Endocrine Journal 2018, **65** (4), 499-508

NOTE

Increased secreted frizzled-related protein 4 and ficolin-3 levels in gestational diabetes mellitus women

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Abstract. By biochemical and epidemiological similarity with type 2 diabetes mellitus (T2DM), gestational diabetes mellitus (GDM) has some overlap between prediction markers and risk factors of T2DM. The present study aimed to establish that secreted frizzled-related protein 4 (SFRP4) and ficolin-3 levels, which have been linked to insulin resistance and the development of T2DM, are elevated in GDM women. A longitudinal prospective cohort study of 86 GDM and 273 normal glucose tolerant (NGT) pregnant women was performed. The clinical parameters, lipid profiles, and serum SFRP4 and ficolin-3 levels were tested during the early and late second-trimester and third-trimester of pregnancy. Both SFRP4 and ficolin-3 levels were significantly higher in GDM women as compared to the NGT participants at three test points ($p < 0.01$). Spearman's correlation analysis showed that serum SFRP4 levels were significantly positively correlated with ficolin-3 during the early and late second-trimester and third-trimester of pregnancy. The elevated SFRP4 and ficolin-3 concentrations at 16–18 weeks gestation significantly associated with GDM were conformed using binary logistic regression analysis after controlling for other variables [odds ratios (OR) with 95% confidence intervals (CI) for SFRP4: 2.84 (1.78–4.53), $p < 0.01$; for ficolin-3: 2.45 (1.55–3.88), $p < 0.01$]. In Conclusions, increased SFRP4 and ficolin-3 levels are significantly associated with GDM development and might be important risk factors for this pregnancy complication.

Key words: Gestational diabetes mellitus, Secreted frizzled-related protein 4, Ficolin-3

GDM, a state of varying degree of carbohydrate intolerance with onset or first recognition during pregnancy, may result in a rapid global increase in T2DM [1]. This epidemic is associated with a high risk of developing T2DM in both GDM mothers and their children. Due to biochemical and epidemiological similarity with T2DM, GDM shows cross-talk with predictive markers and risk factors of T2DM [2]. A correlation between cytokine levels (adipokines and inflammatory markers) and the risk of T2DM is also extended to GDM [3].

SFRP4 has recently been associated with insulin resistance and the development of T2DM [4]. SFRP4 is one of the Wnt signaling antagonists, which has been newly described to be involved in lipid and glucose metabolism through interaction with the Wnt ligands. The elevated SFRP4 is associated with obesity, type 1 diabetes mellitus (T1DM) and T2DM as demonstrated in previous studies [5-9]. However, the correlations between the changes of soluble SFRP4 and GDM are unclear. Ficolin-3 is a circulating pattern recognition molecule, which is known to activate the lectin complement pathway and remove the late apoptotic cells [10]. It was up-regulated significantly in the serum of T2DM patients as described previously using a proteomic approach [11]. The elevated levels of ficolin-3 were subsequently found in the serum and vitreous fluid of patients with hyperplastic diabetic retinopathy [12]. However, cross-sectional studies and another prospective study report that low concentrations of ficolin-3 are asso-

Submitted Dec. 1, 2017; Accepted Jan. 17, 2018 as EJ17-0508
Released online in J-STAGE as advance publication Feb. 27, 2018
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ciated with diabetic peripheral neuropathy and the incidence of T2DM [13, 14]. Furthermore, ficolin-3 levels significantly decrease both in pregnant women with GDM outcome and in pre-eclampsia (PE) patients compared with normal controls as investigated by quantitative proteomic analysis and enzyme-linked immunosorbent assay (ELISA), respectively [15, 16]. Our previous report showed higher levels of ficolin-3 are associated with risk of GDM [17]. However, the fluctuation of ficolin-3 during pregnancy is not investigated up to date.

Based on these observations and inconsistent ficolin-3 concentrations in T2DM patients, we performed a longitudinal analysis to evaluate the role of SFRP4 and ficolin-3 in GDM. We measured the SFRP4 and ficolin-3 levels during the early and late second-trimester and third-trimester of pregnancy using ELISA. Furthermore, the association with the two possible risk factors and GDM development was assessed using binary regression analysis.

Materials and Methods

Patient enrollment and samples collection

A cohort of 526 consecutively pregnant women who received a routine prenatal examination at 16–18 weeks gestation at the Changzhou Maternity and Child Health Hospital was recruited to our longitudinal prospective study from April 2013 to July 2014. A written informed consent was provided by each participant in our study, which was approved by the ethics committee of Changzhou Maternity and Child Health Hospital (No. ZD201203). The study participants were received routine screening for GDM by a 75 g oral glucose tolerance test (OGTT) at 24–28 weeks gestation. The International Association of Diabetic Pregnancy Study Group (IADPSG) criteria were established for the diagnosis of GDM [18]. GDM was diagnosed if one or more following criteria was fulfilled: (1) fasting glucose ≥5.1 mmol/L; (2) 1 h glucose ≥10.0 mmol/L; (3) 2 h glucose ≥8.5mmol/L. Of the 526 participants, 97 cases were diagnosed with GDM. A low-sugar and low-fat diet, without any antidiabetic drugs, was administered in the GDM subject to monitor the blood glucose for achieving euglycemia. The subject was excluded if any of the following criteria were fulfilled: 1) missed collection of all the programmed blood samples; 2) maternal age >40 or <18 years; 3) multiple pregnancy; 4) gestational weeks <37 weeks; 5) alcohol and/or illicit drug use; 6) other illnesses including pregestational diabetes mellitus (type 1 or 2), PE, chronic hypertension, thyroid disease, active or chronic liver diseases, and renal failure. None of the participants smoked during gestation. 2 GDM and 14 NGT subjects either rejected the Down's syndrome screen test at 16–18 weeks gestation in our hospital or received the test elsewhere and thus, were excluded for missing the first collection of blood samples. Also, 3 GDM and 113 controls were excluded for whom the outcome of pregnancy was unknown as they delivered elsewhere and did not collect blood samples. Furthermore, cases of pregestational diabetes mellitus (PGDM), multiple pregnancy, premature delivery, and PE were also excluded. Finally, 273 NGT and 86 GDM individuals with singleton pregnancy were included in the study (Fig. 1). The PGDM diagnosis was based on self-report and confirmed with medical records. The outcome characteristics of eligible participants were longitudinally studied at 16–18 weeks gestation (examination 1), 24–28 weeks gestation (examination 2), and before delivery (examination 3). The serum samples taken from participants were collected and stored in several aliquots at –80°C for subsequent biochemical analysis.

Laboratory measurements

Samples were randomly organized and analyzed by personnel who were ignorant of the purpose of the present study. Serum lipid levels, plasma glucose and C-reactive protein (CRP) were analyzed using enzymatic procedures on an automated bioanalyzer (Hitachi 7180, Japan). HbA1c concentrations were determined by a high performance liquid chromatography method (BioRad Variant II Turbo HbA1c, USA). SFRP4 and ficolin-3 were investigated in duplicate by an automatic enzyme immunoassay analyzer (Hamilton Medical, Switzerland) using ELISA kits (for SFRP4: SU-B14218, for ficolin-3: SU-B11493, Shanghai Bio-Sh Biotechnology Corporation, China). All of these inter-assay and intra-assay variation for SFRP4 and ficolin-3 were <6%.

Statistical analysis

The data were collected and analyzed by SPSS 19.0 statistical software (SPSS, USA). The results were presented as mean ± SD or median (interquartile range). The continuous variables with normal distribution between individuals with and without GDM were compared by Student's *t*-test. The Mann-Whitney U-test was used to determine the differences of continuous variables with skewed distribution between the two groups. The corre-

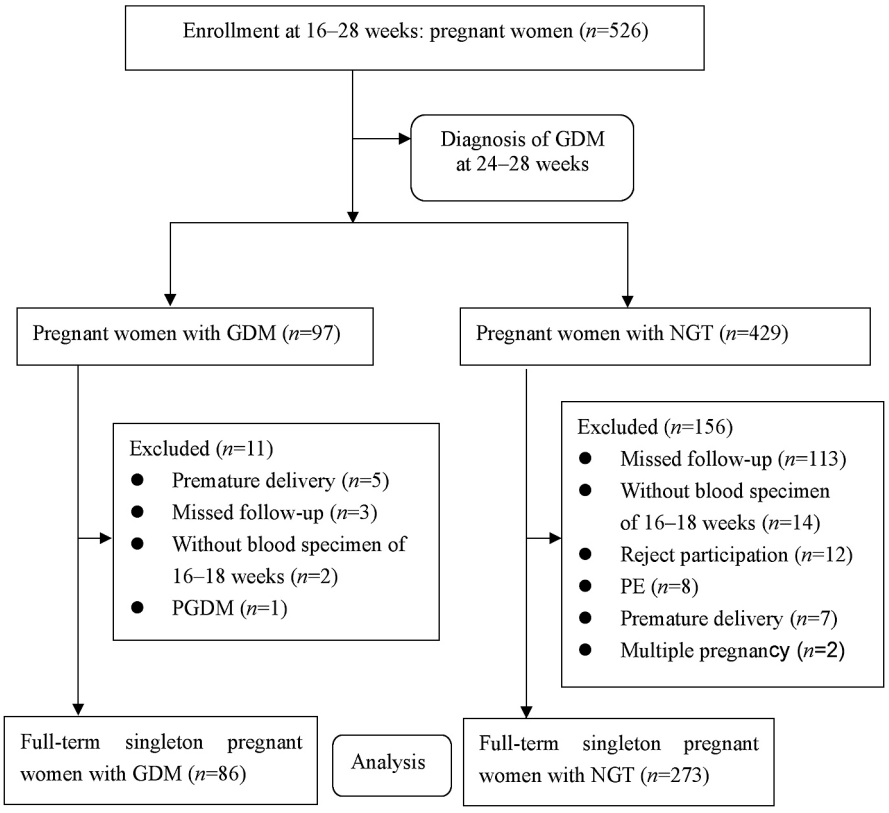

Fig. 1 Flow-chart of patient recruitment and sample collection schedule. GDM, gestational diabetes mellitus; NGT, normal glucose tolerant; PGDM, pre-gestational diabetes mellitus. PE, pre-eclampsia.

lations between SRFP-4 or ficolin-3, and other variables were assessed with Spearman's correlation analysis. ORs with 95% CI were calculated by binary logistic regression analysis. Statistical significance was accepted at $p < 0.05$.

Results

Maternal and pregnancy characteristics

A comparison of maternal and neonatal characteristics between GDM women and NGT individuals was described in Table 1. In the GDM group, median maternal age, BMI, glucose, and HbA1c levels were significantly higher as compared to the NGT group. However, there was no significant difference in the fetal birth weight and length between the two groups.

Differences in SFRP4 and ficolin-3

Table 2 showed that both SFRP4 and ficolin-3 serum levels in the GDM group were significantly higher than in the NGT group at the corresponding checkpoint (for SFRP4: 12.84 *vs.* 10.17 ng/mL, $p < 0.01$; 14.51 *vs.*12.83 ng/mL, $p < 0.01$; 8.80 *vs.* 5.72 ng/mL, $p < 0.01$; for ficolin-3: 10.64 *vs.* 8.24 μg/mL, $p < 0.01$; 14.79 *vs.* 12.81 μg/mL, $p < 0.01$; 9.30 *vs.* 7.60 μg/mL, $p < 0.01$; respectively). Both in the GDM and control groups, serum SFRP4 and ficolin-3 levels increased remarkably from examination 1 to 2 and decreased before delivery as compared to examination 2 (all $p < 0.01$). Serum SFRP4 and ficolin-3 levels at examination 3 were lower than those at examination 1 in women with or without GDM, however, SFRP4 was statistically different (all $p < 0.01$) while ficolin-3 was not ($p = 0.252$ in GDM, $p = 0.798$ in NGT, Table 2).

Correlations analysis and GDM outcome

In order to identify the impact factors of SFRP4 and ficolin-3, Spearman's correlation analysis between SFRP4 or ficolin-3 and other clinical parameters derived from three examinations were carried out. Tables 3, 4 showed serum SFRP4 levels had significantly positive correlation with ficolin-3, maternal age and fasting

Table 1 Clinical characteristics of pregnant women with or without GDM

	GDM	NGT	*p* value
Subjects (*n*)	86	273	
Maternal age (years)	29 ± 4	26 ± 3	<0.001
Gravidity	1.63 ± 0.90	1.52 ± 0.84	0.426
Parity	0	0	
Body mass index (kg/m^2)			
Pre-pregnancy	22.00 ± 3.43	20.98 ± 2.80	0.007
16–18 week	24.80 ± 3.37	22.70 ± 2.79	<0.001
24–28 week	25.84 ± 3.51	23.78 ± 2.92	<0.001
37–41 week	28.02 ± 3.80	25.88 ± 3.18	<0.001
Weight gain (kg)*	9.0 (6.9–12.0)	9.0 (6.0–12.0)	0.435
Fasting glucose (mmol/L)			
16–18 week	4.71 ± 0.74	4.16 ± 0.26	<0.001
24–28 week	5.12 ± 0.81	4.33 ± 0.27	<0.001
37–41 week	5.03 ± 0.79	4.75 ± 0.29	0.002
24–28 week OGTT 1-h glucose (mmol/L)	10.89 ± 1.34	6.92 ± 1.31	<0.001
24–28 week OGTT 2-h glucose (mmol/L)	9.54 ± 1.52	6.02 ± 0.99	<0.001
24–28 week HbA1c (%)	5.24 ± 0.44	4.81 ± 0.37	<0.001
Triglyceride (mmol/L)			
16–18 week	2.05 (1.78–2.81)	1.85 (1.51–2.13)	<0.001
24–28 week	2.85 (2.29–3.78)	2.29 (1.83–2.77)	<0.001
37–41 week	4.09 (3.25–4.65)	3.48 (2.94–4.63)	0.021
LDL-C (mmol/L)			
16–18 week	2.48 ± 0.60	2.50 ± 0.57	0.867
24–28 week	2.60 ± 0.66	2.95 ± 0.65	0.001
37–41 week	2.49 ± 0.80	3.01 ± 0.69	<0.001
HDL-C (mmol/L)			
16–18 week	1.57 ± 0.39	1.58 ± 0.27	0.772
24–28 week	1.56 ± 0.36	1.64 ± 0.28	0.11
37–41 week	1.44 ± 0.26	1.48 ± 0.26	0.325
Total cholesterol (mmol/L)			
16–18 week	5.06 ± 0.78	4.98 ± 0.74	0.487
24–28 week	5.37 ± 0.88	5.56 ± 0.89	0.172
37–41 week	5.68 ± 0.99	5.88 ± 0.95	0.175
CRP (μg/mL)			
16–18 week	3.60 (1.80–6.45)	1.90 (1.10–4.40)	0.004
24–28 week	3.84 (1.91–6.41)	2.27 (1.08–4.52)	0.002
37–41 week	1.53 (0.73–3.55)	1.28 (0.70–3.21)	0.650
Gestation at delivery (weeks)	38.00 (38.00–39.00)	39.00 (39.00–40.00)	<0.001
Fetal birth weight (kilogram)	3.41 ± 0.57	3.49 ± 0.40	0.282
Fetal birth length (centimeter)	49.77 ± 1.31	49.99 ± 0.51	0.147

Data was presented as mean ± SD or median (IQR). SD, standard deviation; IQR, interquartile range; GDM, gestational diabetes mellitus; OGTT, oral glucose tolerance test; HDL-C, high density lipoprotein-cholesterol; LDL-C, low density lipoprotein-cholesterol; CRP, C-reactive protein. * Weight gain in pregnancy from pre-pregnancy to OGTT.

Table 2 SFRP4 and ficolin-3 levels in GDM women and controls

	GDM	NGT
SFRP4 (ng/mL)		
16–18 week	12.84 (8.97–14.85)[a,b,c]	10.17 (7.22–11.68)[b,c]
24–28 week	14.51 (11.92–15.42)[a,c]	12.83 (9.33–14.48)[c]
37–41 week	8.80 (7.49–10.28)[a]	5.72 (3.90–6.83)
Ficolin-3 (μg/mL)		
16–18 week	10.64 (7.50–11.25)[a,b,d]	8.24 (6.34–9.57)[b,e]
24–28 week	14.79 (13.33–15.62)[a,c]	12.81 (10.48–13.81)[c]
37–41 week	9.30 (7.83–9.70)[a]	7.60 (4.94–9.17)

Data was presented as median (IQR). IQR, interquartile range. [a]$p < 0.001$ compared with control group at same weeks of gestation, [b]$p < 0.001$ compared with 24–28 weeks of gestation, [c]$p < 0.001$ compared with 37–41 weeks of gestation, [d]$p = 0.252$ compared with 37–41 weeks of gestation, [e]$p = 0.798$ compared with 37–41 weeks of gestation.

glucose from the early second-trimester to the third-trimester ($p < 0.01$ or 0.05). Furthermore, SFRP4 levels were significantly positively correlated with triglyceride in the second-trimester, and were negatively correlated with low density lipoprotein cholesterol (LDL-C) in the late second-trimester, as well as in the third-trimester ($p < 0.01$ or 0.05). Both SFRP4 and ficolin-3 levels were positively correlated with CRP levels at three examinations ($p < 0.01$ or 0.05).

Using binary logistic regression analysis, both SFRP4 and ficolin-3 levels at three test points were showed as major risk factors of GDM (OR for SFRP4: 2.84, 1.76, 6.71, $p < 0.01$ or 0.05; for ficolin-3: 2.45, 2.76, 2.58, $p < 0.01$; Table 5).

Discussion

Up to date, this is the earlier longitudinal prospective study with respect to SFRP4 and ficolin-3 in GDM women. Herein, we found that the two kinds of protein were significantly higher in the GDM subjects than in the NGT individuals in the second and third stages of pregnancy. Also, SFRP4 and ficolin-3 were found to be high in the GDM group at 16–18 weeks gestation.

Hitherto, there have been fewer published reports on maternal serum SFRP4 and GDM. According to our results, pregnant women with high circulating levels of SFRP4 have an increased risk of GDM. Our findings are consistent with a previous report correlating serum SFRP4 and risk of T2DM [4]. In the current study, we found that GDM women showed significantly high levels of serum SFRP4 compared with NGT individuals, thereby contributing to extending the literature on SFRP4 to GDM. However, it is yet unclear how SFRP4 plays a vital role in the pathogenesis of GDM. Several studies show different physiological and pathological properties of SFRP4. SFRP4 functions as an inhibitor of angiogenesis and tumor growth and participates in the promotion of epidermal differentiation, development of polycystic kidney disease, and PE [19-22]. Recently, the role of SFRP4 in obesity and T2DM has been elucidated [23-25]; SFRP4 promotes adipogenic differentiation in human adipose tissue-derived mesenchymal stem cells and addresses the mechanisms of obesity [26, 27]. Furthermore, SFRP4 acts as an early regulator of pancreatic β-cell dysfunction by declining islet expression of Ca^{2+} channels and inhibiting insulin exocytosis, thereby, correlating with insulin resistance and T2DM. Moreover, the elevated SFRP4 levels are found in the subjects who suffered from T2DM several years later, reflecting the possibility of SFRP4 as a predictive marker [4]. The expression of SFRP4 is negatively regulated by platelet-derived miR-103b in T2DM that impairs Wnt signaling and provides a novel mechanism in diabetic osteopenia by oxidative stress [28, 29].

Ficolin-3 is produced mainly in the liver and lungs and then released into the blood, bile ducts, bronchi, and alveoli, participating in both local and systemic inherent immune responses. The complement system represents a critical branch of inflammation and innate immunity and is constituted of the classical pathway, alternative pathway, and the lectin pathway. Complement activation,

Table 3 Significant correlations between SFRP4 levels and other variables in pregnant women in the second and third trimesters

	r	*p* value
Early second-trimester		
Age	0.115	0.03
Fasting glucose	0.201	0.008
Triglyceride	0.223	0.003
CRP	0.295	<0.001
Ficolin-3	0.542	<0.001
Late second-trimester		
Age	0.154	0.043
Fasting glucose	0.232	0.002
OGTT 1-h glucose	0.28	<0.001
OGTT 2-h glucose	0.25	0.001
Triglyceride	0.16	0.035
LDL-C	–0.212	0.005
CRP	0.346	<0.001
Ficolin-3	0.573	<0.001
Third-trimester		
Age	0.241	0.001
Fasting glucose	0.21	0.005
CRP	0.202	0.008
LDL-C	–0.234	0.002
Ficolin-3	0.599	<0.001

CRP, C-reactive protein; OGTT, oral glucose tolerance test; LDL-C, low density lipoprotein-cholesterol.

Table 4 Significant correlations between ficolin-3 levels and other variables in pregnant women in the second and third trimesters

	r	*p* value
Early second-trimester		
Age	0.141	0.007
Fasting glucose	0.191	0.012
Triglyceride	0.187	0.014
CRP	0.196	0.009
SFRP4	0.542	<0.001
Late second-trimester		
Age	0.226	0.003
Fasting glucose	0.28	<0.001
OGTT 1-h glucose	0.383	<0.001
OGTT 2-h glucose	0.327	<0.001
Triglyceride	0.165	0.03
LDL-C	–0.228	0.002
CRP	0.333	<0.001
SFRP4	0.573	<0.001
Third-trimester		
Age	0.109	0.039
Fasting glucose	0.127	0.016
LDL-C	–0.148	0.005
CRP	0.151	0.047
SFRP4	0.599	<0.001

CRP, C-reactive protein; OGTT, oral glucose tolerance test; LDL-C, low density lipoprotein-cholesterol.

especially by the mannose-binding lectin pathway, has been shown to play a crucial role in the pathogenesis of several diseases including T2DM [30, 31]. The present results further suggest that the over-represented serum ficolin-3 in pregnant women was associated with GDM. Thus, we propose that ficolin-3 stimulates the lectin-complement pathway by the activation of the innate immune system, thereby establishing chronic low-grade inflammation (increased serum inflammatory factors without overt symptoms of inflammation). Ficolin-3 may also play a major role in the development of GDM. Moreover, ficolins are probably responsible for the clinical manifestations of PE and may also have therapeutic potential to alleviate the adverse clinical outcomes and predictive value of this gestational complication [32].

Interestingly, SFRP4 and ficolin-3 initially increased during the second-trimester of pregnancy and then declined before delivery of all the subjects in the present study. However, the underlying mechanism of these changes is unclear. The Wnt signaling pathway plays a role in human placental development and implantation, especially in the trophoblast. A previous study demonstrated that SFRP4 was expressed in the rat uterus with pregnancy, but not in the unpregnant uterus; the expression of SFRP4 mRNA peaked in the uterus on 12th day of pregnancy and then decreased. As previously demonstrated in another study, in which sFRP4 mRNA was generated throughout gestational weeks in the pregnant macaque placenta; the mRNA expression of SFRP4 reached a peak on day 50 of gestation (the duration of

Table 5 Influence of variables on GDM using binary logistic regression analysis

Variables	Univariate			Multivariate		
	OR	95% CI	*p* value	OR	95% CI	*p* value
Early second-trimester						
Maternal age (years)	2.3	1.57–3.38	<0.001	2.14	1.38–3.32	0.001
Body mass index (kg/m^2)	1.93	1.31–2.82	0.001	2.31	1.44–3.70	0.001
Triglyceride (mmol/L)	2.63	1.58–4.40	<0.001	2.17	1.15–4.08	0.017
CRP (μg/mL)	1.72	1.18–2.51	0.005	0.97	0.86–1.10	0.658
SFRP4 (ng/mL)	2.87	1.89–4.35	<0.001	2.84	1.78–4.53	<0.001
Ficolin-3 (μg/mL)	2.34	1.58–3.48	<0.001	2.45	1.55–3.88	<0.001
Late second-trimester						
Maternal age (years)	2.3	1.57–3.38	<0.001	1.95	1.23–3.02	0.003
Body mass index (kg/m^2)	1.9	1.30–2.79	0.001	1.92	1.20–3.08	0.006
Triglyceride (mmol/L)	1.95	1.39–2.73	<0.001	1.69	1.16–2.44	0.006
CRP (μg/mL)	1.14	1.03–1.26	0.011	1.08	0.98–1.19	0.133
SFRP4 (ng/mL)	1.9	1.30–2.79	0.001	1.76	1.14–2.73	0.011
Ficolin-3 (μg/mL)	2.71	1.80–4.09	<0.001	2.76	1.71–4.45	<0.001
Third-trimester						
Maternal age (years)	2.3	1.57–3.38	<0.001	2.29	1.48–3.55	<0.001
Body mass index (kg/m^2)	1.85	1.26–2.72	0.002	1.94	1.23–3.05	0.004
Triglyceride (mmol/L)	1.42	1.12–1.80	0.004	1.49	1.12–1.98	0.006
CRP (μg/mL)	1.04	0.94–1.14	0.442	1.03	0.92–1.15	0.579
SFRP4 (ng/mL)	6.25	3.69–10.6	<0.001	6.71	3.58–12.10	<0.001
Ficolin-3 (μg/mL)	2.39	1.60–3.56	<0.001	2.58	1.61–4.12	<0.001

gestation in the macaque was 165 days). The above-mentioned studies suggest that the SFRP4 mRNA decreased in the rat uterus and macaque placenta during the mid-late pregnancy [33, 34]. The changes in the mRNA levels are earlier compared to changes in the protein expression. Therefore, we speculated that the concentrations of SFRP4 in the circulation of pregnant women was first increased and then decreased along with the increasing of gestational weeks. It was confirmed by our results. A longitudinal study indicated that activity of the lectin pathway and circulating lectin mannose-binding (MBL) levels were increased in normal pregnancy [35]. It might be reasonable to speculate that ficolin-3, another activator of the lectin complement pathway, is also probably influenced by gestation. The activation of the lectin pathway reaching a possible peak in the third-trimester of pregnancy might form a large amount of complex of ficolin-3 and MBL associated serine protease 2 (MASP-2) resulting in decreased free ficolin-3 in the circulation [36]. Furthermore, the mechanism of positive correlation between SFRP4 and ficolin-3 in this study is unknown. The previous study reported that SFRP4 concentrations were positively correlated with the levels of high sensitivity CRP both in NGT group and in T2DM group [8]. CRP had also been found to trigger complement system through the classical pathway both in PE and in normal pregnancy [37]. Both SFRP4 and ficolin-3 were positively correlated with CRP in our study, which provided evidence the levels of these proteins in pregnant women were correlated closely with chronic low-grade inflammation. Also, positive correlation between ficolin-3 and CRP in the study suggested that ficolin-3 mediated lectin pathway and CRP mediated classical pathway showed a synergistic action by the activation of the complement system in pregnancy. Additionally, we observed that correlation of

CRP with ficolin-3 and the contribution of CRP to GDM in the early and the late second-trimester seemed stronger than in the third-trimester, while SFRP4 and ficolin-3 were still correlated closely. This may implicate that the observed correlation may have multiple underlying mechanisms in the different pregnant stages.

As discussed in our previous paper [17], this study was not in according with the previous report that pregnant women who developed GDM showed down-expressed ficolin-3 as tested by liquid chromatography mass spectrometry (LC-MS) combined with multiplexed isobaric tandem mass tag (TMT) labeling and Western blot analysis [15]. The discrepancies were also presented in T2DM [11-14]. The main differences in these studies were the utilization of different test methods (*e.g.*, ELISA, LC-MS, Western blot analysis), different types of specimens (serum, plasma) and various sample sizes. Furthermore, using various ELISA kits with different reporting units (ng/mL or μg/mL) without comparison may result in variability [12, 14]. Finally, the conjunction and releasing of complex of ficolin-3 and MBL associated serine protease 2 (MASP-2) may change some epitopes of ficolin-3 and contribute to discrepancy of conjunction between ficolin-3 and its antibody used in different tests. Standardization of the tests and reports on ficolin-3 and other biomarkers are very important to the further investigations into GDM.

However, several limitations of the present longitudinal study should also be taken into consideration. Herein, we excluded a certain number of pregnant women, those without consecutive collection of blood samples, which can result in statistical bias. We may include some subjects in this study who might have glucose tolerance or undiagnosed diabetes in early pregnancy. Also, we cannot exclude the possibility of confounding by unmeasured variables. Our study lacks information on pre- and early pregnancy about SFRP4 and ficolin-3 levels and thus cannot identify whether some differences were observed during the first-trimester of gestation or before pregnancy. Therefore, an improved prospective study design with larger populations will be necessary to further study the development of SFRP4 and ficolin-3 levels during a longer observation period in pregnant women and to identify their predictive value in GDM.

In conclusion, circulating concentrations of SFRP4 and ficolin-3 were significantly higher in pregnant women with GDM than in the control individuals during mid- and late pregnancy. Even at 16–18 weeks gestation, the increased levels of SFRP4 and ficolin-3 that might contribute to the development of GDM were observed.

Acknowledgments

This study was funded by Changzhou municipal bureau of science and technology (NO: CJ20130041, CJ20159055, CE20175021). The funders did not play a role in study design, samples collection and analysis, date statistics, or manuscript preparation.

Disclosure

The authors declare that there is no conflict of interests regarding the publication of this paper.

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