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Do free thyroxine levels influence the relationship between maternal serum ferritin and gestational diabetes mellitus in early pregnancy?

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ABSTRACT

Purpose: The objective of this study was to estimate the combined effect of serum ferritin (SF) concentration and free thyroxine (fT4) levels on the risk of gestational diabetes mellitus (GDM).

Methods: Women presented for antenatal care at a tertiary hospital in Shanghai, China were included in this study from December 2012 to March 2014. Women were divided into six groups according to the SF and fT4 level. Multiple logistical regression model was used to estimate odds ratio (OR) among different groups. Relative excess risk of interaction (RERI), the attributable proportion (AP) of the interaction and the synergy index (SI) were applied to evaluate the additive interaction of SF concentration and fT4 level.

Results: A total of 6542 qualifying pregnant women were included in this study. We observed that a high SF concentration in early pregnancy was related to an increased risk of GDM (OR = 1.21, 95%CI: 1.02–1.43); while a low fT4 level was not (OR = 1.18, 95%CI: 0.89–1.58). There is no addictive interaction between SF and fT4 level on the presence of GDM.

Conclusions: The study suggests that only high serum ferritin concentration is associated with an increased risk of GDM in early pregnancy. The level of fT4 in early pregnancy might have no effect on the association between high SF and risk of GDM.

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1. Introduction

Gestational diabetes mellitus (GDM) is attributed to glucose intolerance identified for the first-time during pregnancy. It is one of the most common pregnancy complications, with approximately 1–14% of pregnancies affected every year worldwide [1]. GDM is associated with an increased risk of series of adverse perinatal outcomes, such as macrosomia, preterm birth, and it has a long-term adverse effect on the health of mothers and their offspring [2]. It also has been

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suggested that GDM may impact on epigenetic modifications both in the mother and offspring [3], while the etiology of GDM is still unclear.

Although iron is an essential element in normal human physiology, gross iron overload or excessive iron store is toxic and associated with many pathophysiological conditions, including diabetes mellitus [4–8]. Serum ferritin (SF) as an iron storage protein is widely used for determining body iron stores. A meta-analysis has suggested that the risk of GDM development in pregnant women was positively associated with increased SF concentration [9]. The potential mechanism is that elevated fasting SF might induce decreased insulin sensitivity and impair beta-cell function [10], which is also implied mechanism for diabetes mellitus.

Thyroid hormone was also involved with the process of the pancreas to secrete insulin [11]. Our previous study suggested that thyroid dysfunction in early pregnancy is related to an increased risk of GDM [12]. An experimental study has suggested that thyroid status changes always accompany SF level changes, indicating that SF could be a thyroid function marker [13]. Animal experimental studies have indicated that the liver ferritin synthesis speed increased by 38%, as thyroid hormones thyroxine 3(T3) and fT4 increase in hyperthyroid rats compared with healthy rats [14]. Iron metabolism in Graves' hyperthyroidism patients undergoes dynamic changes that are similar to an acute-phase reaction, and thyroid hormones have a direct effect on the expression of hepcidin in hepatocytes, which is a key regulator of human iron metabolism [15]. When hypothyroidism occurs, glucose uptake in muscle and fat tissue is resistant to insulin [16]. There is little evidence about the role of fT4 in the association of SF concentration and risk of GDM in human beings. We hypothesized that free thyroxine levels might influence the relationship between maternal serum ferritin and GDM in early pregnancy.

2. Subjects and methods

2.1. Study population

A total of 9,091 women who were presented for antenatal care at the hospital from December 2012 to March 2014 were invited to participate in the Thyroid-GDM study [12]. The study was performed at a tertiary hospital in Shanghai, China (Register No.: NCT02555332). Demographic information of the population was collected by questionnaire, including age at delivery, last menstrual period, maternal smoking status, pre-pregnancy weight and history of chronic diseases. Biological monitoring index was extracted from hospital information system and laboratory information management system, including TSH, fT4, TPOAb and SF concentrations. The following exclusion criteria were applied: an estimated gestational age older than 20 weeks (n = 450), an abnormal TSH level (n = 716), thyroid peroxidase antibody (TPOAb) positive (n = 797), other medical histories (n = 29), a lack of medical history (n = 180), a history of thyroid diseases (including hyperthyroidism, hypothyroidism, and thyroid cancer) before pregnancy (n = 78), smokers (n = 15), as well as those lost to follow-up (n = 284). A total of 6,542 women were enrolled in the final analyses.

2.2. Exposure

Venous blood samples were gathered from the women at the first time of antenatal care for TSH, fT4, and TPOAb measurements, which were depicted in detail in our published paper [12]. All women's venous blood sample was centrifuged to obtain serum. A maternal blood sample was collected at the first antenatal visit and was centrifuged (10 min with rethawing cycles at 3,000 rpm) to obtain serum. TSH, fT4 and TPOAb measurements were carried out using ADVIA Centaur instruments and kits (Siemens, Munich, Germany). SF measurements were carried out using UniCel DxI 800 immunology analyzer and kits (Beckman coulter, California, America). According to the recommendations of American Thyroid Association guidelines [17], we set up reference intervals for fT4 and TSH, according to trimester, at our institution. The reference interval of fT4 was 13.00–20.21 pmol/L during the first trimester and 12.23–19.69 pmol/L for the second trimester. Isolated hypothyroxinemia was defined as fT4 levels below the 5th percentile, with normal TSH levels and TPOAb negative (<60 U/ml). SF concentration data were examined continuously and as quartiles. There is no standard cut-off value for high SF levels at present, therefore, we used 25th percentile and 75th percentile as a cut-off value according to the previous study [18]. The 25th and 75th percentile value of SF concentration were 18.6 ng/ml and 63.3 ng/ml respectively. The SF concentration was categorized as low concentration (<18.6 ng/ml), normal concentration (18.6–63.3 ng/ml) and high concentration (≥63.3 ng/ml).

All women were divided into six groups, according to the SF concentration and fT4 level: (1) women with low SF concentrations and low fT4 levels [SF(L)fT4(L), N = 91(1.39%)]; (2) women with high SF concentrations and low fT4 levels [SF(H)fT4(L), N = 65(0.99%)]; (3) women with normal SF concentrations and low fT4 levels [SF(N)fT4(L), N = 204(3.12%)], (4) women with low SF concentrations and normal fT4 levels [SF(L)fT4(N), N = 1465(22.39%)], (5) women with high SF concentrations and normal fT4 levels [SF(H)fT4(N), N = 1581 (24.17%)]; and (6) women with normal SF concentrations and fT4 levels [SF(N)fT4(N), N = 3136(47.94%)].

2.3. Diagnosis of GDM

According to the International Association of Diabetes and Pregnancy Study Groups (IADPSG) criteria, GDM is diagnosed by 75 g oral glucose tolerance test (OGTT) [19]. Blood glucose was measured using the glucose oxidase (Roche Diagnostics, Indianapolis, IN), and glycosylated hemoglobin levels were assessed by high-performance liquid chromatograph (Bio-Rad, Hercules, CA, USA). Any one of the parameters was abnormal, including fasting blood glucose level ≥5.1 mmol/L, 1 h blood glucose level ≥10.0 mmol/L, or 2 h blood glucose level ≥8.5 mmol/L, the women was diagnosed as GDM.

2.4. Statistical analysis

The statistical results of demographic characteristic were presented as frequency and percentage. Chi-squared test (or Fisher exact test where appropriate) was used to compare

them among the six groups. The logistic regression model was used to estimate the risk of GDM among different groups. Women with normal SF and fT4 were set as reference group. Maternal age, pre-pregnancy BMI, gravidity and gestational age of the collected blood were adjusted as potential confounders in multiple logistic regression models. The category for these confounders was presented in Table 1. Meanwhile, in order to evaluate interactive effect of SF and fT4 levels on the presence of GDM, we also calculated the relative excess risk due to interaction (RERI), the attributable proportion due to interaction (AP), and the synergy index (SI) using an Excel spreadsheet by Andersson et al. [20]. SAS version 9.2 statistical software packages (SAS Institute, Inc., Cary, NC, USA) was used for data analyses. P value < 0.05 was considered to be statistically significant.

3. Results

The general characteristics of the study population based on fT4 and SF levels are presented in Table 1. There were no significant differences in pre-gestational BMI, gravidity and mode of pregnancy among the six groups. We observed that women with low fT4, regardless of SF concentration, is more likely with advanced age (≥35 years). More than 90% women were primipara, particularly in the [SF(H)fT4(L)] and [SF(H)fT4(N)] groups.

Logistical regression model showed that high SF concentration is associated with an increased risk of GDM (adjusted odds ratio (aOR) = 1.21, 95% confidence interval (95%CI):1.02–1.43) (Table 2). There is no association between low fT4 level and risk of GDM (aOR = 1.18, 95%CI: 0.89–1.58) (Table 3). When dividing the subjects into six groups according to the SF concentration and fT4 levels, we observed an increased risk of GDM amongst women with higher SF concentrations and normal fT4 levels [SF(H)fT4(N)] compared with women with normal SF concentrations and fT4 levels [SF(N)fT4(N)] (aOR = 1.25, 95%CI: 1.05–1.49), but not among women with higher SF concentrations and low fT4 levels (aOR = 0.97, 95%CI: 0.49–1.95) (Table 4).

Table 5 shown the estimates for evaluating the additive effect of SF and low fT4 on risk of GDM. RERI, AP and S values were −0.49 (95%CI: −1.39 to 0.46), −0.45(95%CI: −1.53 to 0.64) and 0.16 (95%CI: 0.00–460.40), respectively, for the interaction between high SF concentration and low fT4 level, and −0.05 (95%CI: −0.87 to 0.77), −0.04 (95%CI: −0.73 to 0.65) and 0.81 (95%CI: 0.02–28.67) for the interaction between low SF concentration and low fT4 level. Both RERI and AP include 0 or SI includes 1, which indicated there is no biological interaction

Table 2 – Risk of GDM in three groups, according to SF levels during early pregnancy.

	N	%	OR(95%CI)	aOR(95%CI)[a]
SF(Low)	228	23.24	1.03 (0.87–1.22)	1.01 (0.85–1.21)
SF(Normal)	479	48.83	1.00 (ref)	1.00 (ref)
SF(High)	274	27.93	**1.19 (1.02–1.40)***	**1.21 (1.02–1.43)***

OR: odds ratio; aOR: adjusted odds ratio; SF: serum ferritin; GDM: gestational diabetes mellitus; CI: confidence interval.
[a] Adjusted for maternal age, maternal pre-gestational BMI, gravity, and the diagnosis of gestational age.
* $P < 0.05$.

Table 1 – The demographic characteristics were analyzed in early pregnancy based on fT4 and SF levels.

	SF(L)fT4(L) N(%)	SF(H)fT4(L) N(%)	SF(N)fT4(L) N(%)	SF(L)fT4(N) N(%)	SF(H)fT4(N) N(%)	SF(N)fT4(N) N(%)	P
Age							0.001**
≤24	6(6.6)	2(3.1)	7(3.4)	66(4.5)	66(4.2)	135(4.3)	
25–29	37(40.7)	19(29.2)	77(37.8)	649(44.3)	749(47.4)	1398(44.6)	
30–34	34(37.4)	28(43.1)	89(43.6)	610(41.6)	624(39.5)	1306(41.7)	
≥35	14(15.4)	16(24.6)	31(15.2)	140(9.6)	142(9.0)	297(9.5)	
BMI							0.3132
≤18.4	3(3.3)	3(4.6)	16(7.8)	165(11.3)	171(10.8)	298(9.5)	
18.5–23.9	76(83.5)	55(84.6)	158(77.5)	1110(75.8)	1214(76.8)	2416(77.0)	
24–27.9	12(13.2)	6(9.2)	28(13.7)	165(11.3)	175(11.1)	377(12.0)	
≥28	0(0)	1(1.5)	2(1.0)	25(1.7)	21(1.3)	45(1.4)	
Gravidity							0.1302
1	43(47.3)	37(56.9)	107(52.5)	808(55.2)	946(59.8)	1831(58.4)	
2	28(30.8)	19(29.2)	57(27.9)	406(27.7)	393(24.9)	797(25.4)	
≥3	20(22.0)	9(13.9)	40(19.6)	251(17.1)	242(15.3)	508(16.2)	
Parity							<0.001**
0	72(79.1)	60(92.3)	175(85.8)	1202(82.1)	1447(91.5)	2697(86)	
1	17(18.7)	4(6.2)	25(12.3)	252(17.2)	131(8.3)	427(13.6)	
≥2	2(2.2)	1(1.5)	4(2.0)	11(0.8)	3(0.2)	12(0.4)	
IVF-ET							0.2557
Yes	3(3.3)	3(4.6)	14(6.9)	48(3.3)	63(4.0)	121(3.9)	
No	88(96.7)	62(95.4)	190(93.1)	1417(96.7)	1518(96.0)	3015(96.1)	

SF: serum ferritin; fT4: free thyroxine; IVF-ET: in Vitro Fertilization Embryo Transfer; BMI: Body Mass Index.
** $P < 0.01$.

Table 3 – Risk of GDM in two groups, according to fT4 levels during early pregnancy.

	N	%	OR(95%CI)	aOR(95%CI)[a]
fT4 (Low)	64	6.52	1.24(0.94–1.64)	1.18(0.89–1.58)
fT4 (Normal)	917	93.48	1.00(ref)	1.00(ref)

OR: odds ratio; aOR: adjusted odds ratio; fT4: free thyroxine; GDM: gestational diabetes mellitus; CI: confidence interval.
[a] Adjusted for maternal age, maternal pre-gestational BMI, gravity, and the diagnosis of gestational age.

Table 4 – Risk of GDM in six groups, according to SF and fT4 levels during early pregnancy.

	N	%	OR (95%CI)	aOR (95%CI) [a]
SF(L)fT4(L)	16	17.58	1.30(0.75–2.26)	1.22(0.69–2.18)
SF(L)fT4(N)	212	14.47	1.03(0.87–1.23)	1.03(0.85–1.23)
SF(N)fT4(L)	38	18.63	1.40(0.97–2.02)	1.39(0.95–2.02)
SF(N)fT4(N)	441	14.06	1.00 (ref)	1.00 (ref)
SF(H)fT4(L)	10	15.38	1.11(0.56–2.20)	0.97(0.49–1.95)
SF(H)fT4(N)	264	16.7	**1.23(1.04–1.45)***	**1.25(1.05–1.49)***

OR: odds ratio; aOR: adjusted odds ratio; SF: serum ferritin; fT4: free thyroxine; GDM: gestational diabetes mellitus; L: low; N: normal; H: high; CI: confidence interval.
[a] Adjusted for maternal age, maternal pre-gestational BMI, gravity, and the diagnosis of gestational age.
* $P < 0.05$.

Table 5 – Additive interaction of SF and low fT4 level on the risk of GDM.

Measure	SF(H)fT4(L)		SF(L)fT4(L)	
	Estimates	95%CI	Estimates	95%CI
RERI	−0.49	−1.39 to 0.42	−0.05	−0.87 to 0.77
AP	−0.45	−1.53 to 0.64	−0.04	−0.73 to 0.65
SI	0.16	0.00–460.40	0.81	0.02–28.67

between SF concentration and low fT4 level on the presence of GDM.

4. Discussion

Our study suggested that elevated SF concentration in early pregnancy was associated with an increased risk GDM, but not for low fT4 level. Compared with women with normal SF concentrations and fT4 levels, those women with higher SF concentrations but normal fT4 levels has a higher risk of GDM; However, no similar pattern of association was observed among women with higher SF concentrations but low fT4 levels. The addictive effect analysis indicated that there is no biological interaction between SF concentration and low fT4 level on the presence of GDM.

Our study is consistent with previous studies that have indicated that elevated SF concentration in early pregnancy was an independent risk factor for GDM [5–8]. The Rawal, S. study suggested that increased iron stores might be involved in the development of GDM early in pregnancy [5]. In addition, maternal plasma ferritin level or serum iron in early pregnancy is also related with an increased risk of GDM [6,7]. Intermittent supplementation with iron is recommended by the WHO for pregnant women without anemia [21]. A random control trial recently suspected the necessity of routine iron supplementary for women with high hemoglobin [22]. Furthermore, a study has also indicated that high iron intake in the pregnancy period might increase the risk of GDM, particularly in women without anemia in early pregnancy [8]. The underline mechanism is that elevated fasting SF levels could damage beta-cell function and decrease insulin sensitivity [10], which is involved in the pathogenesis of GDM. Ferritin is a modifier that affects the relationship between oxidative damage and glucose intolerance in pregnant women [23]. Excess iron accumulation could induce oxidative stress and cause beta-cell damage and apoptosis, which contribute to impaired insulin synthesis and secretion consequently [24,25].

It has been reported that isolated maternal hypothyroxinemia in early pregnancy has no significant adverse effect on GDM [26,27], which is also consistent with our results. However, it is not yet agreed in published studies whether low fT4 is a risk factor for GDM [28,29]. Maouche, N's study has showed that insulin resistance syndrome was involved in both overt hyperthyroidism and hypothyroidism [30]. Abnormal levels of thyroid hormones can be due to either an overdose caused by graves' thyrotoxicosis or a deficiency caused by hypothyroidism, which has profound effects on glucose metabolism and insulin secretion [31].

Serum iron concentration was positively correlated with fT4 level ($r = 0.98$, $p = 0.007$) in goitrous female patients [32]. Iron supplementation is recommended for goitrous women to improve the efficacy of thyroid metabolism [32]. Hence, we hypothesized that fT4 may play a role in the association between SF and GDM. However, our results indicated that there is no biological interaction for the SF and fT4 level on the risk of GDM. The result was imprecise because of sparse data as the confidence interval for the SI is quite wide. It is necessary to increase the sample size to obtain more accurate results in future studies.

As far as we know, this is the first study to assess the combination influence of SF and fT4 on the risk of GDM. However, our study also should be viewed with caution due to the following limitations. First, we did not take inflammation into account. Peripheral circulating ferritin concentration is a good indicator of iron storage but as an acute phase reactant, ferritin levels may also increase with subclinical systemic inflammation, which is associated with insulin resistance [33]. Second, we did not have information on iron supplement during pregnancy. However, iron was recommended to be the first choice for micronutrients intake and consumption of fortified foods to improve the dietary intake of iron in Chinese pregnant women [34]. Misclassification due to iron supplement intake is more likely to be nondifferential and would underestimate the association. Third, demographic characteristics, adjusted in the regression model, were limited in this study as it is based on the electronic medical register system. However, we believed that adding more unmeasured confounder could not make much difference on the estimation as the adjusted factors are not only basic but also

important potential confounders (OR = 2.29 and 1.83 for overweight and advanced maternal age respectively) [35].

In conclusion, our study suggests that only high serum ferritin concentration is associated with an increased risk of GDM in early pregnancy. The level of fT4 in early pregnancy might have no effect on the association between high SF and risk of GDM.

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Compliance with ethical standards

Conflict of interest: The authors declare that they have no conflict of interest.

Ethical approval: The study was approved by the Ethics Committee of Shanghai First Maternity and Infant Hospital, Tongji University School of Medicine (No. 20120234). The study was performed in accordance with the approved guidelines.

Informed consent: Consent has been obtained from each patient after full explanation of the purpose and nature of all procedures used.

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