

Alarin Level Response to an Oral Glucose Tolerance Test in Subjects with Gestational Diabetes Mellitus

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Abstract

Alarin has been reported to ameliorate insulin resistance in type 2 diabetes mellitus. However, until now, there is no report examined the relationship between alarin and gestational diabetes mellitus (GDM). Therefore, the purpose of this study is to examine the level of alarin in subjects with and without gestational diabetes mellitus. 30 healthy and 30 gestational diabetes mellitus subjects were recruited for this study. A 2-hour 75 g oral glucose tolerance test (OGTT) was performed at 24–28 weeks of pregnancy. GDM were diagnosed using nationally endorsed criteria (fasting [FPG], 1- or 2-hour blood glucose [at least one abnormal value]). Plasma alarin level was measured by ELISA. Plasma alarin levels were significantly higher GDM subjects than in healthy individuals (12. 3±1.4 ng/mL vs 7.8±0.8 ng/mL; $p < .05$). 1- (18. 8±1.9 ng/mL) and 2-hour (24. 2±3.1 ng/mL) alarin levels in women with GDM were significantly increased at OGTT compared with women without GDM [1- (9.4±0.9 ng/mL); 2- (9.7±0.6 ng/mL)]. Plasma alarin positively correlated with FBG, HbA1c, HOMA-IR and insulin). The above results suggest that alarin might be associated in the pathogenesis of gestational diabetes mellitus.

Introduction

The prevalence of gestational diabetes mellitus (GDM) is globally on the increase [1]. GDM is a condition linked with a type of pregnancy-specific glucose intolerance that is less severe than overt diabetes [2]. This disease became an important public health problem in this area [3]. Because women with GDM have certain adverse pregnancy outcomes and their offspring have a greater risk of childhood obesity, glucose intolerance, and diabetes in early adulthood [4].

Even though studies in this field provide clues that genetic predisposition, advanced maternal age, family history of diabetes, overweight or obesity and a type of peptides and protein, related glucose intolerance are associated with GDM, but underline mechanism of GDM is not exactly completely known [2,5]. However, researchers have recently studied to find out whether

there is a link between GDM and certain peptides that improve glucose and insulin sensitivity in human and animals [6-9]. Alarin is one of peptides that improve glucose uptake via the increase in GLUT4 content and insulin sensitivity [10].

Alarin is composed of a 25 amino acid that was first isolated from the gangliocytes of human neuroblastic tumors in 2006 [11]. Alarin name came from its C-terminal serine originating as a splice variant of galanin-like peptide (GALP) mRNA [11]. Alarin was synthesized in the locus coeruleus and the arcuate nucleus (ARC) of the hypothalamus in rats and mice and human salivary gland (manuscript in preparation) [12]. This peptide stimulates secretion of luteinizing hormone and gonadotropin-releasing hormone in murine models as well as decreases insulin resistance of adipocytes in type 2 diabetic rats [13,10]. Alarin also has an antidepressant-like and antimicrobial

function [14,15]. Also, peripheral and intracerebroventricular (ICV) injection of alarin elevated food intake and body weight and glucose uptake in skeletal muscle of diabetic rats [16]. Also, it has been recently reported that alarin level are high in patients with type 2 diabetes and elevated by glucagon-like peptide-1 receptor agonist treatment [17]. As known, there are some similarities between women developing type 2 diabetes and women with GDM [18]. However, to date, no study has investigated the relationship between alarin and GDM so far.

Given above the current state of knowledge about alarin there might be an association between alarin and gestational diabetes mellitus. Therefore, this study was conducted to investigate whether there is an association between alarin and gestational diabetes mellitus.

Materials and Methods

The study was approved by the Kafkas University Local Ethics Committee (March 1, 2019; no: 80576354-050-099/53) before commencement. Universal GDM screening using a 75-g oral glucose tolerance test (OGTT) was applied to diagnose GDM [19]. Thirty healthy and 30 patients who met criteria for GDM were included in this study. Questionnaire details and subsequent screening strategy for GDM, including a sample flowchart of our study, were present in a nationwide multicenter prospective study [20]. The exclusion criteria used in this study were based on the guidelines of IADPSG (2015 version) with some modifications [21,22]. Patients were excluded from the study if they had any of the following risk factors or conditions: Multifetal pregnancies, a body mass index (BMI) ≥ 30 kg/m²; treated with oral hypoglycemic, presentational diabetes, age ≥ 35 years and other details were given our previously published article [23]. Homeostatic Model Assessment for Insulin Resistance (HOMA-IR) was calculated according to the formula: fasting insulin (mU/mL) x fasting glucose (mg/dL)/ (405) [24].

Analysis of Alarin

Alarin (galanin-Like peptide) (Sunred Bioscience, Catalog no: 201-12-5592 Shanghai, CHINA) was analyzed by the ELISA method in accordance with the study procedures specified in the catalogs. The Intra-assay coefficient of variation (CV) and the Inter-Assay (CV) values of the alarin kit were $<10\%$, $<12\%$, respectively. The minimum measurable level of alarin ELISA kit was 0.214 ng/mL. The automatic washer Bio-Tek ELX50 (BioTek Instruments, USA) was used for plate washing while concentration of alarin was read by using the ChroMate Microplate ELISA Reader P4300 instrument (Awareness Technology Instruments, USA) [23].

Statistical Analysis

All analyses were performed using SPSS version.22.0 (SPSS Inc., Chicago, IL, USA). All continuous data are expressed as mean \pm standard deviation. The multiple linear regression analysis was used to compare the FBG, HbA1c, HOMA-IR and alarin. P-values less than 0.05 were considered significant for all the analyses.

Results

Table 1 demonstrates fasting plasma glucose, 1-and 2-hour plasma

glucose levels at OGTT compared with and without GDM. The mean FPG level in control was lower compared with GDM. The glucose levels were significantly higher at 1-hour compared to 2-hour and fasting blood glucose in patient with GDM. In women with GDM, 2-hour glucose levels were on average higher than that of Fasting plasma glucose. GDM patients showed higher insulin, HOMA-IR, TG, and LDL-C values than control group (Table 2).

Table 1: Fasting plasma glucose and a 75 g oral glucose tolerance test results at 24-28 weeks' gestation in women with and without GDM

Parameters	Control	GDM
FPG (mg/dL)	98.8 \pm 10.2	126.9 \pm 12.7
1-HPG (mg/dL)	128.4 \pm 9.7	169.64* \pm 19.6
2-HPG (mg/dL)	102.4 \pm 9.7	148* \pm 12.4

FPG: Fasting Plasma Glucose. GDM: Gestational Diabetes Mellitus. HPG: Hour Plasma Glucose. a Control versus GDM ($p < 0.05$).

Table 2: Comparison of demographic and some biochemical parameters of mothers with and without GDM

Parameters	Control	GDM
Age (years)	31.2 \pm 4.8	30.9 \pm 3.6
BMI (kg/m ²)	28.5 \pm 6.8	29.6 \pm 4.4
Parity	2.2 \pm 0.6	2.1 \pm 0.2
Gravida	2.6 \pm 0.7	2.8 \pm 0.9
Gestational age at OGTT (weeks)	23.88 \pm 2.76	24.41 \pm 2.24
Insulin (μ IU/ mL)	11.81 \pm 5.4	18.66* \pm 7.18
HbA1c (%)	5.6	7.9*
HOMA-IR	3.01 \pm 0.19	5.02* \pm 0.81
TG (mg/dL)	133.2 \pm 52.3	166.9* \pm 67.9
LDL-C (mg/ dL)	124.46 \pm 22.6	142.15* \pm 17.9
HDL-C (mg/ dL)	49.22 \pm 7.18	44.92 \pm 3.11

BMI: Body Mass Index. **GDM:** Gestational Diabetes Mellitus. **HDL-C:** High-Density Lipoprotein Cholesterol. **HPG:** Hour Plasma Glucose. **HOMA-IR:** Homeostatic Model Assessment of Insulin Resistance. **LDL-C:** Low-Density Lipoprotein Cholesterol. **OGTT:** Oral Glucose Tolerance Test. **TG:** Triglyceride. a Control versus GDM ($p < 0.05$).

Alarin levels were lower in control and in patient with GDM before glucose loading at 24-28 week' gestation while after glucose loading alarin levels were significantly elevated in control and in patient with GDM compared with before glucose loading at 24-28 week' gestation ($p < .05$). After glucose loading at 24-28 week' gestation, alarin levels were more substantially increased in patient with GDM compared with control values ($p < .05$), Fig. 1. Blood alarin was also positively correlated with FBG, HbA1c, HOMA-IR and insulin.

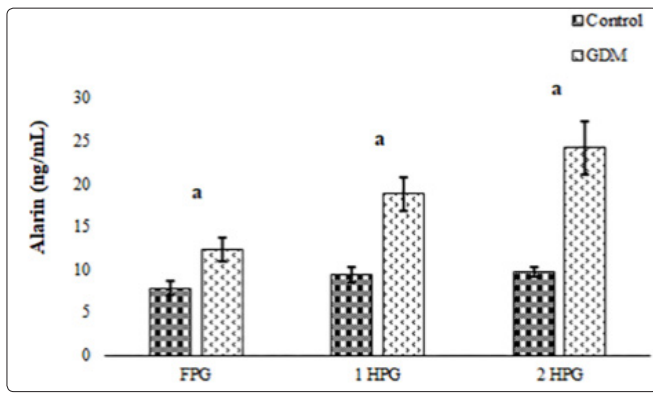


Figure 1: Mean change in alarin level in fasting (FPG), and an oral glucose tolerance test [1-hour (1 HPG) and 2-hour (2 HPG)] at 24–28 weeks' gestation. FPG: Fasting Plasma Glucose. GDM: Gestational Diabetes Mellitus. HPG: Hour Plasma Glucose. aControl versus GDM ($p < 0.05$); a 1-hour (1 HPG) versus 2-hour (2 HPG) ($p < 0.05$).

Discussion

To our knowledge, this is the first study that investigated alarin concentration in response to an oral glucose tolerance test in subjects with gestational diabetes mellitus. FPG levels at 24–28 weeks' gestation were high in subjects with gestational diabetes mellitus. In this study, FPG, a casual plasma glucose or HbA1c of subjects with gestational diabetes mellitus were ≥ 126 mg/dL, ≥ 200 mg/dL, and ≥ 6.5 , respectively. Then, GDM was confirmed with OGTT on a subsequent day. The OGTT were performed after fasting overnight for 10–14 hours. Our glucose results in subjects with gestational diabetes mellitus are in line with previous reports [25]. FPG in subjects with GDM in the later trimesters are generally contradictory, but most previous studies display a decline in FPG by 28–30 weeks' gestation [26–29].

HbA1C levels in subjects with gestational diabetes mellitus have been studied extensively [30–33]. In this work participants with GDM had a higher amount of HbA1C. Based on our present report, and previous reports measurement of HbA1C may be helpful in those with overt diabetes with an HbA1C $> 6.5\%$ [34].

In this study it has been also found those insulin concentration in subjects with gestational diabetes mellitus were higher than that of control subjects. Also, in our study, participants with GDM were more insulin resistant than the control subjects, which gave credit to previous reports [25] according to HOMA-IR values and high insulin levels. As in here if insulin resistance is present, the β -cells attempt to compensate by increasing insulin release, purposing to maintain a normal glucose metabolism [35,36].

Also, it has been previously reported that alarin causes a decrease in insulin resistance of adipocytes in type 2 diabetic rats [10]. Therefore, in this study it was investigated that whether there is any association between alarin and GDM, which is characterized with impaired glucose tolerance and insulin resistance. In this work we measured alarin level in response to a 75 g oral glucose tolerance test in subjects with GDM and without GDM. It was noted that after glucose loading at 24–28 week's gestation, alarin levels were substantially increased in the 1-hour (1 HPG) and 2-hour (2 HPG) in patient with GDM compared with control values ($p < .05$). Alarin is reported to ameliorate insulin resistance and blood glucose in

vivo [10,16]. It is possible that GDM may cause a leading to the increase of alarin secretion to a defensive response, which may represent ability for adaptation to IR or to maintain a normal glucose metabolism in the organism via the increase in GLUT4 content to improve insulin sensitivity [10]. This alarin results are line with previous prediabetes and type 2 diabetes subject studies and women with obese polycystic ovary syndrome (PCOS) subjects [17,37].

The present study has some limitations even though this study is a preliminary study. One of the limitations of this study is that the studied sample size was rather small. Second, all participants were of Turkish origin, which may restrict generalization to other populations.

Conclusion

This is the first study to evaluate alarin in women with GDM. Our results indicated that plasma alarin level was significantly increased after a 75 g oral glucose tolerance test in subjects with GDM and without GDM in the first hour of OGTT and higher in GDM patients than control in both hour of OGTT, indicating a progressive increment of plasma alarin response to glucose concentration. Alarin might be a new sensor of circulated glucose concentration. These findings might help to enlighten the underline mechanism of physiopathology of gestational diabetes mellitus.

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