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The effects of serum granulin levels on anthropometric measures and glucose metabolism in infertile women with different ovarian reserve status

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Abstract

Introduction: Granulin (GRN) is an adipokine with proinflammatory features, which plays important role in glucose metabolism and insulin resistance pathogenesis. It has been reported that granulin precursors were localised in developing follicles in animal studies. The purpose of this study was to evaluate the association of granulin levels with anthropometric features, glucose metabolism, and ovarian reserve.

Material and methods: A total of 109 infertile women were included in this cross-sectional, prospective study, who attended a tertiary clinic. All participants were categorised into diminished ovarian reserve (DOR) and normal ovarian reserve groups (NOR), in accordance with Bologna criteria. The demographic characteristics, including age, BMI, waist-hip circumferences, and biochemical parameters, were recorded. Serum granulin level was determined by enzyme-linked immunosorbent assay.

Results: No significant difference was observed in the GRN levels ($p = 0.229$) between the groups. There was a positive correlation between GRN levels and BMI, WC, HC, and 75 g oral glucose tolerance values in NOR group ($p < 0.01$, $p < 0.05$, $p < 0.01$, and $p < 0.05$, respectively).

Conclusions: Our results suggest that granulin is associated with anthropometric features in infertile patients and might be an important indicator of obesity and impaired glucose metabolism. Elevated levels of granulin may have a diabetogenic effect and predispose women to high glucose levels. **(Endokrynol Pol 2019; 70 (3): 255–259)**

Key words: *granulin; anthropometry; glucose metabolism; ovarian reserve; obesity*

Introduction

Obesity and excessive body weight may affect reproductive health with different mechanisms. In numerous studies it was demonstrated that obesity is associated with poor in vitro fertilisation (IVF) outcomes [1]. It also had a relationship with dyslipidaemia and insulin resistance, as important components of the metabolic syndrome [2]. Furthermore, all of these factors were related with low-grade chronic inflammation process and can lead to cardiovascular diseases [3]. In a recent study, the authors reported that cardiovascular risk markers were increased in patients with diminished ovarian reserve [4]. Decreased oestrogen production due to loss of ovarian functions may increase dyslipidaemia and contribute to adverse effects in glucose and insulin metabolism, body fat distribution, and dysfunction in vascular endothelium [5].

Granulin (GRN), also known as acrogranin and proepithelin, is an adipokine with proinflammatory features. It is thought that elevation of circulating levels may be an important indicator in the development of chronic inflammatory process seen in type 2 diabetes and obesity [6]. It has complex physiological and pathological functions in various conditions including wound healing, tissue remodelling, progression of cell cycle, breast and gynaecological malignancies, and neurodegenerative disorders [7, 8]. In several animal studies it was reported that this glycoprotein possessed oocyte-specific expression, and its precursors were localised in granulosa cells in developing follicles [9]. In a recent study the authors stated that elevated progranulin levels are associated with obesity in infertile women, and progranulin may be a promising therapeutic target to prevent obesity and infertility [10].

As an important adipokine with proinflammatory features and oocyte-specific expression in follicles, we hypothesise that GRN may be associated with glucose metabolism and ovarian reserve in infertile patients. The purpose of this study was to evaluate the levels of granulin in non-polycystic infertile patients and to analyse the relationship between GRN and anthropometric features, glucose metabolism, and ovarian reserve status.

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Material and methods

The cross-sectional study was planned to be carried out at the Hitit University Reproductive Health Centre between January 2016 and September 2016. This study was approved by the Ethics Committee of Ankara Numune Training and Research Hospital and conducted in accordance with the institutional guidelines and Helsinki declaration (reference number: E–15–563). Informed consent was obtained from each of the participants immediately prior to the study.

A total of 109 women seeking fertility assistance were enrolled in this prospective study. In the first step, women with any foreknown systemic chronic diseases (diabetes mellitus, autoimmune and inflammatory diseases), history of pelvic surgery, chemoradiotherapy due to malignancies, current smoking, and drug use that may affect ovarian reserve were excluded. Subsequently, women with PCOS, which may affect anthropometric features and glucose metabolism, were not included in the study. The diagnosis of PCOS was based on the recent Amsterdam ESHRE/ASRM proposal [11].

The participant women who met the inclusion criteria were divided into two groups according to their ovarian status, as normal ovarian reserve (NOR) and diminished ovarian reserve (DOR) groups. The diagnosis of DOR was based on the Bologna criteria of the ESHRE consensus [12]. At least two of the following three characteristics were needed to diagnose the patient as DOR; (i) advanced maternal age (≥ 40 years) or any other risk factor for poor ovarian response; (ii) a previous poor response history (≤ 3 oocytes with a conventional stimulation protocol); (iii) an abnormal ovarian reserve test (i.e. antral follicle counting < 5–7 follicles or anti-Mullerian hormone levels < 0.5–1.1 ng/dL).

First, all anthropometric measurements including height, weight, waist circumference (WC), hip circumferences (HC), and waist-hip circumference ratio (WHR) were performed by the same observer using the same method with the same scale. Body mass index (BMI) was calculated as weight (kg)/height^2 (m^2). Both groups were compared in terms of their anthropometric features.

For assessment of serum follicle stimulating hormone (FSH), luteinising hormone (LH), oestradiol (E2), anti-Mullerian hormone (AMH) and granulin levels, venous blood samples (5 ml) were collected from the participants in the morning, after an approximately eight-hour fasting period on their early follicular phase. Following collection, samples were centrifuged at 3000 rpm for 20 minutes, and separated plasma for AMH and GRN were stored at –80°C until analysis. The samples were analysed on a daily basis for E2, FSH, and LH using electrochemiluminescence immunoassay (ECLIA) method with an auto-analyser (Cobas 6000, E 601 Roche Diagnostics, GmbH, Mannheim, Germany). AMH samples were measured by the same method with an auto-analyser (Cobas 6000, E 601 Roche Diagnostics, GmbH, Mannheim, Germany). The GRN levels were measured using enzyme-linked immunosorbent assay (ELISA) method and granulin assay kits (Biotek Synergy HT, Cloud-Clone Corp., Houston, USA). Comparisons and correlation analyses of all data were performed.

Statistical analyses

All data analyses were performed using SPSS (Statistical Packages for the Social Sciences) software, version 22 (SPSS Inc., Chicago, USA). The Kolmogorov-Smirnov and Shapiro-Wilk tests were used to test normality of distribution. Continuous parameters were presented as mean ± standard deviation (SD). Continuous variables were compared using independent samples t-test due to normally distributed data. Pearson or Spearman correlation analysis, where appropriate, was used to test whether granulin showed any significant linear relationship with other study parameters. A p value less than 0.05 was considered as statistically significant.

Results

Eighty-four women with NOR and 25 women with DOR were included in this study. The demographic and anthropometric characteristics are shown in Table I. As expected, the mean age of the DOR group was higher than the mean age of the control group (37.4 *vs.* 30.0, $p < 0.001$). There was no significant difference between the two groups for the anthropometric features, including BMI, WC, HC, and WHR ($p > 0.05$, for all). While serum E2 and LH levels were comparable between the groups, higher FSH and lower AMH levels were observed in the DOR group (7.21 ± 1.89 *vs.* 9.15 ± 2.68 and 2.93 ± 1.85 *vs.* 1.38 ± 2.12, $p < 0.001$, respectively). No significant difference was observed between the GRN levels ($p = 0.229$) in both groups.

Table I. *Baseline anthropometric and biochemical characteristics of the participant women*

	DOR group (n = 25)	NOR group (n = 84)	p
Age [years]	37.4 ± 3.4	30.0 ± 5.7	**0.000****
BMI [kg/m^2]	25.5 ± 4.3	26.6 ± 5.1	0.443
WC [cm]	89.6 ± 9.1	89.8 ± 11.7	0.947
HC [cm]	105.1 ± 6.7	105.4 ± 10.4	0.878
WHR	0.9 ± 0.1	0.9 ± 0.1	0.789
FSH [IU/mL]	9.2 ± 2.7	7.2 ± 1.9	**0.001****
LH [IU/mL]	6.7 ± 3.0	5.8 ± 2.1	0.240
E_2 [pg/mL]	47.7 ± 25.8	46.8 ± 26.1	0.957
AMH [ng/mL]	1.4 ± 2.1	2.9 ± 1.9	**0.000****
Fasting glucose [mg/dL]	99.3 ± 39.9	91.8 ± 12.2	0.773
75-g OGTT [mg/dL]	129.1 ± 79.3	105.1 ± 32.4	0.119
Granulin [ng/mL]	3.1 ± 1.2	2.7 ± 1.1	0.141

DOR — diminished ovarian reserve; NOR — normal ovarian reserve; BMI — body mass index; WC — waist circumference; HC — hip circumference; WHR — waist–hip ratio; FSH — follicle-stimulating hormone; LH — luteinising hormone; E_2 — oestradiol; AMH — anti-Mullerian hormone; OGTT — oral glucose tolerance test. Values are expressed as mean ± SD; **p < 0.01

Table II. *Correlation analysis of granulin with other parameters according to ovarian reserve status*

	Granulin levels	
	DOR group	**NOR group**
Age [years]	0.301	0.033
BMI [kg/m^2]	0.066	**0.288****
WC [cm]	0.119	**0.324****
HC [cm]	–0.339	**0.266***
WHR	0.383	0.206
FSH [IU/mL]	0.186	–0.113
LH [IU/mL]	0.055	–0.171
E_2 [pg/mL]	0.185	–0.091
AMH [ng/mL]	–0.388	0.053
75-g OGTT [mg/dL]	0.168	**0.254***

DOR — diminished ovarian reserve; NOR — normal ovarian reserve; BMI — body mass index; WC — waist circumference; HC — hip circumference; WHR — waist–hip ratio; FSH — follicle-stimulating hormone; LH — luteinising hormone; E_2 — oestradiol; AMH — anti-Mullerian hormone; OGTT — oral glucose tolerance test

Table III. *Correlation analysis of granulin levels with study parameters*

	r	p
Age [years]	0.154	0.111
BMI [kg/m^2]	**0.191**	**0.047***
WC [cm]	**0.257**	**0.007****
HC [cm]	0.145	0.134
WHR	**0.220**	**0.022***
FSH [IU/mL]	0.021	0.830
LH [IU/mL]	–0.076	0.432
E_2 [pg/mL]	–0.019	0.848
AMH [ng/mL]	–0.107	0.270
75-g OGTT [mg/dL]	**0.228**	**0.017***

BMI — body mass index; WC — waist circumference; HC — hip circumference; WHR — waist–hip ratio; FSH — follicle-stimulating hormone; LH — luteinising hormone; E_2: — oestradiol; AMH — anti-Mullerian hormone; OGTT — oral glucose tolerance test. *$p < 0.05$, **$p < 0.01$

For each group, the relationship of GRN with other parameters were analysed with Spearman-Brown correlation, and the results are shown in Table II. There was a weak positive correlation between GRN levels and BMI, WC, and HC in the NOR group ($r = 0.288$; $p < 0.01$, $r = 0.324$; $p < 0.05$ and $r = 0.266$; $p < 0.01$, respectively). However, no significant correlation was found between these parameters in patients with DOR ($r = –0.066$; $p > 0.05$, $r = 0.119$; $p > 0.05$, $r = –0.339$; $p > 0.05$). In addition, there was a weak positive correlation between serum granulin concentration and 75 g oral glucose tolerance (OGTT) values in the NOR group ($r = 0.254$; $p < 0.05$). However, in the DOR group, no significant correlation was found between glucose tolerance test results and GRN levels ($r = 0.167$; $p > 0.05$).

The correlation analysis of the GRN levels with the parameters in the study independent from ovarian reserve status is shown in Table III. A significant weak positive correlation was found between GRN and BMI, WC, WHR, and 75-g OGTT ($r = 0.191$; $p < 0.05$, $r = 0.257$; $p < 0.01$, $r = 0.220$; $p < 0.01$, $r = 0.228$; $p < 0.05$, respectively). No significant correlation was found between serum GRN level and age.

Discussion

In the present study, we focused on serum GRN concentrations, a novel adipokine with inflammatory and endocrine properties, and its association with anthropometric features, and glucose metabolism in women with non-polycystic infertile patients.

Granulin is a prominent adipokine and its association with reproductive biology has been indicated in several studies. Suzuki et al. reported that GRN acted as an autocrine stimulator during the oocyte maturation process [9]. In another animal study, Vendola et al. suggested that GRN may augment early follicle development, similarly to the androgenic effect in monkeys [13]. Other studies also suggest that GRN and its precursors play a major role in the development of ovarian premature follicle [14]. Although GRN was thought to be effective on ovarian folliculogenesis and embryogenesis, we did not find a significant correlation between granulin and ovarian reserve tests. Similar to our results, Ersoy et al. also did not find any association between GRN and FSH levels in patients with premature ovarian failure (POF) [15]. The authors therefore suggested that the association of GRN with ovarian failure may be encountered as a result of POF, regardless of the impact of FSH or ovarian reserve tests.

In our study, we found a significant relationship between GRN and BMI, WHR, and glucose tolerance test regardless of ovarian reserve status. Recently, it was found that in peripheral tissues, excessive limits of GRN are closely linked to obesity and insulin resistance [16]. In another study, the authors reported that circulating GRN levels were positively correlated with body mass index and glucose levels [17]. As a result, it was thought that levels of this adipokine might increase due to uncontrolled expansion in adipose, and this condition might provoke obesity by stimulating angiogenesis and promoting growth in target tissue [16]. Although measurement of GRN levels from these studies seems useful in predicting inflammation and obesity-related metabolic diseases, further studies are

needed to determine if measuring GRN levels will be useful as a reliable biomarker of metabolic syndrome.

Interestingly, the present results also demonstrated that serum GRN levels did not have any relationship with age, regardless of ovarian reserve status. Similar to our results, Ersoy et al. also did not find a correlation between age and serum GRN levels [15]. When the relationship between GRN levels and age was examined according to ovarian reserve status, there was a significant relationship between age and granulin in normoresponder patients. Considering that patients with normal ovarian reserve are younger than poor responders, it is thought that granulin may be involved in ovarian response physiology, especially in early age. As shown in plants, GRN levels gradually decrease with aging [18]. In light of these findings, Ersoy et al. proposed that GRN levels may be an indicator for senescence of the human ovary, rather than human life senescence [15]. However, the role of GRN in the mechanism of this topic remains unclear.

The relationship between GRN and PCOS and its possible metabolic effects has been previously evaluated in many studies. It is known that insulin resistance plays an important role in PCOS pathophysiology. In a recent study from Turkey, higher progranulin levels in women with PCOS were found compared with non-PCOS patients [19]. The authors proposed that cysteine-rich growth factors like progranulin may cause excessive early follicular growth as androgens. Zhou et al. also investigated the possible effects of progranulin in PCOS patients and found higher progranulin levels in follicular fluid [20]. These findings suggest that progranulin may be used as a marker for insulin resistance in the follicular compartment in polycystic patients.

The strengths of our study include its prospective and multivariable design, being the first to compare serum GRN levels in patients with different ovarian reserve status, and evaluating the effects of GRN on glucose metabolism and anthropometric features regardless of ovarian reserve. Our findings are limited by its relatively small sample size, and further studies with larger groups are warranted. In addition, it is possible to criticise why insulin level measurements and lipid profile-related values are not investigated in order to calculate homeostatic model assessment for the insulin resistance (HOMA-IR) index. Because these data were not investigated, the feasibility of GRN levels in predicting cardiovascular risk factors could not be investigated.

Conclusions

We demonstrated a positive correlation between serum GRN concentration and anthropometric measures in the NOR group. However, our findings did not support any correlation of GRN with these measures in women with DOR. Interestingly, there was a positive correlation between serum GRN level and 75-g OGTT only in the NOR group. In other words, it is possible to propose that elevated GRN levels may have a diabetogenic effect and predispose women to high serum glucose levels. Further large-sized prospective studies are needed to elucidate the role of GRN on glucose metabolism.

Conflict of interest

The authors declare that they have no conflict of interest.

Authors' contributions

OK and UG analysed the data. OK wrote the paper. UG conceived, designed, and performed the experiments. UG critically reviewed the final version of the article. All authors read and approved the final manuscript.

Ethical approval

This study was approved by the local Ethics Committee and the Institutional Review Board.

Informed consent

Written, informed consent was obtained from all participants.

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