milk of normal-weight women and may influence the energy balance via activating of orexigenic or anorexigenic pathways with possible impact on nutritional programming in the infant and appetite regulation.[8] Proinsulin levels were also low in pregnant women with diabetes and even lower in pre-term vs. at-term births. Both ghrelin and proinsulin levels were lower in pregnant women with diabetes and HbA1c of <6.5%. Recent studies showed that high interleukin (IL)-6 secretion may aggravate insulin resistance in pregnancy and participate in the GDM pathogenesis. IL6 concentrations were significantly higher in women with GDM compared with control women at the time of GDM screening with similar results obtained two months post-partum, where IL-6 levels remained significantly higher in women with GDM compared with control women[9] Markedly, IL6 was found to be significantly associated with insulin resistance markers in GDM,[10] with more emphasis on its association to glucose metabolism during pregnancy.[11] Besides, increasing evidence suggest that migration inhibitory factor (MIF) plays a central role in glucose homeostasis and in the development of type 1 and type 2 diabetes (T1D and T2D, respectively). Evidently, serum levels of MIF are significantly elevated in patients with GDM.[12] Monocyte chemoattractant protein 1 (MCP-1) has been implicated as a key factor in the recruitment and activation of peripheral blood leukocytes in atherosclerotic lesions and adipose tissue. Elevated levels of circulating MCP-1 have been found in patients with T1D and T2D, as well as with coronary artery disease. In GDM patients MCP-1 levels were markedly lower than those found in non-pregnant women and correlated significantly with fasting glucose, insulin and HOMA-IR, HbA1c as well as with prepregnancy and current BMI.[13] Despite the lack of significant differences in omentin and thrombospondin (TSP-1) levels between subjects with GDM and nonGDM controls; positive correlation between serum omentin and TSP-1 in GDM was defined with no comparable correlation with insulin resistance indices.[14] Furthermore, a higher log leptin pregnancy baseline concentration and a lower high density lipoprotein cholesterol (HDL-C) rate of change during pregnancy were associated with higher odds of having a large-for-gestational age (LGA) newborn. LDL-C rate of change throughout pregnancy was positively associated with body weight (BW) Z-score. Log triglycerides and log adiponectin were not significantly associated with BW Z-score or LGA birth.[15] Higher levels of inflammatory mediators such as as IL16 and IL18 are special risk constellation for GDM development with a cross-linkage to BMI in pregnant women.[16-17] Vejrazkova et al.[18] could not define either any substantial discrepancy in plasminogen activator inhibitor-1 (PAI-1) concentrations in GDM women vs. nonGDM controls. Meanwhile, fatty acid-binding protein 4 (FABP4) is mainly expressed in adipocytes and macrophages and is demonstrated to be elevated in diabetes patients. Changes in serum adipocyte fatty acid-binding protein were closely related to obesity, insulin resistance, and leptin resistance in pregnancy and were major risk factors for GDM.[19] In addition to its roles in assisting parturition and lactation; oxytocin, as a metabolic modulator via a multiplicity of molecular action mechanisms,[20] was appreciably involved alterations of metabolism of lipids, protein, and sugar. Specifically oxytocin-produced uterine contractions generated successfully acceleration of fetal maturation, and delivery between 34 and 36 weeks, was proved effective in prevention of fetal hyperinsulinemia consequences, namely hypertension and obesity, in pregnancies complicated by GDM.[21] Further therapeutic implications of oxytocin in obesity, diabetes mellitus, and related disorders are gaining momentum.[22] Considerably, in MetS patients (regardless of glycemic status); plasma level of oxytocin, but not oxyntomodulin, were substantially reduced in comparison to lean normoglycemic control.[23] Invariably, oxytocin (OXT) reduced intolerance of both glucose and insulin; decreased food intake and adiposity, and lowered blood pressure and cardiac oxidation/inflammation, henceforth affording cardioprotection in both diabetes and obesity.[24-25] Reportedly, oxytocin could rectify obese mice metabolic dysregularities via induction of white adipose tissue browning whilst stimulating brown adipose tissue thermogenesis.[26]

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AIM

A number of lifestyle interventional trials including diet, exercise, and breast feeding as well pharmacological/ nonpharmacological interventions that aimed to ameliorate modifiable risk factors, succeeded in reducing the incidence of postpartum diabetes, weight retention, and other obesity-related morbidities.[27] Nevertheless, none could elucidate the discrepancies seen in pregnant and non-pregnant subjects around the diversity of metabolism/inflammation/oxidation-glycation-related biomarkers. In effect, this interventional randomized controlled trial aimed to investigate principally the impact of clinical pharmacist on management of pregnancy hyperglycemia-related maternal markers and neonatal outcomes. In addition, well-designed prospective studies as such with longitudinal assessment of adipokines during pregnancy are needed to understand the trajectories and dynamic associations of biomarkers, namely, oxytocin, MCP1, TSP1, LAR, IL6, MIF, and insulin, with GDM risk, early prediction and severity, implementing preventive/modulation measures.

To compare the changes in biomarkers between the recruited patient groups in plasma samples AT LONGITUDINAL STUDY TIME POINTS

To study the SUBSTANTIAL impact of clinical pharmacist on management outcomes in relation to changes in levels of biomarkers in three different groups.

Ethics approval (informed consent statement and institutional review board approval)

The research was approved by the Scientific Research Committee at the School of Pharmacy and the Deanship of Scientific Research at the University of Jordan (JU). In addition, ethical approval has been obtained from the IRB (Institutional Review Board) committee at the National Centre for Diabetes, Endocrinology, and Genetics (NCDEG) in Amman, Jordan. The study was conducted prospectively at the National Center for Diabetes, Endocrinology, and Genetics (NCDEG) in Amman, Jordan (No.1/2015) and the School of Pharmacy/JU.

METHODS

Study Design

This study is a randomized controlled clinical trial. It was implemented between 8/2018 and 9/2021. It consisted of three arms; control group (normoglycemic pregnant study group); hyperglycemic pregnant women on conventional therapy, and hyperglycemic pregnant women on conventional therapy in addition to clinical pharmacist intervention. Randomization was conducted using www.randomization.com web link. The clinical pharmacist counseled the GDM patients in the intervention group regarding their medications and lifestyle modifications. In more details, each intervention group participant was thoroughly educated about GDM, insulin and injection rotation, how to deal with hypo- and hyperglycemia and any other concern about other health and pregnancy issues. Pregnant patients were assessed during their appointments in the NCDEG and through direct contact with the clinical pharmacist. Each pregnant was handed an educational material to ensure a good perception concerning their illness.

Clinical settings and duration, colorimetric ELISA assays of plasma levels of biomarkers

Pregnant women were approached by a clinical pharmacist to participate in the study in accordance with the inclusion criteria. Those who agreed to participate had the research goals and methods explained to them and were asked to sign an informed consent form in Arabic.

Inclusion Criteria

Pregnant patients of gestational age 20–28 weeks with diabetes mellitus (type 1, type 2, or GDM)

Normoglycemic pregnant females for control

Patient provided written informed consent to their participation in the research

This study was based on the measurements of the outcomes regarding fasting plasma glucose, and changes in plasma levels of cardiometabolic risk and related pharmacotherapy in 2nd and 3rd pregnancy trimesters. Harvested plasma (from lithium heparin collection tubes centrifuged at 4000 rpm for 10 minutes) were immediately stocked at -80^{o}C until analysis. Colorimetric ELISA assays of oxytocin, MCP1, thrombospondin 1, leptin, and insulin were procured from Abcam (Cambridge, MA, USA) while those of interleukin 6 (IL6), macrophage migration inhibitory factor (MIF), and adiponectin were obtained from MyBiosourse, Inc. (San Diego, CA, USA). Plasma levels of biomarkers were assayed according to manufacturers' instructions with intra- and interassay precisions of <10-<12%.

Statistical analysis

We obtained anthropometric, clinical, laboratory and biomarkers levels at the study baseline (second trimester of pregnancy). The anthropometric, clinical, laboratory data and biomarkers levels were also obtained at the third pregnancy trimester. Categorical variables were presented as frequencies and percentages, while continuous variables were presented as means and standard deviations (SD). Normality of data distribution was checked using histograms and Shapiro-Wilk test. Comparison of categorical variables was conducted using Chi square or Fisher exact test as appropriate with pairwise comparisons. The analysis of normally distributed continuous variables was performed using one-way ANOVA with post hoc Bonferoni test while for those not normally distributed, Kruskal-Wallis test was used.

RESULTS

Demographic and clinical characteristics and current medications of study participants (Tables 1 and 2)

The study included 89 pregnant females distributed into three groups: hyperglycemic with clinical pharmacist intervention (n=36), hyperglycemic without clinical pharmacist intervention (n=32) and normoglycemic control (n=21) (Figure 1).

Table 1. Demographic and clinical characteristics of study participants at the baseline (n=89)

Continuous parameters

Parameter	Total sample, mean (SD), n=89	Hyperglycemic intervention group, mean (SD), n=36		Hyperglycemic non-intervention group, mean (SD), n=32		Normoglycemic control, n=21		$H(_2)$*	$p_{overall}$	p_1	p_2	p_3
		Mean (SD)	Mean rank	Mean (SD)	Mean rank	Mean (SD)	Mean rank					
Age	32.7 (5.3)	35.5 (5.1)		32.1 (4.6)		28.7 (3.8)		-	**<0.001**	**0.011**	**<0.001**	**0.032**
Number of previous pregnancies	3.6 (2.2)	3.5 (2.2)	44.62	4.2 (2.3)	52.28	2.7 (1.7)	34.55	*6.166*	***0.046***	*0.647*	*0.448*	***0.039***
Number of abortions	1.7 (1.9)	2.8 (2.9)	23.12	1.7 (0.5)	24.54	1.0 (0.2)	13.15	*10.010*	***0.007***	*1.000*	*0.077*	***0.011***
Number of life births	2.3 (1.4)	2.5 (1.2)	38.46	2.8 (1.2)	41.79	1.4 (1.4)	22.14	*11.313*	***0.003***	*1.000*	***0.022***	***0.004***
Number of previous CSs	1.4 (1.1)	1.9 (1.0)	27.07	1.2 (0.8)	30.12	0.4 (0.8)	10.97	*21.652*	***<0.001***	*1.000*	***<0.001***	***<0.001***
Weight (kg)#	79.4(16.5)	84.2 (19.0)	-	77.7 (15.7)	-	73.5 (10.1)	-	*5.795*	*0.055*	-	-	-
BMI#	31.4 (9.1)	34.4 (12.6)	-	29.9 (5.4)	-	28.2 (3.1)	-	*6.917*	***0.031***	*0.253*	***0.036***	*1.000*
SBP (mm Hg)#	113.2(14.8)	112.2 (19.3)	-	115.7 (11.7)	-	111.5 (9.0)	-	*1.935*	*0.380*	-	-	-
DBP (mmHg)#	71.9 (7.3)	71.9 (7.5)	-	71.1 (7.5)	-	72.8 (7.2)	-	*0.399*	*0.844*	-	-	-
FPG (mg/dL)#	91.9(23.5)	95.5 (17.3)	-	100.3 (25.2)	-	80.5 (22.7)	-	*8.484*	***0.014***	*1.000*	*0.068*	***0.022***
HbA_1c (%)#	5.51(0.93)	5.35 (0.63)	-	5.85 (1.07)	-	4.63 (0.29)	-	8.077	***0.018***	*0.748*	*0.120*	***0.014***

Categorical parameter

	Total sample, N (%)	Hyperglycemic intervention group, N (%)	Hyperglycemic non-intervention group, N (%)	Normoglycemic control, N (%)	p[&]
Type of DM T1DM T2DM GDM Nondiabetic	 11 (12.4) 8 (9.0) 49 (55.1) 21 (23.6)	 3 (8.3) 4 (11.1) 29 (80.6) 0	 8 (25.0) 4 (12.5) 20 (62.5) 0	 0 (0) 0 (0) 0 (0) 21(100)	 *0.162*[$]
Physical activity	78 (87.6)	36 (100)	32 (100)	10 (47.6)	**<0.001**
Proper water intake	60 (78.7)	34 (94.4)	23 (71.9)	13 (61.9)	***0.009***[$]
Adhering to a special diet plan	63 (70.8)	33 (91.7)	25 (78.1)	5 (23.8)	**<0.001**
Smoking during pregnancy	9 (10.1)	1 (2.8)	6 (18.8)	2 (9.5)	*0.093*[$]
Elevated blood pressure during pregnancy	12 (13.5)	6 (16.7)	5 (15.6)	1 (4.8)	*0.405*[$]
PCOS	25 (28.1)	12 (33.3)	6 (18.8)	7 (33)	0.340
Hypothyroidism	29 (32.6)	9 (25)	12 (37.5)	8 (38.1)	0.453
History of obstetric complications	40 (44.9)	16 (44.4)	12 (37.5)	12 (57.1)	0.371
History of GDM	27 (30.3)	13 (36.1)	12 (37.5)	2 (9.5)	*0.063*[$]
History of IVF	7 (7.9)	5 (13.9)	1 (3.1)	1 (4.8)	*0.215*[$]
Family history of DM	73 (82.0)	31 (86.1)	27 (84.4)	15 (71.4)	0.345
Recurrent UTIs during current pregnancy	37 (41.6)	10 (27.8)	15 (46.9)	12 (57.1)	0.071

*$H(_2)$ by Kruskal-Wallis test.
#at the initial visit
^by independent-sample t-test
[&]by Chi-square test (for the type of DM, only two hyperglycemic groups were compared).

[5]by Fisher exact test
Significant p-values are shown in **Bold**; P-values shown in *Italic* are calculated by nonparametric tests (Kruskal-Wallis test for continuous variables and Fisher exact test for categorical ones), those calculated by parametric tests (ANOVA test for continuous variables and Ch-square test for categorical variables) are shown in regular fonts.
p_1: hyperglycemic with intervention vs. hyperglycemic without intervention; p_2: hyperglycemic with intervention vs. normoglycemic control; p_3: hyperglycemic without intervention vs. normoglycemic control.
CS: cesarean section; DBP: diastolic blood pressure; DM: diabetes mellitus; FPG: fasting plasma glucose; GDM: gestational DM; IVF: in-vitro fertilization; PCOS: polycystic ovarian syndrome; SBP: systolic blood pressure; SD: standard deviation; T1DM: type 1 DM; T2DM: type 2 DM; UTI: urinary tract infection.
P^1 is intervention vs. nonintervention groups
P^2 is intervention vs. controls
P^3 is nonintervention vs. controls

Table 2. The most common current medications administered by study participants (n=89)

Medication	Total sample, N (%)	Hyperglycemic intervention group, N (%)	Hyperglycemic non-intervention group, N (%)	Normoglycemic control, N (%)	p
Folic acid before pregnancy	50 (56.2)	19 (52.8)	11 (34.4)	20 (95.2)	**<0.001**
Omega-3 fatty acids	17 (19.1)	11 (30.6)	5 (15.6)	1 (4.8)	***0.047***
Dexamethasone for fetal lung maturation	33 (37.1)	20 (55.6)	10 (31.3)	3 (14.3)	***0.005***
Thyroid hormones	30 (33.7)	10 (27.8)	12 (37.5)	8 (38.1)	0.193
Progestin	19 (21.3)	9 (25.0)	4 (12.5)	6 (28.6)	*0.297*
Metformin	62 (69.7)	33 (91.7)	29 (90.6)	0 (0)	0.880*
Insulin	51 (58.0)	27 (75.0)	24 (75)	0 (0)	1.000*
Aspirin	28 (31.5)	11 (30.6)	10 (31.3)	7 (33.7)	0.976
Iron	74 (83.1)	30 (83.3)	24 (75.0)	20 (95.2)	0.157
Calcium	72 (80.9)	28 (77.8)	23 (71.9)	21 (100)	**0.032**
Vitamin D	71 (79.8)	28 (77.8)	24 (75.0)	19 (90.5)	0.362
Multivitamins	24 (27.0)	8 (22.2)	2 (6.3)	14 (66.7)	***<0.001***

Significant p-values are shown in **Bold**; P-values shown in *Italic* are calculated by Fisher exact test, those calculated by Ch-square test are shown in regular fonts.
*Comparison with normoglycemic control was not performed, as there was 0 number of cases in those cells.

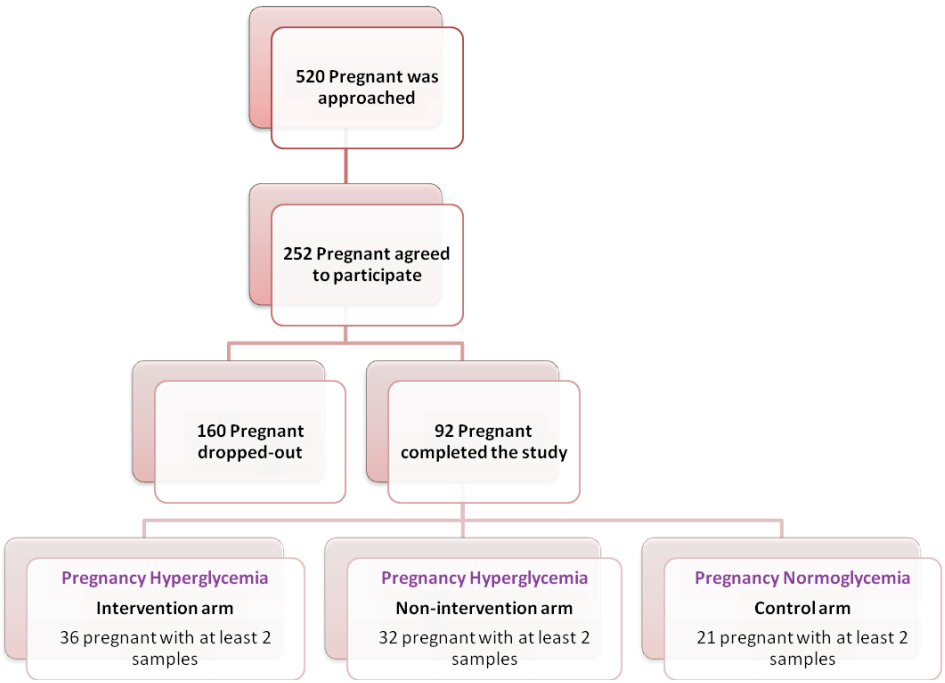

Figure 1. Recruitment Flowchart

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Participants' demographic, clinical and laboratory data at the baseline (second semester of pregnancy) are shown in Table 1. Patients at the intervention PHG group were older (35.5±5.1 years) than those without intervention (32.1±4.6 years) (p=0.011) and patients in both PHG groups were older than the PNG participants (28.7±3.8 years) (p<0.001 for the intervention group and p=0.032 for the non-intervention group). The total number of patients with type 1 diabetes mellitus (T1D) was 11 (12.4%), with type 2 diabetes mellitus (T2D) 8 (9.0%) and 49 (55.1%) had gestational diabetes mellitus (GDM). There was no significant difference in the prevalence of DM types between the two PHG groups (p=0.162). Compared to the PNG group, both hyperglycemic groups had significantly higher number of previous live births (2.5±1.2 and 2.8±1.2 vs. 1.4±1.4; p=0.022 and 0.004, respectively) and previous cesarian sections (CSs) (1.9±1.0 and 1.2±0.8 vs. 0.4±0.8, respectively; p<0.001 for both comparisons). Nevertheless, in a striking dissimilarity to the intervention hyperglycemic pregnancies; the hyperglycemic non-intervention group had significantly higher number of pregnancies and abortions (4.2±2.3 vs. 2.7±1.7; p=0.039 and 1.7±0.5 vs. 1.0±0.2; p=0.011, respectively), greater fasting plasma glucose (FPG; mg/dL) (100.3±25.3 vs. 80.5±22.7; p=0.022), and glycated hemoglobin (HbA1c% 5.85±1.07 vs. 4.63±0.29; p=0.014) compared to the normoglycemic control pregnancies). Distinctively, BMI of the interventional hyperglycemic pregnancies was greater (34.4±12.6 vs. 28.2±3.1; p=0.036) compared to the normoglycemic pregnancies. BMIs of both non-interventional hyperglycemic- and normoglycemic- pregnancies were comparable. Oddly, there were no differential variations between body weights of both hyperglycemic pregnancy groups vs. those of normoglycemic pregnancies (Table 3).

As for the lifestyle factors, all patients in the PHG intervention (n=36; 100%) and in the PHG non-intervention (n=32; 100%) groups claimed having at least some physical activity, as opposed to less than half in the PNG control group (n=10; 47.6%). The majority of patients in both PHG groups (n=33; 91.7% in the intervention group and n=25; 78.1% in the non-intervention group) also adhered to a special diet plan recommended by their physician compared to less than quarter (n=5; 23.8%) in the PNG control (p<0.001). The rest of lifestyle characteristics, obstetric history and the family history of DM, PCOS or IVF, hypothyroidism or recurrent UTIs did not score differential discrepancies between the study groups (p>0.05). Table 2 shows the most commonly consumed medications before and during the current pregnancy. There was a significant difference in folic acid intake before pregnancy between both PHG groups and the PNG control group: almost all patients in the PNG control group (n=20; 95.2%) received this supplement while only more than half (n=19; 52.8%) of the hyperglycemic intervention group and more than one-third (n=11; 34.4%) of the PHG non-intervention group received it, p<0.001. Likewise, multivitamins were significantly less frequently consumed by patients in both PHG intervention (n=8; 22.2%) and non-intervention (n=2; 6.3%) groups compared to the PNG control (n=14; 66.7%), p<0.001. In a striking similarity; calcium supplements were prescribed less frequently to both PHG intervention (n=28; 77.8%) and non-intervention (n=23; 71.9%) compared to the PNG control (n=21; 100%), p=0.032. Conversely; substantially higher proportion of patients in the PHG intervention group (n=11; 30.6%) received omega-3 fatty acids compared to the PNG control group (n=1; 4.8%), p=0.047.; Dexamethasone for fetal lung maturation was prescribed to 20 (55.6%) patients in the hyperglycemic intervention group, significantly higher than in both hyperglycemic non-intervention (n=10; 31.3%) and the normoglycemic control (n=3; 14.3%) groups, p=0.005; possibly in linkage to higher prevalence of premature deliveries. This can be linked to. Among the rest; supplements of iron, vitamin D and hormone replacement therapies were dispensed non differentially between normo- and hyper- glycemic pregnant ladies.There was no significant difference in metformin and insulin frequencies between the two hyperglycemic groups (p>0.05).

Table 3. Intergroup comparison of participants' clinical and laboratory parameters and biomarker levels between the second and the third pregnancy trimesters

Parameter	Hyperglycemic intervention group, n=36		Hyperglycemic non-intervention group, n=32		Normoglycemic control, n=21		$H(_2)$*	poverall	p_1	p_2	p_3
	Mean (SD)	Mean rank*	Mean (SD)	Mean rank*	Mean (SD)	Mean rank*					
Clinical characteristic											
$Weight_{2, kg}$	84.2 (19.0)	51.4	77.7 (15,7)	40.9	73.5 (10.1)	35.7	*5.80*	*0.055*	-	-	-
$Weight_{3, kg}$	81.9 (20.7)	45.7	75.6 (16.0)	37.6	71.3 (10.6)	33.9	*3.79*	*0.150*	-	-	-
$_{\Delta}$Weight, kg	-2.2 (6.5)	40.7	-2.3 (6.8)	39.0	-2.2 (5.6)	40.1	*0.07*	*0.963*	-	-	-
SBP_2, mm Hg	112.2 (19.3)	44.2	115.7 (11.7)	47.1	110.5 (9.0)	37.3	*1.94*	*0.380*	-	-	-
SBP_3, mm Hg	**119.2 (10.1)**	**35.0**	**122.6 (12.4)**	**38.5**	**111.8 (8.0)**	**21.7**	***9.90***	***0.007***	*1.000*	***0.042***	***0.011***
$_{\Delta}$SBP, mm Hg	8.8 (22.1)	33.7	8.6 (11.8)	37.2	0.3 (8.2)	24.3	*5.49*	*0.064*	-	-	-
DBP_2, mm Hg	71.9 (7.5)	35.0	71.1 (7.5)	38.5	72.8 (7.2)	46.1	*0.34*	*0.944*	-	-	-
DBP_3, mm Hg	73.5 (6.4)	30.0	75.9 (11.6)	33.1	74.4 (5.6)	31.7	*0.31*	*0.858*	-	-	-
$_{\Delta}$DBP, mm Hg	1.3 (6.8)	-	3.8 (8.8)	-	1.7 (7.8)	-	-	0.542			

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FPG_2, mg/dL	**95.5 (17.3)**	**29.7**	**100.3 (25.2)**	**31.1**	**80.5 (22.7)**	**17.8**	***8.48***	***0.014***	*1.000*	***0.068***	***0.022***
FPG_3, mg/dL	93.6 (12.1)	20.2	115.4 (36.2)	22.4	85.6 (4.6)	13.0	*5.93*	*0.051*	-	-	-
$_{\Delta}$FPG, mg/dL	-9.7 (17.8)	9.1	24.0 (37.6)	16.8	0.3 (9.0)	14.3	*2.92*	*0.232*	-	-	-
HbA_1c_2, %	**5.35 (0.63)**	**18.2**	**5.85 (1.07)**	**22.6**	**4.63 (0.29)**	**5.8**	***8.08***	***0.018***	*0.748*	*0.120*	***0.014***
HbA_1c_3, %	5.70 (0.69)	12.2	6.14 (0.89)	15.7	5.03 (0.32)	6.0	*5.30*	*0.071*	-	-	-
$_{\Delta}HbA_1c$, %	0.44 (0.79)	-	0.76 (0.89)	-	0.37 (0.15)	-	-	0.714			
Plasma Levels of Cardiometabolic risk –based and related- Pharmacotherapy Biomarkers											
$Insulin_2$ (µIU/mL)	335.3 (50.6)	38.2	330.1 (48.1)	45.7	346.5 (31.8)	54.0	*5.06*	*0.080*	-	-	-
$Insulin_3$ (µIU/mL)	345.8 (33.4)	45.7	325.1 (55.1)	40.2	349.8 (37.4)	49.3	*1.70*	*0.428*	-	-	-
$_{\Delta}$Insulin (µIU/mL)	10.50(65.19)	50.1	-5.00 (21.50)	39.1	3.25 (40.70)	43.1	*3.24*	*0.198*	-	-	-
OXT_2 (pg/mL)	550.6(534.3)	42.6	417.5 (277.2)	40.6	507.9 (379.9)	46.2	*0.56*	*0.7565*	-	-	-
OXT_3 (pg/mL)	497.1(458.8)	42.8	514.8 (399.3)	45.6	427.54(488.9)	35.5	*1.85*	*0.396*	-	-	-
$_{\Delta}$OXT (pg/mL)	-53.5(624.3)	-	97.3 (510.5)	-	-80.4 (717.8)	-	-	0.499			
$Thrombospondin_2$ (µg/mL)	29.52 (6.89)	44.7	29.35 (4.03)	38.8	31.77 (3.92)	53.2	*3.92*	*0.141*	-	-	-
$Thrombospondin_3$ (µg/mL)	**28.47 (6.65)**	**45.6**	**26.92 (5.66)**	**35.3**	**31.54 (5.81)**	**57.4**	***0.93***	***0.009***	*0.288*	*0.296*	***0.007***
$_{\Delta}$Thrombospondin(µg/mL)	-1.05 (4.60)	44.9	-2.43 (5.30)	40.3	-0.22 (4.41)	50.4	*1.94*	*0.380*	-	-	-
$Leptin_2$ (ng/mL)	**5.63 (1.16)**	**37.1**	**6.47 (1.52)**	**54.6**	**5.93 (0.85)**	**41.8**	**8.25**	***0.014***	***0.019***	*1.000*	***0.037***
$Leptin_3$ (ng/mL)	**5.61 (1.05)**	**36.5**	**6.17 (1.27)**	**54.2**	**5.87 (0.83)**	**43.4**	***8.20***	***0.017***	***0.013***	*1.000*	***0.407***
$_{\Delta}$Leptin (ng/mL)	-0.01 (1.75)	-	-0.31 (0.96)	-	-0.06 (0.98)	-	-	-	0.644		
$Adiponectin_2$ (µg/mL)	192.8(146.2)	41.2	189.7 (115.1)	43.0	245.9 (163.1)	52.8	*2.79*	*0.248*	-	-	-
$Adiponectin_3$ (µg/mL)	168.7(152.3)	37.3	200.2 (107.5)	49.2	228.1 (160.2)	50.0	*4.88*	*0.087*	-	-	-
$_{\Delta}$Adiponectin (µg/mL)	-24.17 (97.10)	-	10.53 (95.71)	-	-17.83(102.63)	-	-	0.323			
LAR_2	45.28(29.57)	45.3	56.81 (48.58)	48.5	31.16 (13.52)	36.6	*2.77*	*0.250*	-	-	-
LAR_3	54.45(41.38)	49.2	42.66 (28.30)	42.3	39.17 (26.47)	40.0	*2.22*	*0.329*	-	-	-
$_{\Delta}$LAR	9.17 (45.56)	46.3	-14.15 (42.87)	38.5	8.01 (21.87)	50.9	*3.2*	*0.200*	-	-	-
$IL6_2$ (pg/mL)	**14.11 (9.97)**	**54.0**	**8.68 (3.53)**	**45.0**	**5.72 (3.22)**	**26.7**	***14.69***	***0.001***	*0.447*	***<0.001***	***0.036***
$IL6_3$ (pg/mL)	**11.57 (8.90)**	**40.5**	**12.94 (4.41)**	**55.9**	**8.14 (3.45)**	**30.6**	***13.14***	***0.001***	***0.036***	*0.508*	***0.002***
$_{\Delta}$IL6 (pg/mL)	**-2.54 (6.61)**	**29.8**	**4.26 (5.28)**	**57.2**	**2.30 (4.27)**	**48.9**	***20.81***	***<0.001***	***<0.001***	***0.023***	*0.782*
$MCP1_2$ (pg/mL)	121.6 (69.3)	48.5	106.3(32.8)	46.8	90.1 (27.2)	33.6	*4.81*	*0.090*	-	-	-
$MCP1_3$ (pg/mL)	117.7 (57.4)	43.	113.0(41.4)	45.3	107.5 (32.9)	44.4	*0.06*	*0.971*	-	-	-
$_{\Delta}$MCP1 (pg/mL)	-3.92(53.46)	40.1	6.70 (39.67)	43.4	17.31 (28.78)	54.1	*3.95*	*0.138*	-	-	-
MIF_2 (ng/mL)	6.58 (3.07)	51.8	5.33 (2.68)	39.9	5.35 (3.13)	38.7	*5.05*	*0.080*	-	-	-
***MIF_3* (ng/mL)**	***6.32 (2.99)***	***51.3***	***5.64 (3.00)***	***43.7***	***4.59 (2.04)***	***33.6***	***6.21***	***0.045***	*0.672*	***0.039***	*0.491*
$_{\Delta}$MIF (ng/mL)	-0.26 (3.05)	43.5	0.31 (2.27)	49.8	-0.76 (2.69)	37.8	2.80	0.246	-	-	-

*$H(_2)$ by Kruskal-Wallis test.
P-values shown in *Italic* are calculated by Kruskal-Wallis test, those calculated by ANOVA test are shown in regular fonts. Significant p-values are shown in **Bold**;
p_1: hyperglycemic with intervention vs. hyperglycemic without intervention;
p_2: hyperglycemic with intervention vs. normoglycemic control;
p_3: hyperglycemic without intervention vs. normoglycemic control.
DBP: diastolic blood pressure; FPG: fasting plasma glucose; IL6: interleukin 6; LAR: leptin/Adiponectin ratio ; MCP1: Monocyte chemoattractant protein-1; MIF: macrophage migration inhibitory factor ; OXT: oxytocin; SBP: systolic blood pressure; SD: standard deviation. "$_2$": the second trimester; "$_3$": the third trimester; "$_{\Delta}$": difference between the third and the second trimesters.
P^1 is intervention vs. nonintervention groups
P^2 is intervention vs. controls
P^3 is nonintervention vs. controls

Intergroup comparisons of participants' clinical and laboratory parameters and maternal biomarker plasma levels between the 2nd and 3rd pregnancy trimesters (Table 3)

Table 3 demonstrated comparison of clinical and laboratory parameters and biomarker levels, along with their changes between the 2nd and the 3rd pregnancy trimesters among

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the study groups. Among the rest; the 2nd and 3rd pregnancy trimesters plasma levels and their differences of insulin, oxytocin, thrombospondin, adiponectin and LAR, and MCP1 lacked pronounced discrepancies between study participants. Notably, in Table 3 and in concordance with the study longitudinal design, both hyperglycemic groups; at the study baseline (the 2nd pregnancy trimester) but not in the 3rd pregnancy trimester, had substantial greater fasting plasma glucose (FPG) (mg/dL) levels and glycated hemoglobin (HbA1c) (%) vs. normoglycemic controls'. However, the difference was significant only between the hyperglycemic non-intervention group (FPG (mg/dL): 100.3±25.2 vs. 80.5±22.7; p=0.022) and (HbA1c%: 5.85±1.07 vs. 4.63±0.29; p=0.014). Outstandingly, among the rest of cardiometabolic risk-based and related pharmacotherapy biomarkers in the 2nd pregnancy trimester of the hyperglycemic intervention group; leptin plasma level (ng/mL) was markedly lower (5.63±1.16 vs. hyperglycemic non-intervention group's 6.47±1.52, respectively; p=0.019) while IL6 level (pg/mL) was significantly higher (14.11±9.97 vs. normoglycemic controls' 5.72±3.22; p=0.001). Unlike the rest of the 3rd pregnancy trimester parameters; there were significantly higher systolic blood pressure (SBP, mm Hg) but not DBP between both hyperglycemic pregnancy intervention (119.2±10.1) and non-intervention (122.6±12.4) groups (vs. normoglycemic controls' 118.8±8.0; p=0.042 and 0.011, respectively), appreciably higher IL6 levels in both hyperglycemic pregnancies (intervention's 11.57±8.90 vs. non-intervention's 12.9±4.41 p=0.036; and non-intervention's 12.9±4.41 vs. normoglycemic's 8.14±3.45; p=0.002); and considerably greater MIF plasma levels (ng/mL) of hyperglycemic pregnancy intervention (6.32±2.99 vs. controls' 4.59±2.04; p=0.039).

Remarkably in the 3rd pregnancy trimester; thrombospondin level (µg/mL) was lower in the non-intervention (but not in the intervention) hyperglycemic pregnancy group (26.92±5.66 vs. the normoglycemic controls' 31.54±5.81; p=0.007) and leptin circulation concentration (ng/mL) was also less in the PHG intervention's group than in the non-intervention's (5.61± 1.05 vs. 6.17± 1.27; p=0.013). Most notably, during the period between the 2nd and 3rd trimesters, the IL6 level decreased in the hyperglycemic intervention group (-2.54±6.61), in contrast to increase in the hyperglycemic non-intervention (4.26±5.28), p<0.001 and the normoglycemic controls (2.30±4.27), p=0.023.

Comparison of maternal and neonatal outcomes between the participating groups (Table 4)

Postnatal maternal and neonatal outcomes in the study groups are shown in Table 4. None of the assessed outcomes and observations (namely; CS (cesarean section), NICU (neonatal intensive care unit) admission, labor induction, preterm delivery or other post delivery maternal complications, neonatal hypoglycemia and other complications) was found of differential significance between any of three study groups. Exceptionall,y neonatal hyperbilirubinemia was substantially higher in the hyperglycemic intervention group (n=24; 66.7%) compared to the normoglycemic control group (n=5; 28.6%), p=0.015.

Table 4. Comparison of maternal and neonatal outcomes between the study groups (n=89)

Continuous parameters								
Parameter	**Total sample, mean (SD), n=89**	**Hyperglycemic intervention group, mean (SD), n=36**	**Hyperglycemic non-intervention group, mean (SD), n=32**	**Normoglycemic control, mean (SD), n=21**	**$p_{overall*}$**	**p_1**	**p_2**	**p_3**
Neonatal weight (kg)	3.112 (0.483)	2.992 (0.523)	3.154 (0.523)	3.236 (0.333)	0.181	0.669	0.228	1.000
NICU stay (days)	4.77 (3.92)	4.71 (4.07)	4.85 (3.91)	0	-	0.932^	-	-
Categorical data								
	Total sample, N (%)		Hyperglycemic intervention group, N (%)	Hyperglycemic non-intervention group, N (%)	Normoglycemic control, N (%)		p[&]	
CS	44 (57.9)		18 (56.3)	17 (73.9)	9 (42.9)		0.111	
Labor induction	20 (22.5)		11 (30.6)	4 (12.5)	5 (23.8)		*0.202*[$]	
Preterm delivery	6 (6.7)		5 (13.9)	1 (3.1)	0 (0)		*0.085*[$]	
NICU admission	27 (30.3)		14 (38.9)	13 (40.6)	0 (0)[@]		0.884	
Neonatal hypoglycemia	7 (7.9)		2 (5.6)	3 (9.4)	2 (9.5)		*0.800*[$]	
Neonatal hyperbilirubinemia	**44 (49.4)**		**24 (66.7)**	**14 (43.8)**	**6 (28.6)**		**0.015**	
Presence of other neonatal complications	23 (25.8)		12 (33.3)	7 (21.9)	4 (19.0)		*0.402*[$]	
Presence of post delivery maternal complications	52 (58.4)		22 (61.1)	17 (53.1)	13 (61.9)		0.748	

* by one-way ANOVA test. As of
^by independent-sample t-test
[&]by Chi-square test
[$]by Fisher exact test
[@]excluded from comparison due to zero number of cases in the cell
Significant p-values are shown in **Bold**; P-values shown in *Italic* are calculated by Fisher exact test, those calculated by parametric tests (ANOVA test for neonatal

weight, independent-sample t-test for NICU stay and Chi-square test for categorical variables) are shown in regular fonts.
p_1: hyperglycemic with intervention vs. hyperglycemic without intervention;
p_2: hyperglycemic with intervention vs. normoglycemic control;
p_3: hyperglycemic without intervention vs. normoglycemic control.
CS: cesarian section; NICU: neonatal intensive care unit; SD: standard deviation

DISCUSSION

GDM affects 5%-20% of all pregnancies;[27] it is associated with escalating prevalence in relation to increasing incidence of maternal obesity and inactivity as well as increasing rates of women of advanced age becoming pregnant. There is a proportional correlation between increasing insulin insensitivity with oral glucose intolerance testing and risk of cesarean section, macrosomia and infant adiposity among others. A complementary intervention of diet and lifestyle with pharmacological agents can aim at optimizing a normal body mass index of women of childbearing age before and after pregnancy along with controlling incident hyperglycemia.[28] Further potential strategy to prevent neonatal outcomes of developmental programming of diabetes, adiposity, and pubertal onset can be highly advised.[29] Importantly, among urinary biomarkers and exosomes; maternal IL1RA was found of a strong, early, and noninvasive diagnostic/predictive power of GDM based on sensitivity and specificity obtained[30] and possibly spontaneous abortion. Moreover, maternal serum neutrophil gelatinase-associated lipocalin (NGAL), C-reactive protein (CRP) and insulin-like growth factor binding protein 2 (IGFBP2), among many more, were successfully assigned as GDM prediction biomarkers in the first pregnancy trimester.[31-34] Distinctively, obesity, GDM and preeclampsia can share metabolic anomalies in sera as early as the 1st pregnancy trimester.[35-38] Postpartum follow-up metabolomics studies can outline maternal transition from GDM to T2D and decipher the impact of maternal GDM history on offspring and neonatal adverse outcomes.[27] Invariably, women with hypertensive disorders in pregnancy should be routinely screened for MetS within the first year postpartum to reduce cardiometabolic risks.[39] Taken together, Sylvester et al.;[40] in development and validation of weekly baseline ''fingerprinting'' profile of the human pregnancy urine metabolome, underscores robustness of a high-resolution molecular reference for adverse prenatal and postnatal outcomes.

In assessing the impact of the clinical pharmacist on optimizing drug therapy and intensive education on main pregnancy hyperglycemia management outcome measures, six-weeks postpartum reduction in HbA1c values was fundamentally greater in the intervention group (− 0.54% vs. control − 0.08%, p=0.04) with more FPG-controlled patients during pregnancy (94% vs. control 64.7%). The need for caesarian delivery (58.8% vs. control 35.3%) and severe episodes of hypoglycemia (0% vs. control 8.8%) were significantly ($p<0.05$) reduced in the intervention group.[41] Furthermore, Ji et al.,[42] defined greater reductions in fasting plasma glucose, 2 h postprandial plasma glucose and glycated hemoglobin A1c in the intervention group at the end of delivery. Also, markedly lower rate of polyhydramnios and fewer macrosomia in the intervention group were manifested vs. controls'. In this current interventional study, comparable amendment of glycemic control parameters was not obtainable in either 2nd or 3rd pregnancy trimesters despite significantly more adherence to physical activity with a better dietary plans and water intake unlike normoglycemic pregnant women. Neonatal findings lacked pronounced variations among study participants. Recently, GDM women with more exercise times ≥60 minutes/day had a lower percentage of abnormal plasma glucose[43] while high sweets intake was related with a higher GDM risk in women who were not overweight prior to pregnancy.[44]

TSP1

The thrombospondin family can be associated with inflammation, angiogenesis, and regulation of a diversity of physiologic functionalities.[45] TSP1 can promote ovarian cells apoptosis, interact with proangiogenic FGF2, is involved in matrix remodeling and regulated by microRNAs.[46] Exceptionally, unlike our study outcomes in the 3rd pregnancy trimester of lower TSP1 concentrations in non-intervention PHG vs. those of PNG; TSP4 expression levels were increased by 3.4-fold in GDM cases as compared to normoglycemic ones.[47] Conversely, TSP1 increased circulation concentrations substantially correlated with decreased gestational duration.[48] Appreciably, serum TSP1 levels decrease in preeclampsia (PE)-pregnant women; therefore, maternal TSP1 is taken for as a determinant of PE detection and severity.[49] Of important note, the reduction in TSP1 concentration in the 3rd trimester was not observed in the clinical pharmacist intervention group. Interestingly, a thrombospondin-1 inhibitor in experimental model of preeclampsia and in human placental endothelial cells exposed to preeclampsia-like conditions was adequately associated with pro-angiogenic effects.[50] Impressively, TSP1 analog could effectively inhibit endometriosis-related vascularization without affecting trans-generational pregnancy outcomes in mice.[51]

MCP1

MCP-1 in the 2nd and 3rd pregnancy trimesters and in postpartum exceeded in pregnant (with/out GDM) women its levels in non-pregnant and healthy pregnant women.[52] Conversely, MCP-1 circulation concentrations in the 3rd pregnancy trimester (irrespective of their glucose tolerance status) were found to be markedly lower than those in non-pregnant women with positively proportional correlations with BMI, HbA1c%, fasting glycemia and insulin resistance.[13] In a surprising dissimilarity to the previous reports; MCP1 present trajectory lacked any pronouncedly differential variations in the 2nd or 3rd pregnancy trimesters of both PHG groups and PNG. As maternal plasma and placental levels of inflammatory factors (IL-1β, IL-6, and MCP-1) were substantially and incrementally augmented);[53] hyperglycemia was proven proinflammatory in human placental chorionic villous in all gestational hyperglycemic conditions,

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even in hyperglycemia that is less severe than gestational or overt diabetes. Prominently, inflammation [mainly reflected in NLR (neutrophil/lymphocyte ratio) and MPV (mean platelet volume)], ROS (reactive oxygen species)-stress, and related DNA damage were apparently contributing in GDM development and progression independent of obesity.[54] Henceforth, the earliest opportunity of primary prediction-prevention of pregnancy hyperglycemia should not be missed.[3]

Oxytocin

Distinctly of this unprecedented study outcomes; maternal oxytocinplasma levels and their differences of the 2nd and 3rd pregnancy trimesters lacked pronounced discrepancies between all study participants (both GDM and nonGDM alike). A 3-week oxytocin treatment promoted the proliferation of pancreatic β-cells, enhanced glucose-stimulated insulin secretion and increased the β-cell mass in gestating but not in non-gestating mice. It is much needed for β-cell adaptation during pregnancy to maintain β-cell function, and, hence, the lack of oxytocin could be associated with the risk of GDM. Strong evidence suggests that the blood oxytocin levels were lower in GDM patients than in non-GDM healthy pregnant women and were associated with impaired pancreatic β-cell function.[55] OXT receptor suppression can be due to pregnancy hyperglycemia-induced persistent oxidative stress and epigenetic methylation,[56] thereby contributing to social deficits in offspring. Equally, important antagonism of oxytocin receptors by atosiban impaired insulin secretion and induced GDM[57] in gestating but not in non-gestating mice. Mechanistically, oxytocin was found to enhance (first-phase) insulin secretion in a (pre)diabetic setting to prove its value as a therapeutic target.[58] This comes in conflict with early lack of evidence of a role of oxytocin in the alteration of glucose metabolism in GDM women but alterations in oxytocin levels were assigned a possible significance for an impaired glucose tolerance in type 1 diabetic and extremely obese patients.[59]

Insulin and MIF

Though highly unlikely, maternal insulin circulation concentrations and their differences were comparable among all study participants (both GDM and nonGDM alike) in both 2nd and 3rd pregnancy trimesters. Apparently, exacerbation of maternal insulin insensitivity,[60] closely tied with progressive hyperinsulinemia of GDM pathology, were not observed in our study PHG women. Nevertheless, this comprehensive screening practice of maternal biomarkers in mid and late pregnancies could not be linked to the comparable pregnancy and neonatal outcomes; as in CS, NICU admission, labor induction, preterm delivery or other post delivery maternal complications and neonatal hypoglycemia, equally among GDM and nonGDM women.

MIF gene was delineated as a novel candidate gene for T2D[61] and, thus, attenuation of MIF levels improved glucose pathways.[12, 62] MIF expression level in placental tissues of GDM (but not obesity placenta) women was increased[63] and MIF gene polymorphisms correlated proportionally with increased GDM-linked insulin resistance, and risk[61] and with preconception obesity.[62] Most significantly, maternal circulation level of MIF declined starting at the 1st pregnancy trimester and onward (between 12 and 28 weeks of gestation), in line with the reduction in placental expression but differentially altered as a candidate biomarker of pregnancy complications.[64] Nevertheless, and not any earlier than the 3rd pregnancy trimester; considerably greater maternal MIF plasma levels of PHG intervention vs. controls' were recovered and unlike the rest of its assessed parameters. This incurs the lack of any impact of this intervention longitudinal study on MIF trajectory in both GDM and nonGDM participants.

Leptin, adiponectin and LAR

Among sera metabolomics; mannose, 4-hydroxyglutamate, 1,5-anhydroglucitol, and lactosyl-N-palmitoyl-sphingosine (d18:1/16:0) were outlined as novel, externally validated, strongly and independently predictive metabolites with clinical utility for GDM screening instead of only early pregnancy obesity.[65] Normoglycemic pregnancy specific reference values as elevations of CRP and pentraxin (PTX3) during the later phase may take place even in absence of infection. Thus, normal reference intervals may have to be determined during normal pregnancy.[66] Compliance with tight glycemic control altered maternal serum leptin and CRP levels in GDM women and their infants' markers.[67] In our trajectory 2nd and 3rd pregnancy trimesters; markedly lower leptin plasma level was in PHG intervention vs. PHG non-intervention, quite comparable to outcomes of dietary intervention of GDM.[68]

Fundamentally, lower adiponectin levels were detected in pregnant women with obesity and with subsequent GDM in mid- and late- pregnancy.[69] Principally, adiponectin is physiologically implicated in normal pregnancy and in obstetrical complications equally in relation to its physiologic corrective (antidiabetic, antiatherogenic, antiinflammatory and angiogenic) propensities.[69] While increased leptin level was defined as a GDM risk factor, decreased adiponectin level was outlined as a protective factor for GDM.[19] As a proof of concept; preconception leptin levels differed significantly in subsequent pregnancy between control vs. GDM and hypertensive pregnancy groups.[70] Currently neither maternal adiponectin nor LAR (but not leptin) were impressively of differential variations in mid- or late pregnancies of longitudinal study recruits (both GDM and nonGDM women alike); in a striking similarity to Florian et al.[71] outcomes but dissimilarly with lower adiponectin levels in pregnant women with obesity and subsequent mid- and late- pregnancy GDM.[52] While increased leptin level was defined as a predictive GDM risk factor (along with advanced maternal age),[71] decreased adiponectin level was outlined as a protective factor for GDM.[19] Oddly, maternal serum adipokines adiponectin and leptin at late gestation were not associated with newborn birth weights; while maternal inflammatory MCP1 levels were significantly related in GDM and nonGDM mothers with birth weights.[72] Impressively, as early pregnancy adiponectin deficiency has contributed to GDM development; adiponectin upregulation and agonists thereafter improved hyperglycemia in diabetic pregnant mice and rats.[73] This can be collectively coined into a clearer

understanding of how these pathways originate and evolve so that therapeutic targeting can be consequently improved via future replicated and validated studies.

Interleukin 6 (IL6)

As epidemiological mounting data outline the significance of IL6 as a vascular risk biomarker; Anti-IL6 blockers and therapeutics have proven concepts of principle in cardio-cerebrovascular diseases, improving inflammation-induced lipid metabolism impairments, atherosclerotic inflammation, plasminogen activator inhibitor-1 (PAI-1) induction-related endothelial dysfunction and kidney co-morbidities in T2D.[74] Nevertheless, the impact of a clinical pharmacist's combined non/pharmacological IL6-lowering interventions are still considerably unmet clinical needs in management of metabolic disorders and related expression changes and pathological mechanisms of IL6.

As for conflicting reports of biomarkers differential trajectories,[75] no difference was found in IL-6 serum levels in women with and without GDM as determined at 24th -28th gestation weeks.[76] Nevertheless, differentially expressed IL6 in CSF from 13 preeclampsia patients as compared to 14 controls' were underlined.[53] This is presently in line with 2nd pregnancy trimester of IL6 plasma level of both PHG groups reported significantly and differentially higher vs. PNG's levels. Outstandingly in the third trimester, there appeared difference in IL6 level between the PHG intervention group and that of PHG non-intervention group. Moreover differentially expressed proinflammatory IL6 in CSF from 13 preeclampsia patients as compared to 14 controls' were underlined.[77] This is presently in line with either of the 2nd and 3rd pregnancy trimester of IL6 plasma level of either of PHG groups reported significantly and differentially higher as compared to PNG's. Furthermore, as a result of clinical pharmacist intervention the IL6 plasma level was significantly reduced from the 2nd to the 3rd trimester, as opposed to the increase in the PHD non-intervention and the PND groups.

It was hence concluded prospectively and in totality that robust and generalisable biomarkers of candidacy and practical utility can perform substantially better than clinical markers in GDM risk prediction/prevention and management in a clinically meaningful affordable and convenient way if combined with risk factors in an early pregnancy (and maybe preconception) predictive model.[78]

CONCLUDING REMARKS, FUTURE DIRECTIVES and IMPACT OF FINDINGS ON PRACTICE

Conclusively early prediction and, hence, intervention of the risk of GDM are of utmost significance to reduce its adverse pregnancy and postnatal outcomes. Recent advancements and summarizing evidence from studies on the application of GDM metabolomics highlight collectively the aspects of early and differential diagnoses of GDM. Unequivocally plasma IL6 levels can potentially reflect serial changes of pregnancy progression; thus facilitating early diagnosis of pregnancy hyperglycemia-related anomalies and inflammation, with a better understanding of pathogenesis of these conditions and potential development of new therapeutic strategies in these areas. In effect this can offer with a better understanding of pathogenesis of pregnancy hyperglycemia and potential development of new therapeutic strategies.We highlight the need for further research to assess enablers to meet the tighter target recommendations of glycemic control and to assess the impact on predictive relevant robust and generalisable biomarkers. Strikingly the unprescedented impact of clinical pharmacist intervention was detectable at proinflammatory IL6 level indicating the vast utility of this biomarker in the assessment of implementing therapeutic outcomes.

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ETHICAL APPROVAL

This article does not contain any studies with animals performed by any of the authors.

DATA AVAILABILITY STATEMENT

Data are provided upon direct request to corresponding author.

COMPETING INTERESTS

The authors declare that there are no conflicts of interest.

AUTHOR CONTRIBUTIONS

All authors contributed equally to this manuscript.

ABBREVIATIONS

BMI (body mass index); CRP (C-reactive protein); CS (cesarean section); BW (body weight); Fatty acid-binding protein 4 (FABP4); FPG (fasting plasma glucose); GDM (gestational diabetes mellitus); HbA1c % (glycated hemoglobin); HDL-C (high density lipoprotein cholesterol); IGFBP2 (insulin-like growth factor binding protein 2); IL (interleukin); LAR (leptin/adiponectin ratio); LGA (large-for-gestational age); MCP1 (monocyte chemoattractant protein); MIF (macrophage migration inhibitory factor); MetS (metabolic syndrome); NGAL (maternal serum neutrophil gelatinase-associated lipocalin); NICU (neonatal intensive care unit); PAI-1 (plasminogen activator inhibitor-1); PHG (pregnancy hyperglycemia); PCOS (polycystic ovarian syndrome); PE (preeclampsia); ROS (reactive oxygen species); SBP (systolic blood pressure); T1D (type 1 diabetes mellitus); T2D (type 2 diabetes mellitus); TSP-1 (thrombospondin 1)

References

1. Capula C, Chiefari E, Vero A, et al. Prevalence and predictors of postpartum glucose intolerance in Italian women with gestational diabetes mellitus. Diabetes Research & Clinical Practice. 2014; 105(2):223-230. https://doi.org/10.1016/j.diabres.2014.05.008
2. Freebairn L, Atkinson J, Qin Y, et al. Diabetes in Pregnancy Modelling Consortium. 'Turning the tide' on hyperglycemia in pregnancy: insights from multiscale dynamic simulation modeling. BMJ Open Diabetes Research Care. 2020; 8:e000975. https://doi.org/10.1136/bmjdrc-2019-000975
3. Masood, SN, Baqai S, et al. GDM Guidelines Committee, Society of Obstetricians & Gynaecologists of Pakistan. Guidelines for Management of Hyperglycemia in Pregnancy (HIP) by Society of Obstetricians & Gynaecologists of Pakistan (SOGP)#. Journal of Diabetology. 2021; 12(1): 83-98. https://doi.org/10.4103/ jod.jod_88_20
4. Law KP, Zhang H. The pathogenesis and pathophysiology of gestational diabetes mellitus: Deductions from a three-part longitudinal metabolomics study in China. Clinica Chimica Acta. 2017; 468:60-70. https://doi.org/10.1016/ j.cca.2017.02.008
5. Amudha P, Nithya D, Pradeeba S, et al. Correlation between first trimester uric acid level and subsequent development of gestational diabetes mellitus. International Journal of Reproduction, Contraception, Obstetrics and Gynecology. 2017; 6(2):606-610. https://doi.org/10.18203/2320-1770.ijrcog20170391
6. Bao W, Baecker A, Song Y, et al. Adipokine levels during the first or early second trimester of pregnancy and subsequent risk of gestational diabetes mellitus: A systematic review. Metabolism. 2015; 64(6):756-64. https://doi.org/10.1016/j.metabol.2015.01.013
7. Mousa A, Abell SK, Shorakae S, et al. Relationship between vitamin D and gestational diabetes in overweight or obese pregnant women may be mediated by adiponectin. Molecular Nutrition & Food Research. 2017; 61(11): 1700488. https://doi.org/10.1002/ mnfr.201700488
8. Badillo-Suárez PA, Rodríguez-Cruz M, Nieves-Morales X. Impact of Metabolic Hormones Secreted in Human Breast Milk on Nutritional Programming in Childhood Obesity. Journal of Mammary Gland Biology and Neoplasia. 2017; 22(3):171-191. https://doi.org/10.1007/s10911-017-9382-y
9. Morisset AS, Dubé MC, Côté JA, et al. Circulating interleukin-6 concentrations during and after gestational diabetes mellitus. Acta Obstetricia et Gynecologica Scandinavica. 2011; 90(5):524-530. https://doi.org/10.1111/j.1600-0412.2011.01094.x
10. Nergiz S, Altınkaya ÖS, Küçük M, et al. Circulating galanin and IL-6 concentrations in gestational diabetes mellitus. Gynecological Endocrinology. 2014; 30(3):236-40. https://doi.org/10.3109/09513590.2013.871519
11. Mac-Marcjanek K, Zieleniak A, Woźniak L, et al. Comparison of leukocyte IL6 expression in patients with gestational diabetes mellitus (GDM) diagnosed by the Polish Diabetes Association (PDA) 2011 and 2014 criteria. Endokrynologia Polska. 2017; 68(3):317-325. https://doi.org/10.5603/EP.a2017.0014
12. Yilmaz Ö, Küçük M, Kebapçilar L, et al. Macrophage migration-inhibitory factor is elevated in pregnant women with gestational diabetes mellitus. Gynecological Endocrinology. 2012; 28(1):76-9. https://doi:10.3109/ 09513590. 2011.588757
13. Telejko B, Kuzmicki M, Zonenberg A, et al. Circulating monocyte chemoattractant protein-1 in women with gestational diabetes. Folia Histochemica et Cytobiologica. 2007; 45 Suppl 1:S153-6. PMID: 18292825
14. Lewandowski K, Nadel I, Lewinski A, et al. Positive correlation between serum omentin and thrombospondin-1 in gestational diabetes despite lack of correlation with insulin resistance indices. Ginekologia Polska. 2010; 81(12):907-12. PMID: 21391440
15. Farias DR, Poston L, Franco-Sena AB, et al. Maternal lipids and leptin concentrations are associated with large-for-gestational-age births: a prospective cohort study. Scientific Reports. 2017; 7(1):804. https://doi.org/10.1038/s41598-017-00941-y
16. Wang Q, Würtz 1, Auro K, et al. Metabolic profiling of pregnancy: cross-sectional and longitudinal evidence. BMC Medicine. 2016; 14(1):205. https://doi.org/10.1186/s12916-016-0733-0
17. Tarnowski M, Wieczorek A, Dziedziejko V, et al. IL16 and IL18 gene polymorphisms in women with gestational diabetes. Ginekologia Polska. 2017; 88(5):249-254. https://doi.org/10.5603/GP.a2017.0047
18. Vejrazkova D, Lischkova O, Vankova M, et al. Distinct response of fat and gastrointestinal tissue to glucose in gestational diabetes mellitus and polycystic ovary syndrome. Physiology Research. 2017; 66(2):283-292. https://doi.org/10.33549/physiolres.933366
19. Zhang Y, Zhang HH, Lu JH, et al. Changes in serum adipocyte fatty acid-binding protein in women with gestational diabetes mellitus and normal pregnant women during mid- and late pregnancy. Journal of Diabetes Investigation. 2016; 7(5):797-804. https://doi.org/10.1111/jdi.12484
20. Kerem L, Lawson EA. The Effects of Oxytocin on Appetite Regulation, Food Intake and Metabolism in Humans. International Journal of Molecular Sciences. 2021; 22(14):7737. https://doi.org/10.3390/ijms22147737
21. Gojnic M, Pervulov M, Petkovic S, et al. Acceleration of fetal maturation by oxytocin-produced uterine contraction in pregnancies complicated with gestational diabetes mellitus: a preliminary report. Journal of Maternal-Fetal & Neonatal Medicine. 2004; 16(2):111-4. https://doi.org/10.1080/14767050400005715
22. (a)Tsingotjidou AS. Oxytocin: A Multi-Functional Biomolecule with Potential Actions in Dysfunctional Conditions; from Animal Studies and beyond. Biomolecules. 2022; 12:1603. https://doi.org/10.3390 /biom12111603
(b). Barengolts E. Oxytocin - an emerging treatment for obesity and dysglycemia: review of randomized controlled trials and

cohort studies. Endocrine Practice. 2016; 22(7):885-94. https://doi.org/10.4158/ EP151192.RA
23. Naserallah R, Kasabri V, Naffa R, et al. The Levels of Oxytocin and Oxyntomodulin, Adiposity and Blood Indices in Pharmacotherapy Naive Diabetic and Non-Diabetic Patients with Metabolic Syndrome. Jordan Journal of Pharmaceutical Sciences. 2018;11(3):105-117
24. Jankowski M, Broderick TL, Gutkowska J. Oxytocin and cardioprotection in diabetes and obesity. BMC Endocrine Disorders. 2016; 16:34. https://doi.org/10.1186/s12902-016-0110-1
25. Lorenzo-Almorós A, Hang T, Peiró C, et al. Predictive and diagnostic biomarkers for gestational diabetes and its associated metabolic and cardiovascular diseases. Cardiovascular Diabetology. 2019; 18:140. https://doi.org/10.1186/ s12933-019-0935-9
26. Yuan J, Zhang R, Wu R, et al. The effects of oxytocin to rectify metabolic dysfunction in obese mice are associated with increased thermogenesis. Molecular & Cellular Endocrinology. 2020; 514:110903. https://doi.org/10.1016/ j.mce.2020.110903
27. (a) Huvinen E, Lahti J, Klemetti MM, et al. Genetic risk of type 2 diabetes modifies the effects of a lifestyle intervention aimed at the prevention of gestational and postpartum diabetes. Diabetologia. 2022; 65(8):1291-1301. https://doi.org/10.1007/s00125-022-05712-7
(b) Moon JH, Kwak SH, Jang HC. Prevention of type 2 diabetes mellitus in women with previous gestational diabetes mellitus. Korean Journal of Internal Medicine. 2017; 32(1):26-41. https://doi.org/10.3904/kjim.2016.203
(c)Vladu IM, Clenciu D, Mitrea A, et al. Maternal and Fetal Metabolites in Gestational Diabetes Mellitus: A Narrative Review. Metabolites. 2022; 12(5):383. https://doi.org/10.3390/metabo12050383
28. Farrar D. Hyperglycemia in pregnancy: prevalence, impact, and management challenges. International Journal of Women Health. 2016; 8:519-527. https://doi.org/10.2147/IJWH.S102117
29. Bianco ME, Josefson JL. Hyperglycemia during Pregnancy and Long-Term Offspring Outcomes. Current Diabetes Reports. 2019; 19(12):143. https://doi.org/10.1007/s11892-019-1267-6
30. (a).Guo Y, Han Z, Guo L, et al. Identification of urinary biomarkers for the prediction of gestational diabetes mellitus in early second trimester of young gravidae based on iTRAQ quantitative proteomics. Endocrine Journal. 2018; 65(7):727-735. https://doi.org/10.1507/ endocrj.EJ17-0471
(b). Liu X, Wang X, Sun H, et al. Urinary metabolic variation analysis during pregnancy and application in gestational diabetes mellitus and spontaneous abortion biomarker discovery. Scientific Reports. 2019; 9:2605. doi.org/10.1038/s41598-019-39259-2
(c.) Gutaj P, Matysiak J, Matuszewska E, et al. Maternal serum proteomic profiles of pregnant women with type 1 diabetes. Scientific Reports. 2022; 12:8696. doi.org/10.1038/s41598-022-12221-5
(d). Wang X, Zhao M, Guo Z, et al. Urinary proteomic analysis during pregnancy and its potential application in early prediction of gestational diabetes mellitus and spontaneous abortion. Annals of Translational Medicine. 2022; 10(13):736. https://doi.org/10.21037/atm-21-3497
(e). Zhang M, Yang H. Perspectives from metabolomics in the early diagnosis and prognosis of gestational diabetes mellitus. Frontiers in Endocrinology (Lausanne). 2022; 13:967191. https://doi.org/10.3389/fendo.2022.967191
(f). Xie J, Li L, Xing H. Metabolomics in gestational diabetes mellitus: A review. Clinica Chimica Acta. 2023; 539:134-143. https://doi.org/10.1016/j.cca.2022.12.005
31. (a).Liu X, Sun J, Wen X, et al. Proteome profiling of gestational diabetes mellitus at 16-18 weeks revealed by LC-MS/MS. Journal of Clinical Laboratory Analysis. 2020; 34(9):e23424. https://doi.org/10.1002/jcla.23424
(b). Lu L, Li C, Deng J, et al. Maternal serum NGAL in the first trimester of pregnancy is a potential biomarker for the prediction of gestational diabetes mellitus. Frontiers in Endocrinology. 2022; 13:977254. https://doi.org/10.3389/ fendo.2022.977254
32. (a). Alesi S, Ghelani D, Rassie K, et al. Metabolomic Biomarkers in Gestational Diabetes Mellitus: A Review of the Evidence. International Journal of Molecular Sciences. 2021; 22(11):5512. https://doi.org/10.3390/ijms22115512
(b). Zhu Y, Barupal DK, Ngo AL, et al. Predictive Metabolomic Markers in Early to Mid-pregnancy for Gestational Diabetes Mellitus: A Prospective Test and Validation Study. Diabetes. 2022; 71(8):1807-1817. https://doi.org/10.2337/db21-1093
(c). Khan RS, Malik H. Diagnostic Biomarkers for Gestational Diabetes Mellitus Using Spectroscopy Techniques: A Systematic Review. Diseases. 2023; 11(1):16. https://doi.org/10.3390/diseases11010016
33. Sakurai K, Eguchi A, Watanabe M, et al. Exploration of predictive metabolic factors for gestational diabetes mellitus in Japanese women using metabolomic analysis. Journal of Diabetes Investigation. 2019; 10(2):513-520. https://doi.org/10.1111 /jdi.12887
(b) Diboun I, Ramanjaneya M, Majeed Y, et al. Metabolic profiling of pre-gestational and gestational diabetes mellitus identifies novel predictors of pre-term delivery. Journal of Translational Medicine. 2020; 18:366. https://doi.org/10.1186/s12967-020-02531-5
(c) Meng X, Zhu B, Liu Y, et al. Unique Biomarker Characteristics in Gestational Diabetes Mellitus Identified by LC-MS-Based Metabolic Profiling. Journal of Diabetes Research. 2021; Article ID 6689414, 15 pages. https://doi.org/ 10.1155/2021/6689414
(d) Heath H, Luevano J, Johnson CM, et al. Predictive Gestational Diabetes Biomarkers with Sustained Alterations throughout Pregnancy. Journal of Endocrine Society. 2022; 6(12):bvac134. https://doi.org/10.1210/jendso/bvac134
34. Palomo M, Youssef L, Ramos A, et al. Differences and similarities in endothelial and angiogenic profiles of preeclampsia and COVID-19 in pregnancy. American Journal of Obstetrics and Gynecology. 2022; 227(2):277.e1-277.e16. https://doi.

org/10.1016/j.ajog.2022.03.048
35. Vakhtangadze T, Gakhokidze N, Khutsishvili M, et al. The link between hypertension and preeclampsia/eclampsia-life-long cardiovascular risk for women. Vessel Plus. 2019; 3:19. https://doi.org/10.1016/j.preghy.2015.04.001
36. (a). Kivelä J, Sormunen-Harju H, Girchenko PV, et al. Longitudinal Metabolic Profiling of Maternal Obesity, Gestational Diabetes, and Hypertensive Pregnancy Disorders. Journal of Clinical Endocrinology and Metabolism. 2021; 106 (11): e4372–e4388. https://doi.org/10.1210/clinem/dgab475
(b). Agarwal NR, Kachhawa G, Oyeyemi BF, et al. Metabolic profiling of serum and urine in preeclampsia and gestational diabetes in early pregnancy. Medicine in Drug Discovery. 2022; 16: 100143. https://doi.org/10.1016/j.medidd.2022.100143
37. Lee SM, Kang Y, Lee EM, et al. Metabolomic biomarkers in mid trimester maternal plasma can accurately predict the development of preeclampsia. Scientific Reports. 2020; 10:16142. https://doi.org/10.1038/s41598-020-72852-4
38. (a).Cho GJ, Jung US, Sim JY, et al. Is preeclampsia itself a risk factor for the development of metabolic syndrome after delivery? Obstetrics & Gynecology Science. 2019; 62(4):233-241. https://doi.org/10.5468/ogs.2019.62.4.233
(b).Alonso-Ventura V, Pérez-López FR. Preeclampsia negatively affects future maternal metabolic and endocrine outcomes. Gynecological Endocrinology. 2021; 37:9, 773-774. https://doi.org/10.1080/09513590.2021.1948996
39. Ishaku SM, Karima T, Oboirien KA, et al. Metabolic syndrome following hypertensive disorders in pregnancy in a low-resource setting: A cohort study. Pregnancy Hypertension. 2021; 25:129-135. https://doi.org/10.1016/ j.preghy. 2021.05.018
40. Sylvester KG, Hao S, Li Z, et al. Gestational Dating by Urine Metabolic Profile at High Resolution Weekly Sampling Time points: Discovery and Validation. Frontiers in Molecular Medicine. 2022; 2:844280. https://doi.org/10.3389/fmmed.2022.844280
41. Batta RA, Kasabri V, Akour A, et al. Impact of clinical pharmacists' intervention on management of hyperglycemia in pregnancy in Jordan. International Journal of Clinical Pharmacy. 2018; 40:48–55. https://doi.org/10.1007/s11096-017-0550-3
42. Ji C, Sun LJ, Li LT, et al. Impact of clinical pharmacist intervention on blood glucose control and perinatal outcomes in gestational diabetes mellitus through a diabetes management system. Clinical and Experimental Obstetrics & Gynecology. 2020; 47(5):645–652. https://doi.org/ 10.31083/ j.ceog.2020. 05.2212
43. Wang R, Yang Q, Sun T, et al. Physical Exercise is associated with Glycemic Control among Women with Gestational Diabetes Mellitus: Findings from a Prospective Cohort in Shanghai, China. Diabetes, Metabolic Syndrome and Obesity. 2021; 14:1949-1961. https://doi.org/10.2147/DMSO.S308287
44. Wen L, Ge H, Qiao J, et al. Maternal dietary patterns and risk of gestational diabetes mellitus in twin pregnancies: a longitudinal twin pregnancies birth cohort study. Nutrition Journal. 2020; 19(1):13. https://doi.org/10.1186/s12937-020-00529-9
45. Stenina-Adognravi O, Plow EF. Thrombospondin-4 in tissue remodeling. Matrix Biology. 2019; 75-76:300-313. https://doi.org/10.1016/j.matbio.2017.11.006
46. Farberov S, Basavaraja R, Meidan R. Thrombospondin-1 at the crossroads of corpus luteum fate decisions. Reproduction. 2019; 157(3):R73-R83. https://doi.org/10.1530/REP-18-0530
47. Mavreli D, Evangelinakis N, Papantoniou N, et al. Quantitative Comparative Proteomics Reveals Candidate Biomarkers for the Early Prediction of Gestational Diabetes Mellitus: A Preliminary Study. In Vivo. 2020; 34(2):517-525. https://doi.org/10.21873/invivo.11803
(b) Liu X, Sun J, Wen X, et al. Proteome profiling of gestational diabetes mellitus at 16-18 weeks revealed by LC-MS/MS. Journal of Clinical Laboratory Analysis. 2020; 34(9):e23424. https://doi.org/10.1002/jcla.23424
48. Viklund F, Hallingström M, Kacerovsky M, et al. Protein Concentrations of Thrombospondin-1, MIP-1β, and S100A8 Suggest the Reflection of a Pregnancy Clock in Mid-Trimester Amniotic Fluid. Reproductive Sciences. 2020; 27: 2146–2157. https://doi.org/ 10.1007/ s43032-020-00229-z
49. Ulu İ, **Çekmez** Y, Yıldırım Köpük Ş, et al. Maternal serum thrombospondin-1 is significantly altered in cases with established preeclampsia. Journal of Maternal-Fetal & Neonatal Medicine. 2019; 32(15):2543-2546. https://doi.org/10.1080/14767058.2018.1441279
50. Marc C, Achille A, Frégeau G, et al. Abstract 004: Pro-angiogenic Effects of the Thrombospondin-1 Inhibitor Lskl in Preeclampsia Models. Hypertension. 2022; 79:A004. https://doi.org/10.1161/ hyp.79.suppl_1.004
51. Nakamura DS, Edwards AK, Ahn SH, et al. Compatibility of a Novel Thrombospondin-1 Analog with Fertility and Pregnancy in a Xenograft Mouse Model of Endometriosis. PLoS ONE. 2015; 10(3): e0121545. https://doi.org/ 10.1371/ journal.pone.0121545
52. Klein K, Satler M, Elhenicky M, et al. Circulating levels of MCP-1 are increased in women with gestational diabetes. Prenatal Diagnosis. 2008; 28(9):845-51. https://doi.org/10.1002/pd.2064
53. Corrêa-Silva S, Alencar AP, Moreli JB, et al. Hyperglycemia induces inflammatory mediators in the human chorionic villous. Cytokine. 2018; 111: 41-48. https://doi.org/10.1016/j.cyto.2018.07.02057
(b) Olmos-Ortiz A, Flores-Espinosa P, Díaz L, et al. Immunoendocrine Dysregulation during Gestational Diabetes Mellitus: The Central Role of the Placenta. International Journal of Molecular Sciences. 2021; 22(15):8087. https://doi.org/10.3390/ijms22158087
54. Basu J, Datta C, Chowdhury S, et al. Gestational Diabetes Mellitus in a Tertiary Care Hospital of Kolkata, India: Prevalence, Pathogenesis and Potential Disease Biomarkers. Experimental and Clinical Endocrinology & Diabetes. 2020; 128(4):216-223. https://doi.org/10.1055/a-0794-6057
55. Gu P, Lin Y, Wan Q, et al. Oxytocin signal contributes to the adaptative growth of islets during gestation. Endocrine Connections.

2021; 10(7):694-706. https://doi.org/10.1530/EC-21-0043
56. Liu J, Liang Y, Jiang X, et al. Maternal Diabetes-Induced Suppression of Oxytocin Receptor Contributes to Social Deficits in Offspring. Frontiers in Neuroscience. 2021;15:634781-95. https://doi.org/10.3389/fnins.2021.634781
57. Ko HJ, Hong SY, Bae JY. Pregnancy and neonatal outcomes of hyperglycemia caused by atosiban administration during pregnancy. Clinical and Experimental Obstetrics & Gynecology. 2021; 48(2):257–262. https://doi.org/10.31083/ j.ceog.2021.02.2364
58. Scerbo MJ, Gerdes JM. Bonding With β-Cells—A Role for Oxytocin in Glucose Handling. Diabetes. 2017; 66 (2): 256–257. https://doi.org/10.2337/dbi16-0053
59. Stocks S, Bremme K, Uvas-Moberg K. Is Oxytocin Involved in the Deterioration of Glucose Tolerance in Gestational Diabetes? Gynecologic and Obstetric Investigation. 1993; 36:81–86. https://doi.org/10.1159/000292601
60. McElwain CJ, McCarthy FP, McCarthy CM. Gestational Diabetes Mellitus and Maternal Immune Dysregulation: What We Know So Far. International Journal of Molecular Sciences. 2021; 22(8):4261. https://doi.org/10.3390/ijms22084261
61. Li C, Qiao B, Qi W, et al. Association of Macrophage Migration Inhibitory Factor Polymorphisms with Gestational Diabetes Mellitus in Han Chinese Women. Gynecologic and Obstetric Investigation. 2016; 81(1):84-9. https://doi.org/10.1159/000398796
62. Aslani S, Hossein-nezhad A, Maghbooli Z, et al. Genetic variation in macrophage migration inhibitory factor associated with gestational diabetes mellitus and metabolic syndrome. Hormone and Metabolic Research. 2011; 43: 557-561. https://doi.org/10.1055/s-0031-1275706
63. Radaelli T, Lepercq J, Varastehpour A, et al. Differential regulation of genes for fetoplacental lipid pathways in pregnancy with gestational and type 1 diabetes mellitus. American Journal of Obstetrics and Gynecology. 2009; 201(2):209.e1-209.e10. https://doi.org/10.1016/j.ajog.2009.04.019
(b) Zheng L, Li C, Qi WH, et al. Expression of macrophage migration inhibitory factor gene in placenta tissue and its correlation with gestational diabetes mellitus. Zhonghua Yi Xue Za Zhi. 2017; 97(43):3388-3391.https://doi.org/10.3760/cma.j.issn.0376-2491.2017.43.006
64. (a)Napso T, Zhao X, Lligoña MI, et al. Placental secretome characterization identifies candidates for pregnancy complications. Communications Biology. 2021; 4:701. https://doi.org/10.1038/s42003-021-02214-x
(b). Wei W, He Y, Wang X, et al. Gestational Diabetes Mellitus: The Genetic Susceptibility behind the Disease. Hormone & Metabolism Research. 2021; 53(8):489-498. https://doi.org/10.1055/a-1546-1652
65. Raczkowska BA, Mojsak P, Rojo D, et al. Gas Chromatography-mass spectroscopy-based metabolomics analysis reveals potential biochemical markers for diagnosis of gestational diabetes mellitus. Frontiers in Pharmacology. 2021; 12:770240. https://doi.org/10.3389/ fphar.2021.770240
(b). Sovio U, Clayton GL, Cook E, et al. Metabolomic Identification of a novel, externally validated predictive test for gestational diabetes mellitus. Journal of Clinical & Endocrinology Metabolism. 2022; 107(8): e3479–e3486. https://doi.org/10.1210/clinem/dgac240
66. Wirestam L, Pihl S, Wetterö J, et al. Plasma C-Reactive protein and pentraxin-3 reference intervals during normal pregnancy. Frontiers in Immunology. 2021; 12:722118. https://doi.org/10.3389/fimmu.2021.722118
67. Hofer OJ, Alsweiler J, Tran T, et al. Glycemic control in gestational diabetes and impact on biomarkers in women and infants. Pediatric Research. 2023; 1-11. https://doi.org/10.1038/s41390-022-02459-0
68. Jack-Roberts C, Maples P, Kalkan B, et al. Gestational diabetes status and dietary intake modify maternal and cord blood allostatic load markers. BMJ Open Diabetes Research & Care. 2020; 8(1):e001468. https://doi.org/10.1136/ bmjdrc-2020-001468
69. Quotah OF, Poston L, Flynn AC, et al. Metabolic Profiling of Pregnant Women with Obesity: An Exploratory Study in Women at Greater Risk of Gestational Diabetes. Metabolites. 2022; 12(10):922. https://doi.org/10.3390/metabo12100922
70. Peltokorpi A, Irina L, Liisa V, et al. Preconceptual leptin levels in gestational diabetes and hypertensive pregnancy. Hypertension in Pregnancy. 2022; 41(1):70-77. https://doi.org/10.1080/10641955.2022.2033763
71. Florian AR, Cruciat G, Pop RM, et al. Predictive role of altered leptin, adiponectin and 3-carboxy-4-methyl-5-propyl-2-furanpropanoic acid secretion in gestational diabetes mellitus. Experimental and Therapeutic Medicine. 2021; 21: 520. https://doi.org/10.3892/etm.2021.9951
72. Saucedo R, Valencia J, Moreno-González LE, et al. Maternal serum adipokines and inflammatory markers at late gestation and newborn weight in mothers with and without gestational diabetes mellitus. Ginekologia Polska. 2021; 93(2):126–133. https://doi.org/10.5603/GP.a2021.0083
73. Gázquez A, Rodríguez F, Sánchez-Campillo M, et al. Adiponectin agonist treatment in diabetic pregnant rats. Journal of Endocrinology. 2021; 251(1):1-13. https://doi.org/10.1530/JOE-20-0617
(b). Moyce Gruber BL, Dolinsky VW. The Role of Adiponectin during Pregnancy and Gestational Diabetes. Life. 2023; 13(2):301. https://doi.org/10.3390/ life13020301
74. (a)Dimosiari A, Patoulias D, Kitas GD, et al. Do Interleukin-1 and Interleukin-6 Antagonists Hold Any Place in the Treatment of Atherosclerotic Cardiovascular Disease and Related Co-Morbidities? An Overview of Available Clinical Evidence. Journal of Clinical Medicine. 2023; 12(4):1302. https://doi.org/10.3390/jcm12041302
(b).Kang S, Tanaka T, Inoue H, et al. IL-6 trans-signaling induces plasminogen activator inhibitor-1 from vascular endothelial

cells in cytokine release syndrome. Proceedings of the National Academy of Sciences of the United States of America. 2020; 117(36):22351-22356. https://doi.org/10.1073/ pnas.2010229117
(c). Koshino A, Schechter M, Sen T, et al. Interleukin-6 and cardiovascular and kidney outcomes in patients with type 2 diabetes: new insights from CANVAS. Diabetes Care. 2022; 45 (11): 2644–2652. https://doi.org/10.2337/dc22-0866
(d). Kostina, D A, Pokrovskaya TG, et al. Interleukin-6 is a potential target for a correction of endothelial dysfunction associated with low-grade systemic inflammation. Research Results in Pharmacology & Clinical Pharmacy. 2017; 3(3):89-96. https://doi.org /10.18413/2313-8971-2017-3-3-89-96
(e).Ridker PM, Rane M. Interleukin-6 Signaling and Anti-Interleukin-6 Therapeutics in Cardiovascular Disease. Circulation Research. 2021; 128(11):1728-1746. https://doi.org/10.1161/CIRCRESAHA 121.319077
(f). Su JH, Luo MY, Liang N, et al. Interleukin-6: A Novel Target for Cardio-Cerebrovascular Diseases. Frontiers in Pharmacology. 2021; 12: 745061. https://doi.org/10.3389/fphar.2021.745061
(g). Denga TM, Gunter S, Fourie S, et al. Interleukin-6 blockers improve inflammation-induced lipid metabolism impairments but induce liver fibrosis in collagen-induced arthritis. Endocrine Metabolic Immune Disorders-Drug Targets. 2023; 23(4):548-557. https://doi.org/10.2174/ 1871530323666221017153157

75. (a).Amirian A, Mahani MB, Abdi F. Role of interleukin-6 (IL-6) in predicting gestational diabetes mellitus. Obstetrics & Gynecology Science. 2020; 63(4):407-416. https://doi.org/10.5468/ogs.20020
(b). Spence T, Allsopp PJ, Yeates AJ, et al. Maternal Serum Cytokine Concentrations in Healthy Pregnancy and Preeclampsia. Journal of Pregnancy. 2021; 2021:6649608. https://doi.org/10.1155/ 2021/ 6649608
(c) Vilotić A, Nacka-Aleksić M, Pirković A, et al. IL-6 and IL-8: An Overview of Their Roles in Healthy and Pathological Pregnancies. International Journal of Molecular Sciences. 2022; 23(23):14574. https://doi.org/10.3390/ijms232314574
(d). Bogdanet D, Reddin C, Murphy D, et al. Emerging protein biomarkers for the diagnosis or prediction of gestational diabetes-a scoping review. Journal of Clinical Medicine. 2021; 10(7):1533. https://doi.org/10.3390/jcm10071533
(e). Xiang LL, Chen C, Wang QY, et al. Impact of inflammatory factors, hemoglobin A1c, and platelet parameters in gestational diabetes mellitus. Archives of Gynecology & Obstetrics. 2023; 307(2):439-446. https://doi.org/10.1007/s00404-022-06528-x
(f). Omazić J, Viljetić B, Ivić V, et al. Early markers of gestational diabetes mellitus: what we know and which way forward? Biochemical Medicine (Zagreb). 2021; 31(3):030502. https://doi.org/10.11613/ BM.2021.030502
76. Braga FO, Negrato CA, Matta MFBD, et al. Relationship between inflammatory markers, glycated hemoglobin and placental weight on fetal outcomes in women with gestational diabetes. Archives in Endocrinology & Metabolism. 2019; 63(1):22-29. https://doi.org/10.20945/2359-3997000000099
77. Ciampa E, Li Y, Dillon S, et al. Cerebrospinal Fluid Protein Changes in Preeclampsia. Hypertension. 2018; 72(1):219-226. https://doi.org/10.1161/ HYPERTENSION AHA.118.11153
78. Madhu SV. Prediction of gestational diabetes mellitus: are we ready for a biomarker lead screening strategy for GDM? International Journal of Diabetes in Developing Countries. 2022; 42:573–575. https://doi.org/10.1007/ s13410-022-01146-4
(b). Karami M, Mousavi SH, Rafiee M, et al. Biochemical and molecular biomarkers: unraveling their role in gestational diabetes mellitus. Diabetology & Metabolic Syndrome. 2023; 15(5):5. https://doi.org/10.1186/s13098-023-00980-8
(c). Zou. J, Liu. Y, Shen. J, et al. The role of 25(OH)D3 and circRNAs in early diagnosis of gestational diabetes mellitus. Journal of Clinical Laboratory Analysis. 2023; 37:e24826. https://doi.org/10.1002/jcla.24826
(d). Khan RS, Malik H. Diagnostic Biomarkers for Gestational Diabetes Mellitus Using Spectroscopy Techniques: A Systematic Review. Diseases. 2023; 11(1):16. https://doi.org/10.3390/diseases1101001