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MATERNAL-FETAL MEDICINE

Maternal serum screening marker levels in twin pregnancies affected by gestational diabetes

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Abstract

Purpose To investigate the levels of maternal serum screening markers in the first and second trimester twin pregnancies, which subsequently developed gestational diabetes mellitus (GDM).

Methods 145 twin pregnancies were recruited in the first trimester. Stored blood samples were retrospectively tested for pregnancy-associated plasma protein (PAPP)-A, human chorionic gonadotrophin (hCG), placental growth factor (PlGF), placental protein (PP)13, α-fetoprotein (aFP) and inhibin A. Values were expressed in multiples of the gestation-specific median (MoMs) in singletons, adjusted for maternal weight and parity, as appropriate.

Results Twenty samples of first and second trimester were available from 11 twins who subsequently developed GDM and 219 samples from unaffected twins. The median PAPP-A level in the affected twins was 3.61 MoM compared with 2.46 MoM in unaffected twins ($P < 0.001$, Wilcoxon rank sum test, two tailed); significant results were found in both trimesters. The median PP13 was also increased but to a lesser extent. It was only statistically significant overall ($P < 0.05$) and in second trimester samples ($P < 0.02$). No other marker differed significantly. Logistic regression found that combining PAPP-A and maternal weight had a 55% detection rate for a 10% false-positive rate.

Conclusions Early prenatal marker evaluation in twin pregnancies can be also useful for predicting the risk for developing GDM and should be further investigated.

Keywords Screening · Maternal serum biomarkers · Gestational diabetes · Twins · PAPP-A · PP13

Introduction

Gestational diabetes mellitus (GDM) is a major pregnancy complication associated with elevated risks for pre-eclampsia, perinatal mortality, fetal macrosomia, and shoulder dystocia often requiring delivery by cesarean section [1]. Prevalence is continuously increasing and a large international study estimated that it is currently 17.8% with a range of 9.3–25.5% [2]. GDM is associated with maternal morbidity due to after delivery development of obesity, development of type 1 and 2 diabetes mellitus (T1 DM and T2 DM), and of cardiovascular disorders (CVDs) [1–6]. Macrosomic newborns are also at risk of developing obesity, T1 and T2 DM, and CVDs [7], and there are additional maternal and infant effects [8].

Several studies have investigated placental-associated plasma protein (PAPP)-A levels as a potential marker of GDM in singleton pregnancies [9–22]. On average, the level was reduced in the first trimester in all but one of the fourteen published studies. Most of them also included maternal serum human chorionic gonadotrophin (hCG), intact, free-β or total, and it was generally found that this marker was also reduced, albeit to a smaller extent than for PAPP-A [9–16, 20, 22]. Three of the studies also reported on first trimester maternal serum placental growth factor (PlGF) [19], placental protein (PP)13 [21], and inhibin A [10] levels in GDM. Another study estimated that a detection rate of 80% can

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be achieved in singletons by combining standard biophysical and biochemical markers with additional non-standard metabolic markers that can be chemically tested together with blood hemoglobin, sugar and other standard tests [23].

Our group focuses on screening markers among twin pregnancies, a population widely neglected in major screening studies, while being a sub-group of pregnant women who experience a high frequency of pregnancy complications. We have previously evaluated several serum markers that are used to screen for trisomy 21 or pre-eclampsia in twin pregnancies and compared them with singletons. Six maternal serum markers—PAPP-A, free β-hCG, PlGF, PP13, α-fetoprotein (AFP) and inhibin A were considered, and the marker profile in twins was compared with singletons [24–27]. In the present study, we expanded this evaluation to determine the distribution of these markers in twin pregnancies, which subsequently develop GDM compared with singletons.

Methods

The cohort

Twin pregnancies were recruited prospectively from women aged 18 or more, attending a tertiary referral clinic in our medical center, for targeted scanning of twins, between September 2011 and December 2013. Upon recruitment to the study, women signed an informed consent agreeing to blood sampling, determination of biophysical markers, telephone interview and anonymous disclosure of pregnancy and medical information.

Patients were informed that marker levels were to be used for research purposes only, that they would not be informed of the results, and that no interventions would be based on them. Subsequently, women were referred to their obstetrician for further prenatal care according to Israeli standard guidelines for twins, including them under close surveillance.

The study was approved by the Institutional Ethics Committee of the Yitzhak Shamir Medical Center (formerly Assaf Harofeh Medical Center), Zerifin, Israel (ethical committee permit #217/13).

Inclusion criteria were both fetuses are alive, a CRL between 45 and 84 mm in both fetuses corresponding to 11–14-week gestation, regular cycles and/or in patients with assisted reproduction technology (ART)—a CRL consistent with the date of fertilization [28]. Exclusion criteria included ultrasound suspicion of an anomaly or nuchal translucency (NT) > 3.5 mm, application of any fetal reduction techniques, and maternal cardiovascular disease or medication. Patients with placentation support hormonal treatment for in vitro fertilization (IVF) were only included after discontinuing treatment.

Results in twins were compared with a consecutive series of 109 viable singleton pregnancies in women scanned prospectively in our institution during the same period [24–27]. The singleton controls were not individually matched to the twins.

The characteristics of the twin study population in GDM and unaffected pregnancies are shown in Table 1.

Serum samples

Two blood samples were taken—at the time of recruitment (11–14 weeks) and at a second trimester visit (21–27 weeks). After having been left for clotting at a room temperature for 30–90 min, first and second trimester blood samples were centrifuged for 15 min at 3000*g*. Serum was collected, stored at − 20 °C for up to 4 days, transferred to local − 70 °C storage. At the end of the study period, samples were shipped on dry ice to a central laboratory abroad, where they were assayed for five markers except for PP13 that was tested in Hy-Laboratories, Rehovot, Israel, as previously described [24–27]. Samples from twins or singletons were tested with the same batches of kits and laboratory technicians who were blinded to the sample type and the outcome of pregnancy.

Marker serum level of each twin-pregnancy serum marker was expressed as a multiple of the normal gestational week-specific medians (MoM) for CRL derived from singleton pregnancies referred to our center, based on regression analyses spanning all included gestational weeks of first and second trimester values [24–27]. The singleton cohort comprised 109 subjects presenting at our clinics during the same period as the twin enrolment. In Israel, women with singleton pregnancy seldom attend medical centers, but are routinely evaluated and managed in community clinics. Nonetheless, the median MoMs for different markers were the same as previously published studies [24–27] and indicated that the series was sufficiently large to establish stable and reproducible MoMs for the biochemical markers.

PAPP-A, PP13 and AFP MoMs were adjusted for maternal weight, and the MoM of free β-hCG was adjusted for parity [24–27].

GDM diagnosis

GDM was diagnosed using our departmental two-step protocol which follows published guidelines [29]. At 24–28-week gestation, a 1-h glucose challenge test (50 g) was performed and if the glucose level exceeds 140 mg/dL, a 3-h oral glucose tolerance test (OGTT, 100 g) was carried out. GDM was diagnosed in women who had two or more abnormal OGTT results: fasting ≥ 95 mg/dL, 1-h ≥ 180 mg/dL, 2-h ≥ 155 mg/dL, and 3-h ≥ 140 mg/dL. The GDM group

Table 1 Characteristics of the study population affected versus unaffected by GDM

Parameter	Twin pregnancies		*P* value*
	GDM (11)	Unaffected (134)	
Enrolment			
Gestation (median [95% CI]), (days)	84 [82–86]	85 [84–86]	0.31
Maternal weight (median [95% CI]), (Kg)	82 [67–100]	64 [62–66]	< 0.0005
BMI > 30 (%)	64%	16%	< 0.001
Maternal age (median [95% CI]), (years)	32 [30–34]	31 [30–32]	0.24
Nulliparous (%)	36%	41%	1.00
Conception by ART (%)	36%	36%	1.00
Smoking (%)	18%	18%	1.00
Delivery			
Gestation (median [95% CI]) (days)	258 [249–268]	255 [249–250]	0.66
Birth weight[a] (median [95% CI]) (g)	2510 [2252–2798]	2332 [2182–2494]	0.40
Birth weight[b] > 95th centile (%)	18%	9%	0.29
Cesarean delivery (%)	64%	59%	1.00

CI confidence interval

*Wilcoxon rank sum tests for continuous and Fisher's exact test for categorical variables

[a]Average of both fetuses

[b]At least one fetus based on Israeli charts [52]

was subsequently managed either by diet control (GDMA1) or treated by insulin (GDMA2). This procedure was according to the guidelines of Israel Society of Obstetrics and Gynecology, which were routine at the time of enrolment, according to a previous world consensus [29] and before WHO and FIGO recommendation of a one-step 75 g glucose challenge test was introduced with glucose range limits according to the HAPO study [2, 30].

Statistical methods

Significant differences in the marker distributions between GDM and unaffected twin pregnancies were assessed using the non-parametric rank sum test, with $P < 0.05$. Statistically significant markers were entered into a logistic regression analysis after log transformation and where appropriate with prior risk factors. The modeled screening performance of each logistic regression formula was assessed by the area under the receiver operation characteristic curve (AUC) and the observed detection rate for a 5% and 10% false-positive rate.

Results

A total of 190 twin pregnancies were enrolled, of which 19 were lost to follow-up, 21 were excluded due to fetal reduction from triplets, increased nuchal translucency or fetal anomalies and five had incomplete data. The remaining cohort comprised 145 twins, 11 which subsequently developed GDM, a rate of 7.6%. There were 94 twin pregnancies with samples—both at recruitment and in the second trimester—and 11 had GDM. In twin pregnancies, which subsequently developed GDM, the median maternal weight at enrolment was significantly higher than in unaffected twins and the proportion with BMI exceeding 30 was more than doubled (Table 1). At delivery, the median birth weight was higher in patients who subsequently developed GDM, and the proportion with birth weight exceeding the 95th centile (based on Israeli charts [29]) was double for GDM, but these differences did not reach significance.

The individual marker levels in 20 maternal serum samples of the first and second trimesters obtained from the 11 twins with GDM are shown (Table 2), together with the gestational age when each sample was drawn. On the longitudinal table axis, the table displays cross-sectional data of each given marker indicating the range of the alpha diversity across the tested population. At the horizontal axis, the values of each marker are displayed for a given patient (beta diversity), showing that there was no clear pattern for individual markers, and no obvious systematic direction of changes for a set of these markers among the different GDM patients.

Table 3 compares, for each marker, the median level in twin pregnancies affected by GDM with unaffected twins, according to trimesters and for the two combined allowing to see if changes are temporary, trimester sensitive or longitudinally consistent, and evaluate the value of late (second trimester) booking.

Table 2 Twin GDM pregnancies: individual marker levels (MoM)

Case	GDM	Gestation (weeks)	PAPP-A	Free β-hCG	PlGF	PP13	AFP	Inhibin
1*	1	12	7.78	3.65	0.70	ND	6.06	2.84
		23	17.59	4.28	0.66	ND	5.37	2.57
2	2	11	5.22	1.72	2.09	ND	1.37	1.34
		23	3.56	2.93	0.88	1.45	1.63	1.65
3	2	12	2.49	2.33	1.06	1.29	1.14	ND
4**	2	11	3.49	1.25	2.41	2.08	6.19	ND
		24	7.54	3.20	3.76	2.28	2.33	4.62
5	1	12	2.98	4.13	0.80	1.15	2.58	2.30
		27	3.73	9.06	1.92	1.21	1.67	3.23
6	1	12	2.02	2.31	1.64	2.12	3.39	2.66
		26	3.51	2.40	0.88	1.94	1.49	2.28
7	1	13	1.90	0.71	0.89	1.50	3.28	1.37
8*	2	12	6.41	1.38	1.26	2.82	4.37	1.12
		24	10.29	2.69	0.66	3.03	2.70	1.20
9	1	12	2.21	1.60	0.89	2.07	3.11	2.22
		24	2.37	2.30	1.36	1.78	1.57	2.22
10	1	11	2.30	0.46	1.22	1.71	2.71	1.43
		25	4.74	0.47	0.83	2.26	2.26	1.87
11	1	11	3.97	1.89	2.19	1.79	1.82	1.78
		24	3.66	2.06	2.07	1.84	0.94	1.11

GDM gestational diabetes, *ND* not done, biomarkers: *PAPP-A* pregnancy-associated placental protein A, *hCG* human chorionic gonadotrophin, *PlGF* placental growth factor, *PP13* placental protein 13

*Pre-eclampsia; **monochorionic

PAPP-A There was a 47% increase in median PAPP-A level in the GDM twin group, and the difference was highly significant ($P < 0.001$, Wilcoxon rank sum test, two tailed). A significant increase was found when evaluating data of both trimesters. Median maternal serum PP13 was also significantly increased by 21% in GDM twin pregnancies ($P < 0.05$) for the values of the two trimesters, but reached significance only for the second trimester. There were no significant differences in the levels of the remaining four markers according to trimesters or for the two combined.

Table 4 summarizes the 15 published studies of maternal serum PAPP-A levels in GDM-affected and -unaffected singleton pregnancies. The ratio between the mean or median value in GDM and unaffected pregnancies ranged from 0.58 to 1.04, and in half the studies it was below 0.90. In contrast, the ratio of the medians between GDM-affected and -unaffected twin pregnancies in the current study was 1.47 and the 95% confidence interval was 1.02–2.11, which only overlaps with the ratio in one of the singleton studies.

β-hCG In singleton pregnancies the published studies of maternal serum free β-hCG levels showed a ratio between the means or medians level of GDM affected and unaffected of: 0.77 [9], 0.87 [10], 0.90 [11], 0.93 [16], 0.95 [12], 0.95 [22], 0.96 [15], 0.98 [13], 1.03 [20], 1.05 [14] and 1 [23]. The median levels of maternal serum free β-hCG levels in GDM-affected and -unaffected twin pregnancies were 1.10 with 95% confidence interval 0.69–1.73. Hence, the twin and singleton results are consistent.

PlGF, PP13, and *inhibin A* The ratio of serum levels for GDM/unaffected for twins and singletons, respectively, for these three markers were PlGF: 0.81 and 1.05 [19]; PP13: 1.21 and 0.75 (based on concentration units rather than MoMs) [21], and inhibin A: 0.99 and 0.87 [10], indicating a larger ratio in twin pregnancies for PP13 and inhibin and a lower one for PlGF.

Logistic regression on PAPP-A alone yielded an AUC of 0.73 ($P < 0.005$) (Fig. 1a), whilst for PP13 alone it was 0.65 and not statistically significant ($P = 0.06$). Using both biochemical markers, the AUC was reduced to 0.71. Combining PAPP-A with maternal weight, the AUC increased to 0.83 ($P < 0.0001$) (Fig. 1b). Using the latter, a regression curve for the observed detection rate for GDM was 55% for a 10% false-positive rate and 35% for a 5% false positive.

Discussion

General GDM is associated with elevated risks for multiple pregnancy complication, including but not limited to, pre-eclampsia, perinatal mortality, fetal macrosomia,

Table 3 Median multiple of the median (MoM) of serum biomarkers in twin pregnancies with gestational diabetes (GDM) versus unaffected twins)

Marker	GDM		Unaffected		A/B	*P* value (two tail)
	Samples (N)	Median (A)	Samples (N)	Median (B)		
All samples						
PAPP-A	20	3.61	219	2.46	1.47	< 0.001
Free β-hCG	20	2.30	219	2.10	1.10	0.73
PlGF	20	1.14	219	1.40	0.81	0.20
PP13	17	1.84	187	1.52	1.21	< 0.05
AFP	20	2.45	219	2.14	1.14	0.35
Inhibin A	18	2.04	217	2.06	0.99	0.77
First trimester						
PAPP-A	11	2.98	134	2.05	1.45	< 0.02
Free β-hCG	11	1.72	134	1.92	0.90	0.57
PlGF	11	1.23	134	1.34	0.92	0.72
PP13	9	1.79	107	1.67	1.07	0.64
AFP	11	3.11	134	2.37	1.31	0.18
Inhibin A	9	1.78	132	2.07	0.86	0.39
Second trimester						
PAPP-A	9	3.74	85	3.07	2.22	< 0.05
Free β-hCG	9	2.69	85	2.37	1.14	0.36
PlGF	9	0.88	85	1.71	0.51	0.14
PP13	8	1.89	80	1.40	1.35	< 0.02
AFP	9	1.67	85	1.83	0.91	0.81
Inhibin A	9	2.22	85	2.00	1.11	0.64

GDM gestation diabetes mellitus, *MoM* multiple of the medians, biomarkers: *PAPP-A* pregnancy-associated placental protein A, *hCG* human chorionic gonadotrophin, *PlGF* placental growth factor, *PP13* placental protein 13, *AFP* alpha fetoprotein

Table 4 Levels of PAPP-A in singleton pregnancies with gestational diabetes (GDM) versus unaffected singleton pregnancies cited in 14 previous studies

Studies	GDM		Unaffected		A/B
	Cases	Median/mean (A)	Cases	Median/mean (B)	
Beneventi et al. [11]	228	0.70	228	1.20	0.58
Farina et al. [21]	12	0.70	60	1.10	0.64
Lovati et al. [14]	307	0.90	366	1.30	0.69
Kulaksizoulu et al. [15]	60	0.77	60	0.97	0.79
Ong et al. [9]	49	0.85	4297	1.05	0.81
Beneventi et al. [17]	112	1.06	112	1.22	0.86
Xiao et al. [22]	599	0.88	986	1.01	0.87
Spencer and Cowans [16]	870	0.91	6559	1.00	0.91
Wells et al. [18]	364	0.91*	1282	1.00	0.94
Savvidou et al. [12]	779	0.94	41,007	1.00	0.94
Syngelaki et al. [16]	787	0.95	30,428	1.00	0.95
Tul et al. [10]	27	0.98	1109	1.01	0.97
Cheuk et al. [20]	169	0.97	351	0.99	0.98
Husslein et al. [13]	72	1.17	216	1.13	1.04
Gabbay-Benziv [23]	63	1.05	861	1.07	0.98

Weighted geometric average of median in cases diagnosed after 22 weeks (0.94 MoM in $n = 301$) and in earlier cases (0.79 MoM in $n = 63$)

A Average of both fetuses, *B* at least one fetus based on Israeli charts [24], *CI* confidence interval

*Wilcoxon rank sum tests for continuous and Fisher's exact test for categorical variables

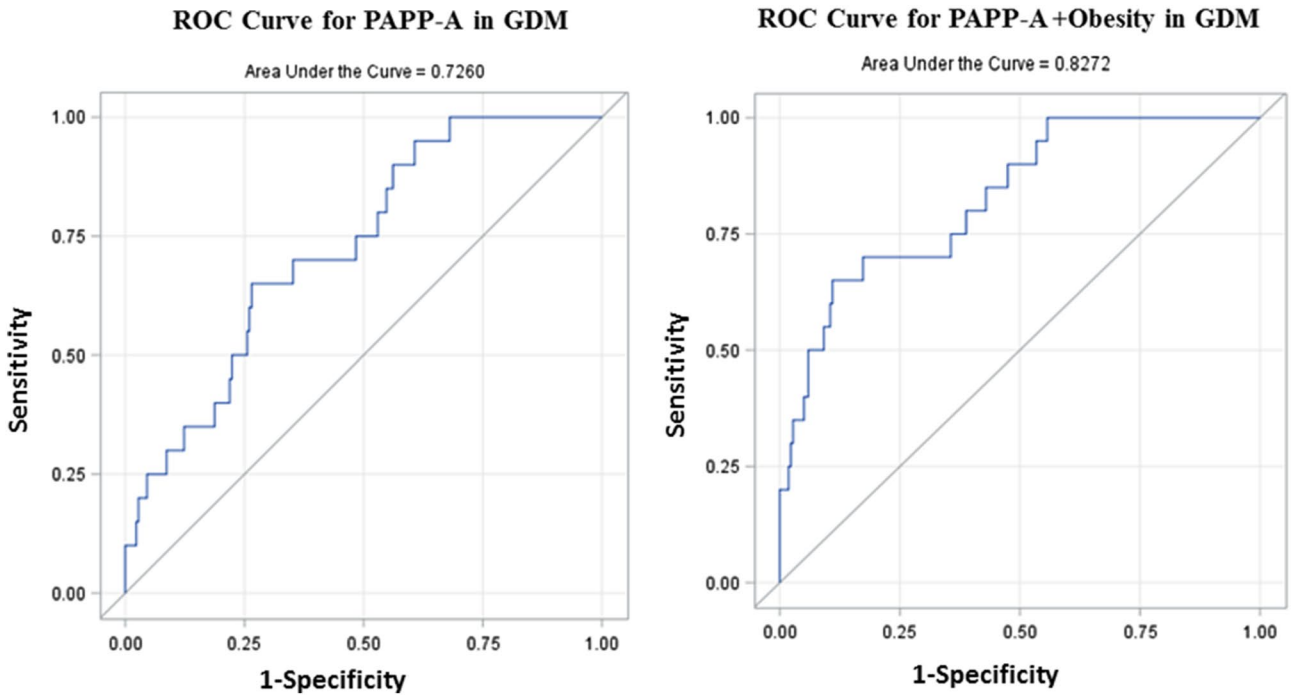

Fig. 1 Receiver operating characteristics (ROC) curve for gestational diabetes (GDM). **a** ROC curve for PAPP-A alone for twin GDM pregnancies yielded area under the curve (AUC) of 0.7260. **b** ROC of PAPP-A combined with maternal body mass index (BMI) > 35 yielded an AUC of 0.8272

shoulder dystocia or cesarean section delivery. Later in life, women who experienced GDM have increased maternal hyperinsulinemia, dyslipidemia, type-2 DM, hypertension, and CVDs and the newborns are at increased risk for DM and obesity. Thus, developing early screening is important, but has only been investigated in singleton pregnancies. Therefore, it makes sense to assess early screening methods for GDM in twins prior to the development of the disorder [1, 2].

Principal findings In this study, we have shown that in twin pregnancies the level of maternal serum PAPP-A is higher in twin GDM pregnancies in the first and the second trimester. Combining PAPP-A and maternal weight, and applying logistic regression, we found a 55% detection rate for GDM with a 10% false-positive rate.

The increased PAPP-A in the first trimester in twin pregnancies is detected ten or more weeks before the time of routine GDM screening at 24–28 weeks, and may help identifying GDM in earlier stages of twin pregnancies. A 55% detection rate for PAPP-A and obesity, though, might be too low to allow for screening. Future study may evaluate combining these markers with metabolic markers as suggested by Gabbay-Benziv et al. [23] including blood glucose, triglycerides, LDH, LDL, or other lipids, phosphates, cations and also hemoglobin A1C. Subsequent studies should evaluate this approach given that in singleton pregnancies this combination yielded an 80% detection rate [23].

The clinical implications are very important. Today, there are 17 meta-analyses and single randomized trials showing that early lifestyle changes (e.g., physical exercise, dietary regimen, pharmacological and psychological interventions) can improve maternal and neonatal outcome parameters in singleton pregnancies with or without maternal obesity [31]. At least one of these studies also included twins [32]. All these studies provided evidence for how far early dietary advice, physical activity or other tools might work to reduce GDM prevalence in general, and also in twin pregnancies, without increasing any harm. In view of the above, introduction of an early screening method as described here has further importance given the potential to follow it with lifestyle changes to reach better outcomes.

PAPP-A in twin pregnancy with GDM It is widely believed that GDM develops when the maternal pancreas is unable to manage the increasing glucose load of pregnancy. PAPP-A encodes a secreted metalloproteinase which cleaves insulin-like growth factor-binding protein. Following cleavage, insulin growth factors are dissociated and bind to their receptors, resulting in activation of the insulin growth factor pathway [33–35].

The increased first trimester PAPP-A levels in twin pregnancies, which subsequently develop GDM, is in contrast to the reduced PAPP-A levels in GDM singleton pregnancies [9–23]. In GDM-affected singleton pregnancies, the initially reduced PAPP-A levels are subsequently sharply elevated

with the increase in blood glucose levels at the active stage of the condition [36]. In our study, the increased PAPP-A level in twin pregnancies is already detected in the first trimester. Thus, it appears that there is an accelerated increase of PAPP-A in twin pregnancies who subsequently develop GDM. This is consistent with a heavier burden of pregnancy in twin compared to singleton pregnancies.

HCG level in twin pregnancies with GDM Most published studies in singleton pregnancies have shown a reduced level in GDM singleton pregnancies [9–23]. In the current study, there was an overall increase in median free β-hCG level among twin GDM pregnancies comprising of a reduction in the first trimester and an increase in the second trimester. However, none of these differences was significant, presumably due to the small size of the cohort.

PP13 in twin pregnancy with GDM The median PP13 level in GDM twin pregnancies was increased by 21%, whilst in the only published singleton study levels were reduced on average in affected pregnancies [21]. Elevated PP13 is anticipated to vasodilate the blood vessels and improve blood flow to the placenta, thereby supporting the pregnancy. PP13 increase in GDM twin pregnancies could serve as a rescue pathway to provide more blood to the placenta in GDM [37].

Early biomarkers in GDM and in pre-eclampsia In both pregnancy complications, the changes in biomarkers followed the same direction for the first (GA 11-13) and second (GA 16-18 or 21-23) trimesters. In GDM, the best prediction was obtained combining PAPP-A and maternal weight, with no value for biophysical markers and other serum biomarkers, whilst in pre-eclampsia all markers were beneficial [26, 27]. These patterns may serve for future differential diagnosis.

Research implications Mothers with multiple pregnancies represent a high-risk group for whom it is crucial to introduce early screening for pregnancy complication. The Australian twin study [38] has already revealed that gene expression and that DNA methylation in twin pregnancies have a wider range of epigenetic discordance at birth than was previously found. The shared uterus (maternal) environment is influencing genetic factors that differentially affects each twin and its tissue and contribute to an increased proportion of epigenetic variations at birth.

The frequency of GDM in twins in this study (7.6%) was more than double the frequency reported for singletons in Israel (3%) [39], a finding comparable to other populations [40–43], though other studies reported even higher frequencies [2, 32]. Twin pregnancies are more likely to have glucose intolerance than singletons [33–35]. The differences in frequency and in marker profiles may be a result of the increased body mass index in twin pregnancies, which may affect the gut microbiome and the liver enzymes, both of them contributing to insulin intolerance during pregnancy [44, 45]. Other studies have pointed to abnormalities in the placental structure, which may affect the placental transport of glucose and metabolic factors in twin compared to singleton pregnancies [46, 47].

Strength and weaknesses Our study weakness is its small cohort, but it demonstrates which future parameters should be tested in larger series. As was already shown by Gabby-Benziv et al. [23], combining serum markers with metabolic parameters (triglycerides, cholesterol, hemoglobin A1C, etc.) could improve screening accuracy. The adoption of their approach for twin pregnancy may generate sufficient accuracy for designing earlier management by diet or insulin [23, 48–50]. Our study has indicated that maternal pre-pregnancy BMI is a valuable parameter, and future studies may need to evaluate whether dietary advice or reduced weight gain prior or during pregnancy can improve maternal and neonatal outcomes.

In conclusion, the incidence of GDM in twin pregnancies is increased as compared to singleton pregnancies. GDM is combined with high maternal BMI and fetal macrosomia, and the metabolic consequences are serious and in twin pregnancies they occur in infants who have increased risks up to adulthood. The current study suggests that first trimester pregnancy evaluation is not only suitable for Down's syndrome and pre-eclampsia screening in twin pregnancy, but may also serve for detecting the risk to develop GDM. This approach that was already suggested by the inverted pyramid model for singleton pregnancies [51] appears to be especially true for twin pregnancies. Thus, a shift towards a personalized prenatal management of twin pregnancies is warranted. Additional larger studies are required to validate our results.

Author contributions RM: clinical protocol and project development, data management, data analysis, manuscript writing and editing. HM: clinical protocol development, data analysis, manuscript writing and editing, and others—test biomarkers. RS: protocol development, data collection and management, data analysis, and manuscript writing. EW: data analysis and manuscript writing. HC: project development, data analysis, manuscript writing and editing

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Compliance with ethical standards

Conflict of interest Howard Cuckle is a consultant to PerkinElmer Inc. Hamutal Meiri holds partial patent rights for placental protein 13. Ron Maymon, Ran Svirski, and Eran Weiner have no conflicts of interest to declare. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Upon recruitment to the study, all women signed an informed consent

and the study received our institutional ethical review board permission #217/13.

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