

# Changes of serum zinc- $\alpha$ 2-glycoprotein level and analysis of its related factors in gestational diabetes mellitus:a cross-sectional study

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## Research article

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## **Abstract**

## **Background**

Previous studies have discovered that zinc- $\alpha$ 2-glycoprotein is related to insulin resistance and lipid metabolism. The aim of the study is to explore the change of serum zinc- $\alpha$ 2-glycoprotein(ZAG) and its related factors in gestational diabetes mellitus(GDM).

## **Methods**

Eighty newly diagnosed GDM patients were enrolled in study group, and 80 normal pregnant women were selected as control group. The differences of baseline data between the two groups were compared, and the change of serum ZAG level and its relationship with related indexes was analyzed.

## **Results**

Compared to control group, the level of serum ZAG in the study group decreased [ $(43.94 \pm 14.51)$ mg/L vs.  $(62.57 \pm 19.05)$ mg/L,  $P < 0.001$ ]. Pearson correlation (or Spearman correlation) analysis showed that serum ZAG level was negatively correlated with FPG, FINS, HOMA-IR and TG ( $P < 0.05$ ) and positively correlated with HDL( $P < 0.05$ ). Multiple linear regression showed that HDL, FINS, HOMA-IR were independent factors of serum ZAG( $P < 0.001$ ).

## **Conclusion**

The level of serum ZAG in patients with gestational diabetes mellitus decreased, and HDL, FINS and HOMA-IR are the influencing factors in study group.

## **Trial registration:**

The study registered in the Chinese Clinical Trial Registry(Chi CTR2000028811).

## **Background**

Gestational diabetes mellitus is one of the common complications of pregnancy. The prevalence of GDM ranges from 1 to 18.5%, which globally has a increasing tendency year by year<sup>1-3</sup>. However, the etiology of GDM has not been clear yet so far, and the pathogenesis needs further study. At present, it is generally believed that maternal obesity and lipid metabolism disorder are related to the occurrence of GDM<sup>4,5</sup>. Nowadays, many specialists focus their eyes on the role of adipokines, which contributes to the metabolic abnormalities in the mother. Some study found statistical difference in adiponectin and zinc- $\alpha$ 2-glycoprotein between GDM patients and normal pregnant women<sup>6,7</sup>. ZAG is a new type of adipocytokine secreted by adipocytes, which exists in human plasma and other various body fluids including sweat, milk and urine<sup>8,9</sup>. It was found that ZAG is not only involved in the regulation of fat metabolism in obesity, but also related to the occurrence of T2DM lipid metabolism disorder<sup>10</sup>.

The purpose of this study is to explore the change of serum ZAG and its related factors in GDM, thus providing theoretical basis for the metabolism mechanism of GDM patients.

## **Participants And Methods**

### **Participants**

All subjects of gestation age between 24 weeks and 28 weeks were recruited in the study in the Third Affiliated Hospital of Zhengzhou University from July 2018 to June 2019. They all undertook 75 g oral glucose tolerance test(OGTT) during their prenatal examination. Newly diagnosed gestational diabetes mellitus patients were selected as study group, and normal pregnant women as control group. And all of them were naturally pregnant and single pregnancy. And we excluded the pregnant women with chronic diseases such as diabetes, hypertension, cardiovascular and cerebrovascular diseases, severe liver and kidney diseases, tumors, mental diseases and other pregnancy complications. Written informed consent was obtained from all participants. Ethics Committee of the Third Affiliated Hospital of Zhengzhou University approved the study, and the study registered in the Chinese Clinical Trial Registry(ChiCTR2000028811).

### **Methods**

The demographic and clinical characteristics data were collected, such as height, weight, body mass index(BMI), age, race, residence, occupation, systolic pressure (SBP), diastolic pressure(DBP) and so on. Some hematologic and biochemical index including fasting blood glucose(FBG), blood glucose, triglyceride(TG), high density lipoprotein(HDL), low density lipoprotein(LDL), total cholesterol(TC) were also collected.

Enzyme linked immunosorbent assay (ELISA) was used to detected the level of serum ZAG. Insulin resistance index(HOMA-IR) and pancreatic  $\beta$  cell function index (HOMA- $\beta$ ) were calculated and analyzed with Homeostasis Model Assessment<sup>11</sup>. Calculation formulas are: HOMA-IR = FPG × FINS/22.5 and HOMA- $\beta$  = 20 × FINS/(FPG - 3.5).

### **Diagnostic criteria for gestational diabetes mellitus**

According to Chinese diagnostic criteria of gestational diabetes mellitus, the critical serum glucose values of fasting, 1 hour and 2 hours after taking glucose were 5.1 mmol/L, 10.0 mmol/L and 8.5 mmol/L, respectively. Gestational diabetes can be diagnosed if any of the three outcomes is greater than or equal to the critical value in pregnant women with fasting glucose or 75gOGTT after 24 weeks gestation.

### **Statistical analysis**

The SPSS21.0 statistical package was used to process the data of the study. Firstly, the 1-sample Kolmogorov-Smirnov test was performed to verify the normal distribution of the quantitative variables. And then normal distributed data were expressed as Mean  $\pm$  SD, while the quantitative data of non normal distribution were expressed as median (25th -75th percentile). And qualitative data were expressed by rate(or percentage). Independent samples  $t$  tests, Mann-Whitney U test or Chi square test were used to explore the difference between the two groups. Pearson(or spearman) correlation analysis and multiple linear regression analysis were used to evaluate the association between the indicators. Two-tailed and  $P$  values less than 0.05 were regarded as statistically significant.

## **Results**

### **Baseline characteristics of the two groups**

According to the inclusion criteria and exclusion criteria, total of 160 subjects(80 subjects in each group) were enrolled in the study. The age of study group was  $(30.08 \pm 3.43)$  years old, while the control group was  $(30.50 \pm 3.88)$  years old. And the difference between two groups was not statistically significant( $P > 0.05$ ). HbA1c were  $(6.00 \pm 0.45)\%$  and  $(5.74 \pm 0.33)\%$  in study group and control group, respectively. And the difference between two groups was statistically significant

( $P < 0.001$ ). Statistical analysis showed significant differences in weight, BMI, fat mass and gain weight between the two groups ( $P < 0.05$ ). But there were no significant differences in height, SBP and DBP ( $P > 0.05$ ). As shown in Table 1.

Table 1  
Baseline characteristics of the two groups

	Study group(n = 80)	Control group(n = 80)	t/ $\chi^2/Z$	P
age, years	30.08 ± 3.43	30.50 ± 3.88	-0.734	0.464
height, cm	161.86 ± 4.71	161.35 ± 4.40	-0.740	0.460
weight, kg	62.73 ± 10.65	58.18 ± 7.18	-3.168	0.002
SBP, mmHg	114.21 ± 6.90	113.26 ± 5.41	-0.970	0.334
DBP, mmHg	68.16 ± 7.36	67.50 ± 5.84	-0.621	0.535
BMI, kg/m <sup>2</sup>	24.58 ± 3.93	22.96 ± 2.82	-3.112	0.002
gain weight, kg	6.91 ± 2.14	5.32 ± 1.23	-2.474	0.015
HbA1c, %	6.00 ± 0.45	5.74 ± 0.33	-4.167	< 0.001
fat mass, kg	20.8(16.93, 22.52)	20.3(15.35, 21.98)	-2.273	0.023
percent of body fat, %	34.47 ± 6.01	33.23 ± 4.63	-1.521	0.130
residence			1.604	0.205
city, n(%)	48(60.00)	55(68.75)		
rural, n(%)	32(40.00)	25(31.25)		

## Comparison of related biochemical indexes between the two groups

Table 2 showed that the levels of FPG, FINS, HOMA-IR and HOMA- $\beta$  in the study group were higher than those in the control group. The levels of FPG, FINS, HOMA-IR showed statistic difference between the study group and the control group ( $P < 0.05$ ). However, the results showed no statistic difference in HOMA- $\beta$  ( $P > 0.05$ ).

Compared to the control group, the serum ZAG level in the study group decreased, and the difference was statistically significant ( $P < 0.001$ ) [(43.94 ± 14.51) mg/L vs. (62.57 ± 19.05) mg/L,  $P < 0.001$ ]. The differences of TG and HDL between the two groups were statistically significant ( $P < 0.05$ ), while there was no statistical difference in TC and LDL ( $P > 0.05$ ).

Table 2  
The comparison of related biochemical indexes between two groups

	Study(n = 80)	Control(n = 80)	t/Z	P
FPG, mmol/l	5.03 ± 0.60	4.45 ± 0.35	-7.817	≤0.001
FINS, mU/L	7.56(5.69, 14.4)	4.92(4.01, 10.44)	-4.363	≤0.001
HOMA-IR	1.59(1.28, 3.22)	0.94(0.79, 1.81)	-5.453	≤0.001
HOMA-β	117.66(72.99, 180.27)	106.67(83.74, 229.25)	-1.068	0.285
TC, mmol/L	5.51 ± 1.32	5.59 ± 1.87	0.293	0.770
TG, mmol/L	2.83(2.21, 3.48)	2.58(1.89, 3.36)	-7.152	≤0.001
HDL, mmol/L	1.73 ± 0.47	2.07 ± 0.62	3.943	≤0.001
LDL, mmol/L	3.06 ± 0.69	2.86 ± 0.86	-1.641	0.103
ZAG, mg/L	43.94 ± 14.51	62.57 ± 19.05	6.955	≤0.001

## Correlation analysis in study group

With ZAG as the dependent variable and other clinical indicators as independent variables, correlation analysis was conducted. The results showed that serum ZAG levels of pregnant women in the study group were negatively correlated with FPG, FINS, HOMA-IR and TG( $r = -0.416, -0.167, -0.236, -0.328, P < 0.05$ ). But it was positively correlated with HDL( $r = 0.279, P = 0.012$ ). The level of serum ZAG was not related to age, BMI and fat mass ( $P > 0.05$ ). As shown in Table 3.

Table 3  
Correlation between serum ZAG level and clinical indexes in study group

	age	BMI	fat mass	fat(%)	FPG	FINS	HOMA-IR	HOMA-β	TC	TG	HDL	LDL
<i>r</i>	0.089	-0.219	-0.015	-0.086	-0.416	-0.167	-0.236	0.003	0.018	-0.328	0.279	0.153
<i>P</i>	0.432	0.051	0.895	0.450	<0.001	0.035	0.015	0.980	0.876	<0.001	0.012	0.174

## Multiple linear regression analysis of ZAG and other clinical indexes in study group

FPG, FINS, HOMA-IR, TG and HDL were included in the regression model as independent variables, and the results showed that FINS( $\beta = 0.611, P = 0.004$ ), HOMA-IR( $\beta = -2.687, P = 0.008$ ) and HDL( $\beta = 3.582, P < 0.001$ ) were independent influencing factors of serum ZAG. What's more, the inclusion and exclusion criteria were  $P \leq 0.05$  and  $P \geq 0.1$ , respectively.

## Discussion

ZAG is a soluble glyprotein with molecular weight of 42kD, which was first isolated and purified from human serum by Burgi et al. in 1961<sup>12</sup>. ZAG is widely found in human body fluids, with carrier protein, ribonuclease activity and other functions<sup>9</sup>. In recent years, many studies have shown that ZAG has function of regulating immunity, cell adhesion, and regulating of melanin production<sup>13,14</sup>. Some studies<sup>15-17</sup> also pointed out that ZAG can be used as a biomarker for the early diagnosis of cancer, and its can participate in regulating tumor cell proliferation and glucose metabolism<sup>18</sup>. In addition, in vivo and in vitro experiments confirmed that ZAG can promote fat mobilization, suggesting that ZAG is

involved in fat catabolism<sup>19,20</sup>. At present, most studies on ZAG are limited to type 2 diabetes, but its relationship with GDM is seldom studied.

According to the study, we found that the serum ZAG level of GDM patients was lower than that of the control group, and regression analysis showed that the serum ZAG level of GDM patients was related to FINS, HOMA-IR and HDL. The analysis suggested that ZAG may play a certain role in the metabolism of serum glucose and lipid in GDM patients, and it has some positive significance in reducing insulin resistance in GDM patients. Yang M et al.<sup>18</sup> also showed that ZAG was related to insulin resistance, which was consistent with this study. Naf S et al.<sup>6</sup> also pointed out that serum ZAG level in GDM patients was related to HDL, suggesting that ZAG was involved in lipid metabolism in GDM patients. However, no statistic difference was found between the GDM group and the NGT group in serum ZAG level, which may be caused by factors such as different races, research methods and sample size. In addition, Naf S et al. measured the serum ZAG level before delivery, while our study collected the detection indicators of pregnant women with GDM at 24 to 28 weeks, which may lead to the difference between the two studies.

In summary, the serum ZAG level of GDM patients was lower than that of the control group, and the changes were related to FINS, HOMA-IR and HDL. Serum ZAG level may be a new predictor of lipid metabolism and insulin resistance in patients with GDM, and may provide a potential therapeutic target for improving serum lipid disorder and insulin resistance in GDM patients. However, the sample size of our study was relatively small. Currently, there are few studies on the relationship between serum ZAG and GDM, and more evidence-based medical evidence is still needed. Therefore, it is still necessary to carry out multi-center and multi-area large sample study in the future to further clarify the relationship between serum ZAG and GDM.

## Declarations

### Ethics approval and consent to participate

The study was approved by the ethics committee of the Third Affiliated Hospital of Zhengzhou University and written informed consent was acquired from all study subjects.

### Consent for publication

There was no personal identifying information in this article.

### Competing interests

The authors declare that they have no competing interests.

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### Authors' Contributions

D.M.X., J.Y. and L.L.C. concepted and designed the study; G.X.C., H.L.S., L.Z. and Z.L.L. conducted the study and the data collection; J.Y. and G.L.H. made the statistics analysis; C.Y.F. and J.Y. drafted and revised the manuscript; Finally, C.Y.F.

wrote the article. All authors have approved the final manuscript.

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