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First trimester screening for gestational diabetes mellitus by maternal factors and markers of inflammation

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ABSTRACT

Objective. To examine the potential role of maternal serum levels of tumor necrosis factor-α (TNF-α) and high sensitivity C-reactive protein (Hs-CRP) in the first trimester of pregnancy in the prediction of gestational diabetes mellitus (GDM).

Methods. Maternal serum TNF-α and Hs-CRP concentrations were measured in a case–control study of singleton pregnancies at 11–13 weeks' gestation, which included 200 cases that subsequently developed GDM and 800 unaffected controls. Measured levels of TNF-α and Hs-CRP were expressed as multiples of the median (MoM) after adjustment for maternal characteristics and history. The performance of screening for GDM by maternal factors and MoM values of TNF-α and Hs-CRP was evaluated by the area under the receiver operating characteristic curves (AUROC).

Results. In the GDM group, compared to the normal group, the median TNF-α was significantly increased (1.303 MoM, interquartile range [IQR] 1.151–1.475 vs. 1.0 MoM, IQR 0.940–1.064; p = 0.031) and the median Hs-CRP was not significantly different (1.113 MoM, IQR 0.990–1.250 vs. 1.0 MoM, IQR 0.943–1.060; p = 0.084). In the prediction of GDM, the AUROC for maternal characteristics with TNF-α or Hs-CRP was not significantly different than the AUROC for maternal characteristics alone (p = 0.5055 and p = 0.2197, respectively).

Conclusions. In pregnancies that develop GDM there is no evidence of an inflammatory response at 11–13 weeks' gestation and the levels of serum TNF-α and Hs-CRP are not useful in first-trimester screening for GDM.

Abbreviations: TNF-α, tumor necrosis factor-α; Hs-CRP, high sensitivity C-reactive protein; MoM, multiple of the median; GDM, gestational diabetes mellitus; LGA, large for gestational age; OGTT, oral glucose tolerance test; ROC, receiver operating characteristic; DR, detection rate; FPR, false positive rate; IQR, interquartile range; SD, standard deviation; AUROC, area under receiver operating characteristic curve; CI, confidence interval.

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1. Introduction

The incidence of gestational diabetes mellitus (GDM) has been estimated to be around 5% [1], but nowadays may be as high as 26% depending on the population, method of screening and glucose threshold values [2]. GDM is associated with increased risk of adverse perinatal outcomes [3] and the development of type 2 diabetes mellitus later in life [4]. There is evidence that inflammation is associated with insulin resistance and is a central feature in the development of Type 2 diabetes mellitus [5,6]. Similarly, inflammation has been reported in GDM but the prognostic significance of this remains to be fully elucidated.

C-reactive protein (CRP), an inflammatory marker released by the liver under cytokine stimulation [7] and tumor necrosis factor-α (TNF-α) a pro-inflammatory cytokine synthesized and secreted by adipose tissue as well as placenta [8], have both been extensively examined in women with GDM [9]. Numerous case–control studies, involving 5–124 cases of GDM, provided contradictory evidence that in pregnancies with established GDM serum TNF-α and high sensitivity CRP (hs-CRP) are increased [10–41]. Similarly, there is some limited evidence that altered levels in these biomarkers may precede the clinical onset of the disease [42–45]. We have previously reported a first-trimester prediction model for GDM based on maternal characteristics and medical history, including maternal age, weight, height, racial origin, family history of diabetes mellitus, method of conception, previous history of GDM and previous delivery of macrosomic neonate [46]. Screening by this method can predict 55%, 68% and 84% of cases of GDM at respective false positive rates (FPRs) of 10%, 20% and 40%. The model allows the estimation of the patient-specific a priori risk for GDM which could be combined with potentially useful biomarkers for further improvement in the performance of screening.

The objectives of this study are first, to examine the application of Bayes theorem to combine the prior risk from maternal characteristics and history with serum levels of TNF-α and hs-CRP at 11–13 weeks' gestation in defining the patient-specific risk for GDM and second, to estimate the potential performance of such combined screening for early identification of affected pregnancies.

2. Methods

2.1. Study Population

This study was drawn from a large prospective observational study for early prediction of pregnancy complications in women attending for their routine first hospital visit in pregnancy at King's College Hospital, London, UK. In this visit, which is held at 11^{+0} to 13^{+6} weeks' gestation, we record maternal characteristics and medical history and perform an ultrasound scan to firstly, confirm gestational age from the measurement of the fetal crown-rump length [47], secondly, diagnose any major fetal abnormalities [48] and thirdly, screen for chromosomal abnormalities based on fetal nuchal translucency thickness and maternal serum pregnancy associated plasma protein-A and free β-human chorionic gonadotropin [49,50]. Women attending for this visit were invited to participate in a study on the prediction of pregnancy complications and from those who provided informed written consent serum samples were stored at −80 °C for subsequent biochemical analysis. The study was approved by the National Research Ethics Committee.

Details of maternal characteristics and the findings of the 11–13 weeks assessment were recorded in our database. Data on pregnancy outcome were obtained from the maternity computerized records or the general medical practitioners of the women and were also recorded in our database.

2.2. Maternal History and Characteristics

Patients were asked to complete a questionnaire on maternal age, racial origin (Caucasian, African, South Asian, East Asian and mixed), cigarette smoking during pregnancy, method of conception (spontaneous or assisted conception requiring the use of ovulation drugs), medical history including diabetes mellitus type 1 or 2, family history of diabetes mellitus (first, second or third degree relative with diabetes mellitus type 1 or 2) and obstetric history. The questionnaire was then reviewed by a doctor together with the patient. The maternal weight and height were measured. For the purpose of this study women were classified as parous or nulliparous with no previous pregnancies at or beyond 24 weeks and if parous we recorded whether the last pregnancy was complicated by GDM or resulted in the delivery of a large for gestational age (LGA) neonate, defined as birth weight above the 95th percentile [51].

Screening for GDM in our hospital is based on a two-step approach. In all women random plasma glucose is measured at 24–28 weeks' gestation and if the concentration is ≥6.7 mmol/L, a 75 g oral glucose tolerance test (OGTT) is carried out within the subsequent 2 weeks. The diagnosis of GDM is made if the fasting plasma glucose level is ≥6 mmol/L or the plasma glucose level 2 h after the oral administration of 75 g glucose is ≥7.8 mmol/L [52].

2.3. Case–Control Study

In this study we measured maternal serum TNF-α and hs-CRP concentrations in 200 cases that developed GDM and 800 controls. The cases of GDM were selected at random from our database of stored samples and each case was matched to four controls that were sampled on the same or next day. The controls were normal pregnancies without GDM or other pregnancy complications resulting in live birth after 37 weeks' gestation of phenotypically normal neonates with birth weight between the 5th and 95th percentiles for gestational age [50].

Serum TNF-α was measured by a Quantikine TNF-α ELISA kit (distributed by R&D Systems Europe, Abingdon, UK); the lower limit of detection of the assay was 0.6 ng/L, the intra-assay coefficient of variation at a concentration of 45.6 to 50.6 ng/L was 5.2% and the inter-assay coefficient of variation at a concentration of 42.4 to 49.2 ng/L was 7.4%. Serum hs-CRP was measured by a Cormay hs-CRP assay (kit distributed by P.Z. Lublin, Poland); the lower limit of detection of the assay was 0.01 mg/dL, the intra-assay coefficient of variation at a concentration of 0.046 to 0.981 mg/dL was 2.0% and the inter-assay coefficient of variation at a concentration of 0.047 to 0.976 mg/dL was 3.3%. All samples

were done in duplicates, and samples with a coefficient of variation exceeding 10% were re-analyzed. None of the samples in this study were previously thawed and refrozen.

2.4. Statistical Analysis

The measured concentrations of serum TNF-α and hs-CRP were converted to multiples of the median (MoM) after adjustment for maternal characteristics and history. Essentially, the values were $\log_{10}$ transformed to make their distributions Gaussian and multivariate logistic regression analysis was then carried out to identify factors from maternal characteristics and history with substantial contribution to the $\log_{10}$ transformed values. Backward elimination was used to identify potentially important terms in the model by sequentially removing non-significant ($p > 0.05$) variables. Effect sizes were assessed relative to the error standard deviation (SD) and a criterion of 0.1 SD was used to identify terms that had little substantive impact in model predictions. Residual analyses were used to assess the adequacy of the model. Mann–Whitney U test was used to compare the median MoM values of TNF-α and hs-CRP between the outcome groups.

The a priori risk for GDM was estimated from an algorithm for the prediction of GDM derived from the multivariable logistic regression analysis of maternal characteristics and history in 75,161 singleton pregnancies including 1827 (2.4%) that developed GDM [46]. Bayes theorem was applied to combine the a priori risk of GDM from maternal characteristics and medical history with TNF-α and hs-CRP MoM values. To assess the performance of the markers in the prediction of GDM, detection rates (DRs) for various FPRs were calculated, receiver operating characteristic (ROC) curves were produced and area under the curves (AUROCs) calculated. The AUROCs were compared using DeLong's test.

The statistical software package R was used for all data analyses [53].

2.5. Literature Search

We searched MEDLINE and EMBASE on 15 August 2015 without any time limits to identify articles reporting on circulating maternal serum or plasma levels of TNF-α and hs-CRP in pregnancies complicated by GDM using the following keys words: (tumor necrosis factor AND gestational diabetes mellitus) and (high sensitivity C-reactive protein AND gestational diabetes mellitus).

3. Results

The maternal characteristics and history of the GDM and control groups are presented in Table 1. In the GDM group, the median maternal weight was higher, the median maternal height was lower, there were more women of Afro-Caribbean

Table 1 – Maternal and pregnancy characteristics the case–control study.

Variables	Controls (n = 800)	Gestational diabetes mellitus			
		All cases (n = 200)	Control by diet (n = 33)	Metformin therapy (n = 51)	Insulin therapy (n = 116)
Maternal age in years, median (IQR)	33.0 (29.0–36.1)	33.7 (30.7–37.6)*	35.2 (32.5–38.1)	34.1 (31.8–37.9)	33.1 (29.7–37.3)
Maternal weight in kg, median (IQR)	67.0 (59.7–78.4)	75.0 (64.0–90.3)*	71.5 (63.0–85.0)	72.0 (61.8–86.9)	78.1 (66.0–92.0)*
Maternal height in cm, median (IQR)	165 (160–169)	163 (158–167)*	163 (158–168)	164 (159–166)	162 (158–168)*
Gestation at sampling (days), median (IQR)	12.7 (12.3–13.0)	12.7 (12.3–13.0)	12.8 (12.4–13.3)	12.7 (12.3–13.1)	12.6 (12.2–13.0)
Racial origin					
Caucasian, n (%)	504 (63.0)	86 (43.0)*	20 (60.6)	22 (43.1)*	44 (37.9)*
Afro-Caribbean, n (%)	211 (26.4)	75 (37.5)*	8 (24.4)	17 (33.3)	50 (43.1)*
South Asian, n (%)	35 (4.4)	19 (9.5)*	1 (3.0)	7 (13.7)	11 (9.5)
East Asian, n (%)	26 (3.3)	14 (7.0)	3 (9.0)	3 (5.9)	8 (6.9)
Mixed, n (%)	24 (3.0)	6 (3.0)	1 (3.0)	2 (3.9)	3 (2.6)
Cigarette smokers, n (%)	54 (6.8)	5 (2.5)	1 (3.0)	0	4 (3.4)
Conception					
Spontaneous, n (%)	780 (97.5)	192 (96.0)	31 (93.9)	47 (92.2)	114 (98.3)
Ovulation induction drugs, n (%)	3 (0.4)	3 (1.5)	1 (3.0)	0	2 (1.7)
In vitro fertilization, n (%)	17 (2.1)	5 (2.5)	1 (3.0)	4 (7.8)	0
Family history of diabetes					
1st degree, n (%)	96 (12.0)	69 (34.5)*	9 (27.3)*	16 (31.4)*	44 (37.9)*
2nd degree, n (%)	64 (8.0)	26 (13.0)	4 (12.1)	4 (7.8)	18 (15.5)
Parity					
Nulliparous, n (%)	199 (24.9)	79 (39.5)*	15 (45.5)*	18 (35.3)	46 (39.7)*
Parous with previous GDM, n (%)	5 (0.6)	41 (20.5)*	4 (12.1)*	10 (19.6)*	27 (23.3)*
Parous with previous LGA, n (%)	30 (3.8)	14 (7.0)	0	4 (7.8)	10 (0.9)
Gestation at delivery in wks, median (IQR)	40.2 (39.4–41.0)	38.7 (38.1–39.3)*	39.5 (39.1–40.0)*	38.7 (38.2–39.5)*	38.5 (38.1–38.9)*
Birth weight in grams, median (IQR)	3433 (3210–3641)	3221 (2878–3528)*	3275 (2960–3490)	3182 (2868–3475)*	3219 (2835–3539)*

IQR = interquartile range; GDM = Gestational diabetes mellitus; LGA = Large for gestational age. Comparisons between each outcome group and unaffected controls (χ^2 test and Fisher's exact test for categorical variables and Mann–Whitney test with post hoc Bonferroni correction for continuous variables).

* Critical significance level $p < 0.0125$.

Table 2 – Fitted regression model for log_{10} tumor necrosis factor alpha levels and high sensitivity C-reactive protein in the control group.

Term	Estimate	SE	95% Confidence interval	P value
Tumor necrosis factor alpha				
(Intercept)	0.16312435	0.01565430	0.13244192, 0.19380679	0.0000
Weight in kg – 69	0.00225749	0.00079604	0.00069725, 0.00381774	0.0047
Afro-Caribbean	−0.09414981	0.02825810	−0.14953568, −0.03876393	0.0009
In vitro fertilization	−0.18986348	0.08374798	−0.35400952, −0.02571743	0.0236
High sensitivity C-reactive protein				
Intercept	0.55441666	0.02597523	0.50350521, 0.6053281	0.0000
Weight in kg – 69	0.01333395	0.00107254	0.01123178, 0.01543613	0.0000
(Weight in kg – 69)^2	−0.00007396	0.00003216	−0.00013699, −0.00001092	0.0217
Height in cm – 164	−0.01373594	0.00183163	−0.01732593, −0.01014595	0.0000
Age in years – 35	−0.00495491	0.00218857	−0.00924452, −0.00066531	0.0238
Parous	0.07993425	0.02664396	0.02771209, 0.13215642	0.0028

SE = standard error.

and South Asian racial origins, with family history of diabetes and more women had a previous pregnancy complicated by GDM and/or the birth of an LGA neonate. In the GDM group there were 33 cases that did not require any treatment apart from dietary intervention, 51 cases treated with metformin and 116 cases treated with insulin.

Multivariate regression analysis in the control group demonstrated that for log_{10} TNF-α significant independent contribution was provided by maternal weight, Afro-Caribbean racial origin and conception by in vitro fertilization (Table 2). Multivariate regression analysis in the control group demonstrated that for log_{10} Hs-CRP significant independent contributions were provided by maternal weight, height, age and parity (Table 2).

In each patient we used the models in Table 2 to derive the expected log_{10} TNF-α and log_{10} Hs-CRP and then expressed the observed values as MoM of the expected (Table 3). In the GDM group, compared to the normal group, the median TNF-α was significantly increased (1.303 MoM, interquartile range [IQR] 1.151–1.475 vs. 1.0 MoM, IQR 0.940–1.064; p = 0.031) and the median Hs-CRP was not significantly different (1.113 MoM, IQR 0.990–1.250 vs. 1.0 MoM, IQR 0.943–1.060; p = 0.084). There was a non-significant trend for higher levels in pregnancies with GDM treated by insulin or metformin than in those requiring dietary advice alone.

The SD with 95% confidence intervals (CIs) for log_{10} TNF-α MoM levels in the control and GDM groups was 0.39776 (95% CI 0.37918, 0.41827) and 0.33494 (95% CI 0.30501, 0.37142), respectively. The SD with 95% CI for log_{10} Hs-CRP MoM levels in the control and GDM groups was 0.36138 (95% CI 0.34450, 0.38002) and 0.37875 (95% CI 0.34491, 0.42000), respectively. There was a weak correlation between log_{10} TNF-α MoM and log_{10} Hs-CRP MoM level of 0.053061 (95% CI −0.008963, 0.114678).

3.1. *Estimated Performance of Screening for GDM*

The DRs of GDM, at fixed FPRs of 10%, 20% and 40%, in screening by maternal factors alone, and combination of maternal factors with serum TNF-α and Hs-CRP are given in Table 4 and illustrated in Fig. 1. In the prediction of GDM, the AUROC for maternal characteristics (0.8200) was not significantly improved with the addition of either TNF-α (0.8241; p = 0.5055) or Hs-CRP (0.8224; p = 0.2197).

3.2. *Literature Search*

The data from previous studies comparing maternal TNF-α and Hs-CRP levels in normal pregnancies and pregnancies that developed GDM are summarized in Supplementary Table 1.

4. Discussion

The study has shown that first, first-trimester maternal serum TNF-α levels are increased and maternal serum Hs-CRP levels

Table 3 – Median and interquartile range of serum biomarkers at 11–13 weeks' gestational age in pregnancies that developed gestational diabetes mellitus and controls.

Biomarker	Controls (n = 800)	Gestational diabetes mellitus			
		All cases (n = 200)	Control by diet (n = 33)	Metformin therapy (n = 51)	Insulin therapy (n = 116)
TNF-α MoM	1.000 (0.940, 1.064)	1.303 (1.151, 1.475) *	1.183 (0.872, 1.605)	1.315 (1.029, 1.681)	1.334 (1.133, 1.569)
TNF-α ng/L	1.371 (1.288, 1.46)	1.815 (1.601, 2.057)	1.656 (1.216, 2.254)	1.782 (1.391, 2.284)	1.879 (1.594, 2.215)
HsCRP MoM	1.000 (0.943, 1.06)	1.113 (0.990, 1.25)	0.975 (0.732, 1.299)	1.112 (0.883, 1.400)	1.156 (0.992, 1.347)
HsCRP mg/L	4.150 (3.884, 4.434)	5.826 (5.103, 6.651)	4.508 (3.254, 6.246)	5.37 (4.132, 6.981)	6.501 (5.464, 7.736)

TNF-α = tumor necrosis factor alpha; hsCRP = high sensitivity C-reactive protein.
* Critical significance level at 5%.

Table 4 – Performance of screening for gestational diabetes mellitus by maternal factors and serum biomarkers at 11–13 weeks' gestational age.

Screening test	AUROC	P value	Detection rate (%)		
			FPR 10%	FPR 20%	FPR 40%
Maternal factors	0.8200		52 (45–59)	71 (64–72)	81 (75–86)
Maternal factors plus					
Tumor necrosis factor-α (TNF-α)	0.8241	0.5055	53 (45–60)	70 (63–76)	83 (77–88)
High sensitivity C-reactive protein (Hs-CRP)	0.8224	0.2197	53 (46–60)	72 (65–78)	82 (75–87)
TNF-α and Hs-CRP	0.8257	0.3738	53 (45–60)	71 (64–77)	84 (78–89)

AUROC = area under receiver operating characteristic curve; FPR = false positive rate.

are not significantly altered in women that subsequently develop GDM and second, combination of these metabolites with maternal factors does not improve the prediction of GDM provided by maternal factors alone.

The strengths of the study are firstly, the large number of cases examined, secondly the use of multivariate regression analysis to determine the factors from maternal characteristics and gestation that provided significant contribution in the prediction of $\log_{10}$ TNF-α and Hs-CRP and the expression of these biomarkers as MoMs and thirdly, use of Bayes theorem to combine the a priori risk for GDM based on maternal factors with serum TNF-α and Hs-CRP MoM values. We examined 1000 singleton pregnancies within a narrow gestational age range at 11–13 weeks, asked specific questions to identify known factors associated with GDM and measured maternal weight and height.

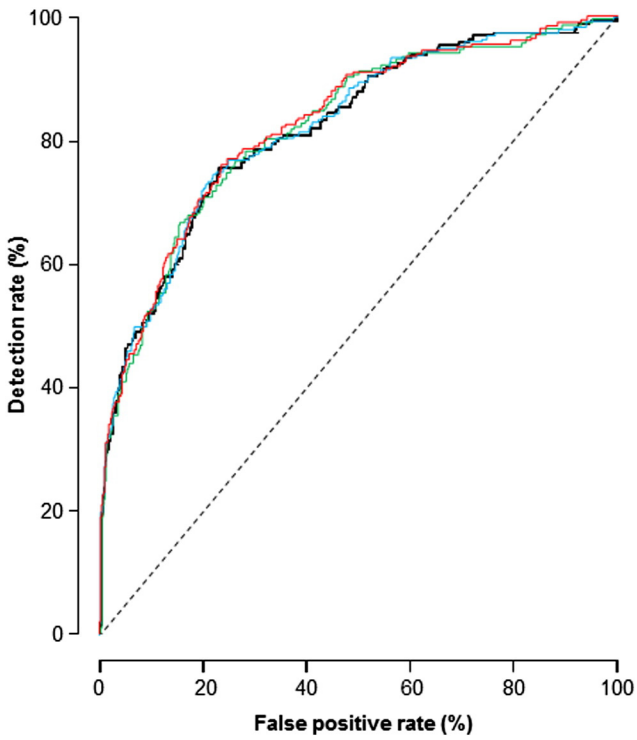

Fig. 1 – Receiver operating characteristic curves for the prediction of gestational diabetes by maternal factors (black), tumor necrosis factor-α (green), high sensitivity C-reactive protein (blue) and tumor necrosis factor-α and high sensitivity C-reactive protein (red).

A limitation of the study relates to the method of identifying the GDM affected pregnancies. The diagnostic OGTT was not carried out in all pregnancies, as recommended by the international association of diabetes and pregnancy study groups [54], but only in those with abnormal results of a random blood glucose level at 24–28 weeks' gestation. It is therefore possible that some of the women included in our non-GDM group actually had GDM and the performance of screening of our method was overestimated.

Three previous case–control studies examined maternal serum TNF-α levels at 11–14 weeks' gestation in 5–-40 GDM cases [11,20,42]. The larger of these studies, reported that TNF-α was increased by 54% in women who subsequently developed GDM [42], whereas the other two found no significant differences [11,20]. Similarly, three previous case–control studies reported that in GDM serum Hs-CRP was increased by 45%–67% [43–45], but in one there was no significant difference between GDM and unaffected pregnancies [55]. All of the previous studies included a small number of GDM cases and this is the most likely explanation for the contradictory results between them and with this study. In addition, none of these studies have examined the performance of screening for GDM by a combination of maternal factors and these biomarkers.

This study cannot fully support the hypothesis that in women who subsequently develop GDM there is evidence of inflammation form 11–13 weeks' gestation; only one of the two inflammatory markers examined was increased. Furthermore, although TNF-α was significantly increased in the cases that subsequently developed GDM, it did not add any value in improving the performance of screening for GDM achieved by screening with maternal characteristics alone.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.metabol.2015.10.029.

Authors' Contribution

AS and KN planned study design; AS, GV, AW and KN prepared, drafted and revised the manuscript; AS and AW performed and revised statistical analysis; AS, KK contributed in data collection.

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Disclosure Statement

The authors have no conflict of interest to disclose.

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