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Original Article

Effects of subclinical hypothyroidism combined with gestational diabetes mellitus on the pregnancy outcome and serum FGF-21 and LCN-2 levels in patients

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Abstract: This study aimed to analyze fibroblast growth factor 21 (FGF-21) and lipocalin-2 (LCN-2) in patients with subclinical hypothyroidism (SCH) and gestational diabetes mellitus (GDM) and the effects of the combined disease on pregnancy outcomes. Altogether 105 patients with SCH and GDM admitted to our hospital and 110 healthy pregnant women during this period were enrolled. The total cholesterol (TC), triglyceride (TG), high density lipoprotein (HDL), and low density lipoprotein (LDL) in the two groups were quantified using an automatic biochemistry analyzer, and the free thyroxine (FT4), thyrotrophin (TSH), and free triiodothyronine (FT3) were quantified using a chemiluminescence method. Additionally, the fasting blood glucose (FBG) and fasting insulin (FINS) were determined using the glucose oxidase method and radioimmunoassay, and the homeostatic model assessment (HOMA) - insulin resistance index (IR) was calculated. Serum FGF-21 and LCN-2 was quantified using the double antibody sandwich method and enzyme-linked immuno-sorbent assay, and the effects of SCH and GDM on pregnancy outcome were analyzed. The observation group showed significantly higher levels of FGF-21, TSH, TC, TG, FPG, FINS, and HOMA-IR, with significantly lower levels of LCN-2 and LDL than the control group, and there was no significant difference between them in serum HDL. Serum FGF-21 was positively correlated with TSH, TC, TG, FPG, FINS, and HOMA-IR, and negatively correlated with FT3, FT4, and LDL, but uncorrelated with HDL, and serum LCN-2 was negatively correlated with TC, TG, FPG, FINS, and HOMA-IR, and positively related with LDL, but not significantly correlated with HDL, TSH, FT3, and FT4. The observation group showed a significantly higher incidence of adverse pregnancy outcomes than the control group. FGF-21 in the serum of patients with SCH and GDM decreased, while LCN-2 increased, so they may be used as indicators for disease assessment. SCH comorbid with GDM significantly increases adverse pregnancy outcomes.

Keywords: Subclinical hypothyroidism, gestational diabetes mellitus, FGF-21, LCN-2, pregnancy outcome

Introduction

Subclinical hypothyroidism (SCH) is a prevalent endocrine disease [1]. Some studies have revealed that the level of thyrotrophin (TSH) in pregnant women was higher than the normal upper reference limit, and hypothyroidism is linked to various pregnancy complications including gestational diabetes mellitus (GDM) [2, 3]. In recent years, the proportion of hypothyroidism comorbid with GDM complications in pregnant women in is increasing, which compromises the growth and development of the fetus and the life and health of the mother, and is also a high risk for premature delivery and abortion [4, 5].

Fibroblast growth factor 21 (FGF-21) is a regulatory factor related to glycolipid metabolism, which can improve the function of islet β cells, promote glucose absorption in adipose tissue, and reduce fat accumulation [6]. Hypothyroidism may give rise to dyslipidemia. Serum FGF-21 is a regulatory factor related to glucose and lipid metabolism. Previous studies have pointed out that the decrease of FGF-21 level is also related to the development of GDM [7, 8]. However, there are few studies on serum FGF-21 in patients with both GDM and SCH. Lipocalin-2 (LCN-2) is a newly discovered adipocytokine and a member of the lipocalin family, which has been adopted as a sensitive indica-

tor of renal injury [9]. In recent years, LCN-2 has gradually captured attention for its role in diabetes mellitus. According to animal experiments in one study, mice with knockout of LCN-2 showed a significant decrease in decreased fasting insulin (FINS) level and a significantly increase in fasting blood glucose (FBG) and insulin sensitivity [10]. Additionally, one study has determined the LCN-2 level in pregnant women with gestation of 9-12 weeks [11], and has found that LCN-2 is significantly and positively correlated with insulin resistance index, and higher LCN-2 level indicates a higher risk of GDM. However, there are relatively few studies on the LCN-2 level in patients with both GDM and SCH.

This study quantified FGF-21 and LCN-2 in the serum of pregnant women with both GDM and SCH, and analyzed the relationship between GDM combined with SCH and pregnancy outcomes.

Materials and methods

A total of 105 pregnant women with both GDM and SCH admitted to the Maternity and Child Care Center of Qinhuang Island from February 2017 to July 2018 were enrolled as an observation group (obs group), and 110 healthy pregnant women were enrolled as a control group (con group). The inclusion criteria of the study: Pregnant women between 21 and 35 years old, with a gestation of 24 to 48 weeks, patients meeting the diagnostic criteria of both SCH and GDM [12, 13], patients with monocyesis, and those with gestational weeks of 24-28 weeks. The exclusion criteria of the obs group: Patients with a history of diabetes mellitus, patients with other pregnancy complications, patients with multiple pregnancies, patients with comorbid malignant tumors, patients accompanied by severe infectious diseases, patients who have taken glucocorticoids, patients with severe liver or kidney dysfunction, and those who refused to participate in our study. All patients and their families who were part of the study agreed to take part in it. The experiment was carried out with permission from the Ethics Committee of the Maternity and Child Care Center of Qinhuang Island, and this study was in line with the Declaration of Helsinki.

Index detection

Fasting venous blood (5 ml) was sampled from each pregnant woman in the two groups, and centrifuged at 3000 r/min for 10 min to separate serum for later analysis. Detection of serum was carried out in strict accordance with instructions. The levels of total cholesterol (TC), triglyceride (TG), high density lipoprotein (HDL), as well as low density lipoprotein (LDL) in the serum were determined using an Siemens AD-VIA1800 automatic biochemistry analyzer, and the levels of free thyroxine (FT4), thyrotrophin (TSH), as well as free triiodothyronine (FT3) in the serum were determined using a chemiluminescence method. In addition, the fasting blood glucose (FBG) and fasting insulin (FINS) in the serum were detected using the glucose oxidase method and radioimmunoassay, respectively. The homeostatic model assessment (HOMA) was insulin resistance index (IR) = (FBG×FINS)/22.5 [14]. The FGF-21 and LCN-2 levels in the serum were determined using the double antibody sandwich method and the ELISA, respectively, and the kits were both purchased from the R&D Company in the United States.

Statistical analyses

In this study, the data were analyzed statistically with SPSS 19.0. Enumeration data were analyzed by the chi-square test, and measurement data were expressed as the mean ± standard deviation. Inter-group comparison was carried out via the t test, and the data were visualized into figures using GraphPad Prism 6. Pearson's correlation coefficient was adopted for correlation analysis. $P<0.05$ indicates a significant difference.

Results

General materials

There was no significant difference in general data including age, body mass index (BMI), and the number of pregnancies between the two groups (all $P>0.05$). **Table 1**.

Expression of serum FGF-21 and LCN-2 in pregnant women in the two groups

The expression of serum FGF-21 and LCN-2 in the obs group was (20.29±5.13) ng/ml and (81.03±10.26) ug/L, respectively, while the expression of them in the con group was (4.16± 1.22) ng/mland (135.71±12.37) ug/L, respectively. The obs group showed significantly high-

Table 1. General data

Factor	The obs group n=105	The con group n=110	t/χ^2	*P*-value
Age (years)			0.003	0.954
≤25	54 (51.43)	57 (51.82)		
>25	51 (48.57)	53 (48.18)		
Pregestational BMI (kg/m^2)			0.003	0.960
≤23	50 (47.62)	52 (47.27)		
>23	55 (52.38)	58 (52.73)		
Education level			0.001	0.976
With junior high school diploma or below	25 (23.81)	26 (23.64)		
With junior high school diploma or above	80 (76.19)	84 (76.36)		
Place of residence			0.018	0.892
Rural area	42 (40.00)	45 (40.91)		
Urban area	63 (60.00)	65 (59.09)		
The number of pregnancies (Times)			0.008	0.927
≤1	76 (72.38)	79 (71.82)		
>1	29 (27.62)	31 (28.18)		
Smoking in the past year			0.054	0.973
Never	61 (58.10)	63 (57.27)		
<4 times/weeks	31 (29.52)	34 (30.91)		
Daily	13 (12.38)	13 (11.82)		
Drinking history			0.045	0.833
Yes	65 (61.90)	69 (62.73)		
No	40 (38.10)	41 (37.27)		

Table 2. Expression of serum FGF-21 and LCN-2 in pregnant women in the two groups

Group	n	FGF-21 (ng/ml)	LCN-2 (ug/L)
The obs group	105	20.29±5.13	81.03±10.26
The con group	110	4.16±1.22	135.71±12.37
t value		32.04	35.19
P-value		<0.001	<0.001

er serum FGF-21 expression and significantly lower serum LCN-2 expression than the con group (both P<0.05). **Table 2**.

Levels of thyroid-related hormones in pregnant women from the two groups

The levels of TSH, FT3, and FT4 in the obs group were (41.58±11.37) mIU/L, (3.15±0.58) pmol/L, and (5.62±0.75) pmol/L, respectively, while the levels of them in the con group were (7.26±1.25) mIU/L, (5.74±1.02) pmol/L, and (14.27±2.11) pmol/L, respectively. The level of serum TSH in the obs group was significantly higher than that in the con group, while the levels of serum FT3 and FT4 in the obs group were significantly lower than those in the con group (all *P*<0.05). **Table 3**.

Comparison of blood lipids and blood glucose-related indexes between the two groups

The levels of serum TC, TG, FPG, FINS, and HOMA-IR in the obs group were significantly higher than those in the con group, and the LDL level in the obs group was significantly lower than that in the con group (all *P*<0.05). Additionally, there was no significant difference between the two groups in the level of serum HDL (P>0.05) **Table 4**.

Analysis of the correlation between FGF-21 and LCN-2 levels and thyroid-related hormones

The level of serum FGF-21 was positively correlated with that of serum TSH (*P*<0.001), and negatively correlated with the levels of FT3 and FT4 (*P*<0.001). There was no significant correlation between LCN-2 and TSH, FT3, and FT4 (all P>0.05). **Figure 1**.

Table 3. Levels of thyroid-related hormones in the pregnant women in the two groups

Group	n	TSH (mIU/L)	FT3 (pmol/L)	FT4 (pmol/L)
The obs group	105	41.58±11.37	3.15±0.58	5.62±0.75
The con group	110	7.26±1.25	5.74±1.02	14.27±2.11
t value		31.46	22.74	39.68
P-value		<0.001	<0.001	<0.001

Analysis of the correlation between FGF-21 and LCN-2 levels and thyroid-related hormones

Serum FGF-21 was positively correlated with TC, TG, FPG, FINS, as well as HOMA-IR (all $P<0.001$), and negatively correlated with LDL ($P<0.001$), but uncorrelated with HDL ($P>0.05$). LCN-2 was negatively correlated with TC, TG, FPG, FINS, as well as HOMA-IR ($P<0.001$), and positively correlated with LDL ($P<0.001$), but uncorrelated with HDL ($P>0.05$). **Figure 2**.

Comparison of pregnancy outcomes between the two groups

In the obs group, there were 10 cases of premature rupture of membranes, 3 cases of fetal growth restriction, 2 cases of placenta previa, 2 cases of placental abruption, and 12 cases of premature delivery. In the con group, there was only 1 case of premature rupture of membranes and 3 cases of premature delivery, so the incidence of adverse pregnancy outcomes in the obs group was significantly higher than that in the con group ($P<0.05$). **Table 5**.

Discussion

SCH is a prevalent thyroid dysfunction during pregnancy, with a rising incidence in recent years [15]. GDM is a prevalent gynecologic and obstetrical disease, mostly occurring in pregnant women in middle or late pregnancy [16]. At present, some pregnant women suffer from both SCH and GDM, which is extremely unfavorable to the life and health of pregnant women and their fetus [17].

The thyroid gland, as an important endocrine gland in human body, it plays an extremely crucial role in maintaining a normal metabolism in the body [18]. During pregnancy, the thyroid gland in a state of stress as it secretes more thyroid hormones to meet the growth and development needs of the fetus, but due to the long-term compensatory state of the thyroid gland, pregnant women are more likely to suffer from hypothyroidism [19, 20]. GDM is mostly caused by the gradual increase of insulin-resistance hormone produced by placenta tissue during pregnancy, and the increase of body mass after pregnancy will lead to the decrease of body sensitivity to insulin, thus giving rise to GDM in the pregnant women [21]. In this study, it was found that the TSH level in the obs group increased, and the FT3 and FT4 levels in the obs group decreased, and the levels of FPG, FINS, as well as HOMA-IR in the obs group were significantly higher than those of the con group, which implied that the obs group suffered from hypothyroidism and insulin resistance due to inflammation.

FGF-21 is a hormone-like fibroblast growth factor and also a regulator of glycolipid metabolism. In the past, it was found to be elevated in the serum of patients with diabetes mellitus and some metabolic syndromes [22]. LCN-2 is a secretory glycoprotein composed of polypeptide chains. Its main function is to transport iron ions into cells and it participates in various metabolic processes of cells [23]. In this study, we found that FGF-21 in pregnant women suffering from both SCH and GDM was up regulated, and LCN-2 was down regulated, which implied that the metabolism of pregnant women with these complications was abnormal. There is one previous study indicating that the expression of FGF-21 may be regulated by the thyroid gland [24]. In our study, we also found that the level of FGF-21 was not only related to thyroid-related hormones, but also significantly related to blood lipid and blood glucose-related indicators, which indicated that FGF-21 may participate in glycolipid metabolism in addition to regulating hormone levels. We also found that LCN-2 had no significant correlation with thyroid-related hormones, which indicated that LCN-2 did not regulate thyroid hormones, but it had significant correlation with blood lipid and blood glucose. One study has explained that for pregnant women with SCH, the basal metabolic rate is slowed down due to insufficient secretion of thyroid hormones, so the renal blood flow, glomerular filtration rate, as well as renal tubular reabsorption ability are all decreased, which results in the decrease of clearance rate of FGF-21 and the increase of serum FGF-21 [25]. One previous study on LCN-2 has revealed that LCN-2 is

Table 4. Blood lipid and blood glucose-related indexes between the two groups

Group	n	TC (mmol/L)	TG (mmol/L)	LDL (pmol/L)	HDL (mmol/L)	FPG (mmol/L)	FINS (mU/L)	HOMA-IR
The obs group	105	6.58±1.37	2.65±0.78	4.62±0.75	1.47±0.58	5.15±1.05	10.35±2.45	2.25±0.46
The con group	110	4.72±0.85	1.52±0.53	3.27±0.32	1.73±0.65	3.86±0.56	6.87±2.11	1.26±0.27
t value		12.02	12.47	17.30	3.090	11.31	11.18	19.35
P-value		<0.001	<0.001	<0.001	0.002	<0.001	<0.001	<0.001

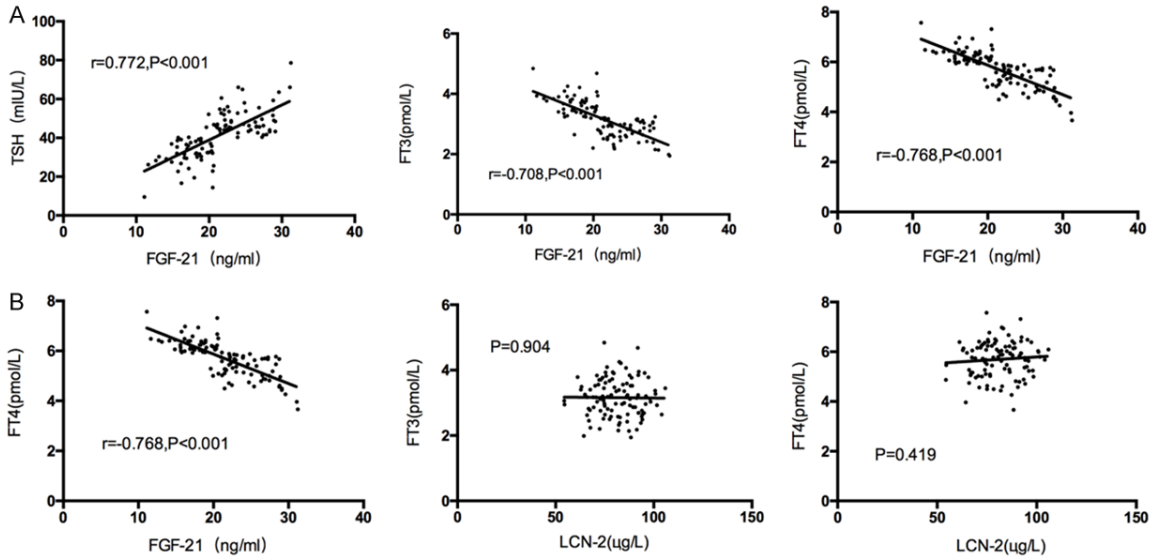

Figure 1. Analysis of the correlation of FGF-21 and LCN-2 levels with thyroid-related hormones. A. Analysis of the correlation of FGF-21 with TSH, FT3, and FT4. B. Analysis of the correlation of LCN-2 with TSH, FT3, and FT4.

significantly up regulated in patients with type 2 diabetes mellitus, and LCN-2 is strongly correlated with insulin resistance [26]. Moreover, one other prospective study has revealed that serum LCN-2 is up regulated in patients with GDM, and positively related to HOMA-IR [27], which is also consistent with our observation results. However, there is no detailed explanation on the relationship between LCN-2 and insulin resistance. Finally, we compared the pregnancy outcomes between the two groups, finding that the incidence of adverse pregnancy outcomes in the obs group was significantly higher than that in the con group, which was consistent with previous studies [28, 29]. The occurrence of SCH or GDM will increase the risk of adverse pregnancy outcomes.

Conclusion

To sum up, FGF-21 is down regulated in the serum of patients with both SCH and GDM, while LCN-2 is significantly up regulated in it, so they may be used as potential indicators for disease assessment. SCH comorbid with GDM will significantly increase the incidence of adverse pregnancy outcomes. However, there are still some deficiencies in this study. For example, we have not explained the possible mechanism of action of FGF-21 and LCN-2 in patients with SCH combined with GDM, which needs further basic experiments to explore it.

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Disclosure of conflict of interest

None.

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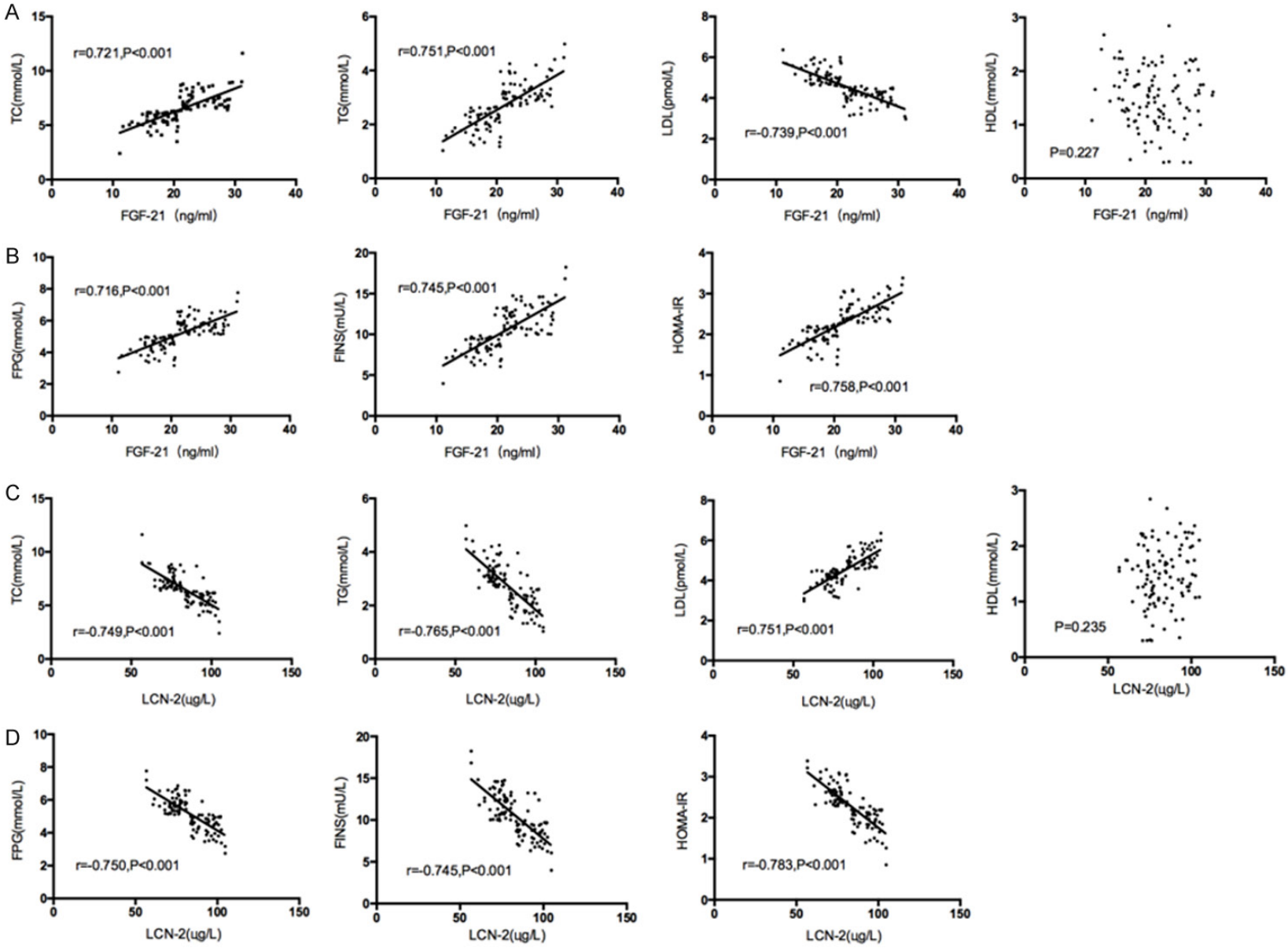
A
TC(mmol/L)
r=0.721,P<0.001
FGF-21 (ng/ml)
TG(mmol/L)
r=0.751,P<0.001
FGF-21 (ng/ml)
LDL(pmol/L)
r=-0.739,P<0.001
FGF-21 (ng/ml)
HDL(mmol/L)
P=0.227
FGF-21 (ng/ml)
B
FPG(mmol/L)
r=0.716,P<0.001
FGF-21 (ng/ml)
FINS(mU/L)
r=0.745,P<0.001
FGF-21 (ng/ml)
HOMA-IR
r=0.758,P<0.001
FGF-21 (ng/ml)
C
TC(mmol/L)
r=-0.749,P<0.001
LCN-2(ug/L)
TG(mmol/L)
r=-0.765,P<0.001
LCN-2(ug/L)
LDL(pmol/L)
r=0.751,P<0.001
LCN-2(ug/L)
HDL(mmol/L)
P=0.235
LCN-2(ug/L)
D
FPG(mmol/L)
r=-0.750,P<0.001
LCN-2(ug/L)
FINS(mU/L)
r=-0.745,P<0.001
LCN-2(ug/L)
HOMA-IR
r=-0.783,P<0.001
LCN-2(ug/L)

Figure 2. Analysis of the correlation of FGF-21 and LCN-2 with blood lipid and blood glucose-related indexes. A. Analysis of the correlation of FGF-21 with TC, TG, LDL, and HDL. B. Analysis of the correlation of FGF-21 with FPG, FINS, and HOMA-IR. C. Analysis of the correlation of LCN-2 with TC, TG, LDL, and HDL. D. Analysis of the correlation of LCN-2 with FPG, FINS, and HOMA-IR.

Table 5. Comparison of pregnancy outcomes between the two groups

Group/Pregnancy outcome	n	Premature rupture of membranes	Fetal growth restriction	Placenta previa	Placental abruption	Premature birth	The total incidence
The obs group	105	10 (9.52)	3 (2.86)	2 (1.90)	2 (1.90)	12 (11.43)	29 (27.62)
The con group	110	1 (0.91)	0	0	0	3 (2.73)	4 (3.64)
X^2 value		8.213	3.187	2.115	2.115	5.815	23.78
P-value		0.004	0.074	0.146	0.146	0.016	<0.001

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