PRACE ORYGINALNE
położnictwo

Serum angiopoietin-related growth factor (AGF) levels are elevated in gestational diabetes mellitus and associated with insulin resistance

Poziom surowiczego czynnika wzrostu związanego z angiopoetyną (AGF) jest podwyższony w cukrzycy ciążowej i związany z insulinoopornością

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Abstract

Objective: *Angiopoietin-related growth factor (AGF) is associated with angiogenesis but it can also affect glucose and energy metabolism. The aim of the study was to determine AGF levels in gestational diabetes mellitus (GDM).*

Materials and Methods: *The study included 44 patients with GDM (GDM group) and 33 non-diabetic, healthy, women in the third trimester of pregnancy (control group). We analyzed serum levels of AGF and other biochemical and anthropometric markers in all subjects.*

Results: *The study revealed that serum AGF levels were significantly higher in patients with GDM (113.30±69.92 ng/ml) than in controls (52.30±35.59 ng/ml), (p-value<0.001). Fasting glucose (117.59 vs. 82.18), homeostasis model of assessment - insulin resistance (HOMA-IR), (2.91 vs. 1.75) diastolic (74.20 vs. 70.00) and mean (89.09 vs. 84.84) blood pressure were found to be significantly higher in the GDM group when compared to the control group (p-value<0.05). There was a significant positive association between AGF and HOMA-IR in the GDM group.*

Conclusions: *Although gestational diabetes mellitus can be a predictor of serum AGF level, further studies are needed to explain the physiologic roles of AGF in glucose metabolism.*

Key words: **Angiopoietin-related growth factor** / **ANGPTL6 protein** / / **human, diabetes** / **gestational** /

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Streszczenie

Cel: *Czynnik wzrostu związany z angiopoetyną (AGF) jest odpowiedzialny za angiogenezy ale może też wpływać na metabolizm glukozy i energii. Celem tego badania była ocena poziomu AGF w cukrzycy ciążowej (GDM).*

Materiał i metoda: *Do badania włączono 44 pacjentki z GDM (tzw. grupa GDM) i 33 zdrowe ciężarne w trzecim trymestrze ciąży (grupa kontrolna). Zbadano poziom surowiczego AGF oraz inne biochemiczne i antropometryczne markery u wszystkich pacjentek.*

Wyniki: *Wykazano, że poziom surowiczego AGF był istotnie wyższy u pacjentek z cukrzycą ciążową (113,3±69,92 ng/ml) niż w grupie kontrolnej (52,3±35,59 ng/ml), (p<0,001). Glukoza na czczo (117,59 vs 82,18), insulinooporność – wskaźnik HOMA (2,91vs 1,75), ciśnienie rozkurczowe (74,2 vs 70) i ciśnienie średnie (89,09 vs 84,84) były istotnie wyższe w grupie GDM niż w grupie kontrolnej (p<0,05). Znaleziono istotne pozytywne powiązanie między AGF a wskaźnikiem HOMA w grupie GDM.*

Wnioski: *Cukrzyca ciążowa może być czynnikiem predykcyjnym poziomu surowiczego AGF, jednak dalsze badania są konieczne aby ustalić rolę AGF w metabolizmie glukozy.*

Słowa kluczowe: **czynnik wzrostu związany z angiopoetyną** / **białko ANGPTL6** / / **cukrzyca ciążowa** /

Introduction

Gestational diabetes mellitus (GDM) is defined as 'any degree of glucose intolerance with onset or first recognition during pregnancy [1]. It is one of the most common pregnancy complications. The overall incidence of 3–6% has steadily increased over time [2, 3]. Although GDM is generally temporary, it is associated with serious health complications, both for mothers and babies [4]. For the mother, higher rates of hypertension and cesarean section are reported [5, 6]. For the infant, immediate risks include macrosomia (birth weight >4kg), low birth weight (<2.5 kg), birth injury and stillbirth [5, 6]. The pathophysiology of gestational diabetes involves abnormalities of insulin-sensitive tissue. Beta-cell sensing of glucose is also abnormal and is manifested as an inadequate insulin response for a given degree of glycemia. Women with gestational diabetes have decreased insulin sensitivity in comparison with weight-matched control groups [7]. Gestational diabetes is associated with increased risks of poor maternal and perinatal outcomes. In population studies, pregnant women with gestational diabetes have increased risk of pregnancy-associated hypertension compared with non-diabetic women [8].

Angiopoietin-related growth factor [(AGF), or angiopoietin-like protein-6 (ANGPTL6)] is known to be a novel hepatokine that modulates angiogenesis and metabolism [9]. AGF directly antagonizes obesity and related insulin resistance. In addition, AGF facilitates increased energy expenditure [10].

The aim of the study is to investigate whether AGF level is affected in patients with gestational diabetes mellitus.

Material and methods

The local Ethical Committee approved the study and written informed consent authors obtained from all the pregnant women between 19 and 39 years of age. Forty-five women diagnosed with gestational diabetes (GDM group) and 35 non-diabetic, healthy, third trimester pregnant women (controls) were included in the study. After determination of serum AGF levels, 1 subject from the GDM group and two subjects from the control group, who showed extremely high or low levels of serum AGF, were excluded. Gestational diabetes was diagnosed according to the Carpenter and Coustan criteria [11].

We used a standardized questionnaire to collect details pertaining to their anthropometrics, family history, medical and obstetric history, and other relevant information. Each subject underwent a complete medical examination, as well as a hematological, hepatic and renal function analysis. None of the patients reported personal history of hypertension, thromboembolic disease, endocrine diseases or cardiovascular events. Body mass index (BMI) we calculated at the time of pregnancy and before pregnancy in all subjects. Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were recorded. Mean arterial pressure (MAP) was calculated as follows: (MAP= DBP+ (SBP-DBP)/3).

Study protocol

In the GDM and the control groups, peripheral venous blood samples were drawn between 8:00 am and 10:00 am after fasting for at least eight hours for measurements of fasting serum insulin, plasma glucose, AGF levels and liver-kidney functions. Sera obtained from all subjects were separated immediately by centrifugation at 4,000 g for 10 min and frozen at -80°C. Serum insulin level was assayed by enzyme-linked immunoassay (ADVIA Centaur, Bayer, Tarrytown, NY, USA). The intra-assay and total coefficients of variation were 6.1% and 7.1% respectively. Insulin resistance score was determined using homeostasis model assessment (HOMA-IR = fasting insulin (mU/L) × fasting glucose (mg/dL)×0.05551/22.5) [12]. Glucose was measured using the hexokinase method at Synchron LX20 systems (Beckman Coulter, Fullerton, Calif) with an intra-measurement coefficient of variance of 0.92–1.54%. Serum levels of AST (aspartate transaminase), ALT (alanine transaminase), BUN (blood urea nitrogen) and creatinine measurements were performed using Synchron LX20 systems (Beckman Coulter, Fullerton, Calif) with the original Beckman Synchron LX system reagents.

The serum ANGPTL6 levels were measured by enzyme-linked immunosorbent assay (Uscn Kit, Life Science Inc., Wuhan, P.R. China). The intra-assay coefficient of variation was 5.4% at 15.6 ng/mL, 4.8% at 62.5 ng/mL, and 3.1% at 125.0 ng/mL. The minimum detectable amount of ANGPTL6 was typically less than 5.8 ng/mL.

Table I. Characteristics of the study population.

	CONTROL group (n=33)	GDM group (n=44)	p-value
Age	27.63±4.44	28.43±4.66	.453
Gravidity	2.33±1.31	2.43±1.53	.768
Parity	1.06±1.08	1.38±1.45	.283
Gestational age, week	33.57±2.82	33.50±2.69	.905
Pregestational weight (kg)	63.84±8.89	66.13±9.81	.296
Gestational weight (kg)	72.36±11.80	77.54±12.25	.066
Pregestational BMI (kg/m2)	24.25±3.17	25.06±3.46	.297
Gestational BMI (kg/m2)	27.46±4.06	29.36±4.23	.051
Systolic blood pressure (mm Hg)	114.55±10.55	118.86±12.52	.114
Diastolic blood pressure (mm Hg)	70.00±8.92	74.20±8.27	.036*
Mean arterial pressure (mm Hg),	84.84±8.65	89.09±9.01	.041*
Fasting glucose(mmol/l)	82.18±11.79	117.59±34.02	.000*
Fasting insulin(U/mL)	8.94±3.66	10.37±6.93	.285
HOMA-IR	1.75±0.63	2.91±2.00c	.002*
AGF(ng/mL)	52.30±35.59	113.30±69.92	.000*
BUN (mg/dL)	13.84±6.38	12.61±4.65	.330
Creatinine (mg/dL)	0.74±0.25	0.79±0.25	.465
Aspartate transaminase (IU/mL)	20.96±6.86	21.38±7.18	.798
Alanine transaminase (IU/mL)	20.18±6.85	20.61±6.71	.783

* Statistically significant, GDM; Gestational diabetes mellitus, BMI; Body mass index,
HOMA-IR; Homeostasis Model of Assessment - Insulin Resistance, AGF; Angiopoietin-related Growth Factor,
BUN; Blood urea nitrogen.

All anthropometric and laboratory parameters were measured at 28-32 weeks. All patients were followed up to the term of the pregnancy; subjects with any diseases except diabetes in the GDM group and subjects with any diseases that complicated the pregnancy (preeclampsia, endocrinologic disease, premature contractions, etc) were excluded.

Statistical Analyses

All data were analyzed using SPSS software version 15.0 (Chicago, IL, USA). The Kolmogorov-Smirnov test we used to determine normal distribution of all variables. All variables are reported as mean ± standard deviation. Differences between groups were evaluated with Student-T test. Correlations were performed using the Pearson's correlation method. Linear regression analysis was also used. A p value of <0.05 was considered as statistically significant in all analyses.

Results

General baseline characteristics of patients with GDM and the control group are were summarized in the Table I. There was no difference between groups with respect to age, parity, gestational age (weeks), weight (kg) in pregnancy and before pregnancy. Mean pre-gestational BMI and gestational BMI were higher in the GDM group when compared to the control group but not significantly (p>0.05). There was no difference between the groups with respect to systolic blood pressure (mm Hg), but diastolic and mean blood pressures were significantly higher in the GDM group than in the control group (p<0.05). Mean fasting blood glucose level (U/ml) and HOMA-IR value were significantly higher in the GDM group than in controls (p<0.05). In addition, mean AGF level (ng/ml) was significantly higher in the GDM group than in the control group (Table I), (p<0.05).

Simple correlations between serum AGF levels and biochemical and anthropometric markers were analyzed by Pearson's correlation test within the control and the GDM groups (Table II). HOMA-IR and insulin showed positive correlation in the GDM group (Table II).

To determine the factors affecting serum AGF levels, linear regression analysis was performed (Table III). Group, HOMA-IR and insulin were analyzed as statistically significant factors affecting the AGF level. AGF level, subjects with gestational diabetes and higher HOMA-IR value showed a positive association, whereas there was no significant association with insulin (p= 0.686), (Table III).

Table II. Correlation between serum AGF levels and biochemical and anthropometric markers.

	CONTROL group (n=33)		GDM group (n=44)	
	r-value	p-value	r-value	p-value
Age	—.063	.729	.173	.262
Gravidity	.044	.810	.133	.389
Parity	—.008	.965	.118	.447
Gestational age	.323	.067	.251	.100
Pregestational weight	—.146	.418	—.173	.260
Gestational weight	—.128	.479	—.102	.509
Pregestational BMI	—.225	.207	—.133	.391
Gestational BMI	—.205	.252	—.056	.717
Systolic blood pressure	.157	.384	.029	.854
Diastolic blood pressure	.298	.092	.020	.896
Mean arterial pressure	.269	.130	.026	.869
Aspartate transaminase	.090	.617	.063	.684
Alanine transaminase	—.078	.667	.050	.745
BUN	—.243	.173	—.093	.549
Creatinine	.140	.438	—.238	.119
Fasting glucose	.073	.687	.214	.162
Fasting insulin	.256	.150	.448*	.002
HOMA-IR	.124	.492	.543*	.000

* Statistically significant, GDM; Gestational diabetes mellitus, AGF; Angiopoietin-related Growth Factor, BMI; Body mass index, BUN; Blood urea nitrogen, HOMA-IR; Homeostasis Model of Assessment - Insulin Resistance,

Discussion

To the best of our knowledge, this is the first study comparing AGF levels between patients with GDM and healthy pregnant controls. In the current study, we demonstrated for the first time that serum AGF levels were significantly higher in patients with GDM than in the control group. Furthermore, statistically significant positive correlation was found between AGF and HOMA-IR and serum insulin level in patients with GDM.

In population studies, pregnant women with gestational diabetes present increased risk of pregnancy-associated hypertension compared with non-diabetic women [8]. Moreover, pregnant patients with hypertension are at increased risk for developing gestational diabetes mellitus [8]. There is no study evaluating the association between GDM and AGF. On the other hand, the relationship between preeclampsia and AGF was evaluated in a single study and serum AGF level was found to be higher in pregnant women with preeclampsia. AGF has an effect on angiogenesis and metabolism [9]. In rats, ablation of AGF has proved to cause accumulation of lipid within the liver and skeletal muscle. These rats presented also higher frequency of insulin resistance and obesity [10]. In transgenic rats, two-fold increase in concentrations of circulating AGF caused elevated energy expenditure and improved insulin sensitivity and lipid profiles [10]. However, contrary to the data obtained from animal studies, an expected favorable effect of AGF on metabolism was not observed in humans. In the studies on patients with diabetes mellitus type 2, serum AGF levels were observed to be higher, although it was expected to be lower in the study hypothesis [14].

In another study, AGF levels were evaluated in 216 individuals with metabolic syndrome. AGF levels were found to

Table III. Linear regression with serum AGF levels.

Independent variable	Unstandardized β	P-value
Gestational diabetes mellitus	18.957	0.006*
Insulin	-0.881	0.686
HOMA-IR	20.995	0.010*

* Statistically significant, AGF; Angiopoietin-related Growth Factor, HOMA-IR; Homeostasis Model of Assessment - Insulin Resistance.

be higher in 69 patients with metabolic syndrome when compared to controls. Another result in that study was a discovered increase in insulin resistance in addition to increased AGF levels in the metabolic syndrome group. It was also shown that an increase in the AGF levels was greater in women [15]. In our study, no assessment was done regarding the relation between AGF and gender, as only women in the third trimester of pregnancy were included. Although an inverse relation was reported between age and AGF in a study evaluating AGF in individuals with metabolic syndrome, no such relation was found in our study. We believe it was caused by the differences between the subjects from that study and from ours, which included only pregnant women of similar ages. Contrary to favorable metabolic effects of AGF in the animal studies, trials on human subjects [14, 15] suggest that there is a negative relationship between insulin resistance and AGF. However, it was shown that there was no association between AGF levels and serum glucose or insulin levels in a study conducted on patients with preeclampsia [13]. In that study, serum AGF levels were found to be higher in preeclamptic patients [13]. The authors proposed a theory that this increase occurs to compensate the effect of impaired angiogenesis and nitric oxide. In that study, another interesting finding was a decrease of the AGF level 6 months after the delivery. In our study, AGF levels were also higher and, actually, impaired glucose tolerance may be the cause of this increase. However, scarcity of data (there have been only 2 studies on this matter so far and they included small numbers of cases) does not allow to draw definitive conclusions.

Namkung et al. reported that HOMA-IR index and BMI showed paradoxical correlation between the groups; being positive in the healthy group and negative in the metabolic syndrome group. They also reported that serum AGF levels were significantly higher in subjects with impaired glucose tolerance than in healthy controls but subjects with diabetes mellitus (β-cell failure) had similar levels compared with controls (data not shown) [15]. In another study, AGF serum levels were significantly increased in diabetic patients. It was also shown that fasting glucose levels had positive predictive effect on AGF levels independent of age, sex, GFR and BMI [14]. Our findings are consistent with these studies. Impaired glucose tolerance is thought to be more primarily effective than ß-cell failure in gestational diabetes mellitus, which is defined as glucose tolerance developing or first recognized during pregnancy [16]. In our study, it was shown that serum AGF levels were significantly higher in subjects with GDM and HOMA-IR value has a positive predictive effect on AGF levels.

Conclusion

There are several limitations to the present study. First, this is a cross-sectional study. Second, sample size is relatively small, particularly in the study group. According to our results, AGF levels were higher in patients with GDM. This does not support the results obtained in animal studies. In order to understand the effects of AGF on metabolism of humans, first one needs to discover the factors affecting AGF levels. After identifying these factors, findings from further studies, including larger sample size, will provide definitive conclusions about metabolic effects of AGF, such as obesity and insulin resistance in humans.

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