J Obstet Gynecol Womens Health 2018
Volume 1: 1

Journal of Obstetrics, Gynecology and Women's Health

Are Serum Granulin Levels Associated with Diminished Ovarian Reserve Status?

Umit Gorkem[1*]
Ferit Kerim Kucukler[2]
Cihan Togrul[1]
Ozgur Kocak[1]
Emine Arslan[1]

[1]*Department of Obstetrics and Gynecology, Faculty of Medicine, Hitit University, Corum Turkey*
[2]*Department of Endocrinology, Faculty of Medicine, Hitit University, Corum Turkey*

Abstract

Background: Only a few studies studies reported that granulin might provide various reproductive functions in the known literature. We aimed to investigate association of serum granulin levels with diminished ovarian reserve status.

Study design: This prospective cross-sectional study included a total of 111 infertile women admitting for fertility problems. The women were categorized into two groups according to their ovarian reserve statuses; 1-Normal ovarian reserve group (NOR, control group, n=72), and 2-Diminished ovarian reserve (DOR, n=39) group. The initial examination included the measurements of weight, height, waist circumference (WC) and hip circumference (HC) to calculate body mass index (BMI), and waist/hip ratio (WHR).

Results: There was no significant difference between both groups for the anthropometric measurements, including BMI, WC, HC and WHR ($p>0.05$, for all). Although the women with DOR had higher FSH levels ($p<0.001$), serum AMH concentrations were higher in the control group ($p<0.001$). The mean granulin level did not differ significantly in DOR and the control groups ($p=0.229$). The serum granulin levels in the normal ovarian reserve group correlated positively and significantly with BMI, WC and HC ($r=0.320$ and $p=0.006$, $r=0.257$ and $p=0.029$, and $r=0.243$ and $p=0.040$, respectively). No correlation between granulin and all study parameters in women with DOR was demonstrated.

Conclusion: There is no relationship of serum granulin level with DOR. Although the granulin level is correlated with anthropometric measurements including BMI, WC and HC in women with NOR, those correlations do not exist in women with DOR.

Keywords: Granulin, Progranulin, Growth factor, Diminished, Ovarian reserve.

Background

Ovarian ageing is revealed by diminished ovarian reserve (DOR), and it has been blamed for age-related decline in fertility [1,2], increase in adverse reproductive outcomes such as miscarriages [3], and aneuploid gestations [4]. Ovarian ageing is one of the main factors that influences the outcome of *in-vitro* fertilization (IVF). Females with DOR usually respond to controlled ovarian stimulation poorly. This results in production of fewer oocytes, poorer quality embryos, and hence reduced implantation and pregnancy rates. The incidence of poor ovarian response, as a measure of reduced ovarian reserve, has been reported between 9 and 24% [5]. However, this rate tends to increase due to overall rise in the number of women that postpone conceiving to their 30s or 40s [6].

Article Information

Article Type: Research
Article Number: JOGWH102
Received Date: 06 September, 2018
Accepted Date: 09 October, 2018
Published Date: 15 October, 2018

***Corresponding author:** Dr. Umit Gorkem, Department of Obstetrics and Gynecology, Faculty of Medicine, Hitit University, Corum Turkey. Fax: +903642251642; Tel: +905333479942; Email: drumitgorkem(at)hotmail.com

Citation: Gorkem U, Kucukler FK, Togrul C, Kocak O, Arslan E (2018) Are Serum Granulin Levels Associated with Diminished Ovarian Reserve Status? J Obstet Gynecol Womens Health Vol: 1, Issu: 1 (07-10).

Granulin is also known as progranulin, acrogranin, proepithelin, and PC cell–derived growth factor. It is a secreted protein, and it has important functions in various processes, including immune response and development of embryo [7]. Granulin is a glycoprotein consisting of 593 amino acids, and its mRNA is expressed in a number of epithelial cells both *in vitro* and *in vivo*. Autosomal dominant mutations of progranulin gene result in frontotemporal dementia [8,9], On the other hand, overexpression of granulin helps invasive progression of some tumors, including breast and brain neoplasms [10]. It was reported that granulin overexpression caused resistance of breast cancer cells to tamoxifen [11]. This suggests that autocrine cancer-cell regulation of progranulin plays a significant role in tumorigenesis of breast cancer [12]. It has also been reported that progranulin mRNA is expressed in the inflammatory infiltrate in transcutaneous murine puncture wounds, and it is highly induced in fibroblasts of skin and endothelium after injury [13]. Granulin also increases accumulation of neutrophils, macrophages, blood vessels, and fibroblasts in cutaneous wounds [14], and this suggests that it may act as a chemotactic protein for myeloid originated cell types, and a factor of angiogenesis.

Only a few studies up to date have investigated associations of granulin with different ovarian reserve patterns. In this study, we aimed to investigate associations of serum granulin levels with DOR status.

Methods

The women included into the current study were the patients that admitted to the Reproductive Endocrinology Department of Hitit University Hospital for fertility problems between January and September 2016. This prospective cross-sectional study was approved by the Institutional Review Board of Ankara Numune Education and Research Hospital in accordance with the Declaration of Helsinki, 2013 Brazil version (E-15-563). All participants provided their written informed consents prior to the study. The exclusion criteria were history of pelvic surgery, chemotherapy and/or radiotherapy, ovarian masses, endometriosis, severe systemic diseases including diabetes mellitus, cardiovascular, renal, hepatic, autoimmune and endocrine disorders, use of drugs that are likely to affect ovarian functions, smoking, alcohol consumption, pregnancy and lactation.

A total of 111 infertile women who met the inclusion criteria were included in this study. The women were categorized into two main groups in relation with their ovarian reserve statuses. 72 women were classified as the normal ovarian reserve group (NOR, control group), and 39 consecutive women were diagnosed as diminished ovarian reserve (DOR) group. The diagnosis of DOR was based on Bologna criteria of ESHRE consensus [15]. At least two of following three characteristics are needed to diagnose the patient as DOR; (i) advanced maternal age (≥ 40 years) or any other risk factor for poor ovarian response (POR); (ii) a previous POR (≤ 3 oocytes with a conventional stimulation protocol; (iii) an abnormal ovarian reserve test (i.e. antral follicle counting < 5-7 follicles or antimullerian hormone <0.5-1.1 ng/dL). We missed the antimullerian hormone item for the study process.

The initial examination of women included the measurements of weight, height, waist circumference (WC) and hip circumference (HC) to calculate body mass index (BMI), and waist/hip ratio (WHR). BMI was calculated as weight (kg) / height^2 (m^2). WC was measured at the mid-point of the lowest margin of 12th rib and the lateral iliac crest during normal expiration. HC was measured at the maximum extension of the major trochantaries. All anthropometric measurements were made with the same scale, and by the same observer.

Venous blood samples were obtained from the antecubital veins in all participants in the morning, between 08 AM and 10 AM, after an eight-hour fasting period, on menstrual days 2–5 for estradiol (E2) and follicle stimulating hormone (FSH), luteinizing hormone (LH), antimullerian hormone (AMH) and granulin. The samples were allowed to clot completely at room temperature, and then they were centrifuged within 30 min at 3000 rpm for 20 min. The serums for AMH and granulin measurements were frozen at -80°C until analysis within 2 h of collecting the blood samples. The serums were analyzed on a daily basis for E2, FSH, LH, using electrochemiluminescence immunoassay (ECLIA) method with an auto-analyzer (Cobas 6000, E 601 Roche Diagnostics, GmbH, Mannheim, Germany). The AMH samples were measured by the ECLIA method with an auto-analyzer (Cobas 6000, E 601 Roche Diagnostics, GmbH, Mannheim, Germany). The granulin levels were measured using enzyme linked immunosorbent assay (ELISA) method and granulin assay kits (Biotek Synergy HT, Cloud-Clone Corp., Houston, USA). The comparisons and correlation analyses of all data were performed.

Statistical Analysis

All data analyses were performed using SPSS (Statistical Packages for The Social Sciences) software, version 21 (SPSS Inc. Chicago, USA). The Kolmogorov-Smirnov test was 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. Spearman correlation 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

The anthropometric and biochemical parameters in DOR and NOR groups are demonstrated in Table 1. As expected, the mean age of DOR group was higher than the mean age of the control group ($p<0.001$). There was no significant difference between two groups for the anthropometric measurements, including BMI, WC, HC and WHR ($p>0.05$, for all). The serum E2 and LH levels were similar in all women ($p=0.070$ and $p=0.061$, respectively). Although the women with DOR had higher FSH levels ($p<0.001$), serum AMH concentrations were higher in the control group ($p<0.001$). The mean granulin level did not differ significantly in DOR and the control groups ($p=0.229$).

Table 1: Comparisons of anthropometric and biochemical characteristics among women with different ovarian reserve patterns (N=111).

	Normal ovarian reserve group (n=72, 64.9%)	Diminished ovarian reserve group (n=39, 35.1%)	p
Age (year)	29.3 ± 5.7	36.0 ± 4.3	0.000*
BMI (kg/m^2)	26.5 ± 5.3	26.0 ± 4.3	0.630
WC (cm)	89.9 ± 12.0	89.3 ± 9.9	0.779
HC (cm)	105.2 ± 10.8	105.3 ± 8.4	0.939
WHR	0.9 ± 0.1	0.9 ± 0.1	0.607
E2 (pg/mL)	43.8 ± 21.0	53.0 ± 32.1	0.070
FSH (IU/L)	6.8 ± 1.2	9.4 ± 2.8	0.000*
LH (IU/L)	5.7 ± 2.0	6.6 ± 2.7	0.061
AMH (ng/dL)	3.3 ± 1.8	1.0 ± 0.4	0.000*
Granulin (ng/mL)	2.7 ± 1.1	3.0 ± 1.0	0.229

Values are expressed as the mean ± standard deviation. BMI: Body Mass Index, WC: Waist Circumference, HC: Hip Circumference, WHR: Waist/Hip Circumference Ratio, E2: Estradiol, FSH: Follicle Stimulating Hormone, LH: Luteining Hormone, AMH: Antimullerian Hormone, PRGN: Progranulin. *p-values indicate statistically significant (p<0.05).

Table 2: Spearman's correlations of Granulin with study parameters in women with normal and diminished ovarian reserve.

	Normal ovarian reserve group		Diminished ovarian reserve group	
	Spearman's rho	p	Spearman's rho	p
Age (year)	0.082	0.494	0.076	0.646
BMI (kg/m^2)	0.320	0.006*	-0.100	0.544
WC (cm)	0.257	0.029*	-0.009	0.956
HC (cm)	0.243	0.040*	-0.245	0.133
WHR	0.145	0.226	0.247	0.130
E2 (pg/mL)	-0.111	0.355	0.154	0.351
FSH (IU/L)	-0.116	0.331	0.159	0.332
LH (IU/L)	-0.118	0.324	-0.004	0.981
AMH (ng/dL)	0.020	0.866	-0.168	0.306

Values are expressed as the mean ± standard deviation. BMI: Body Mass Index, WC: Waist Circumference, HC: Hip Circumference, WHR: Waist/Hip Circumference Ratio, E2: Estradiol, FSH: Follicle Stimulating Hormone, LH: Luteining Hormone, AMH: Antimullerian Hormone, PRGN: Progranulin. *p-values indicate statistically significant (p<0.05).

correlation analysis revealed that the serum granulin levels in the normal ovarian reserve group correlated positively and significantly with BMI, WC and HC (r=0.320 and p=0.006, r=0.257 and p=0.029, and r=0.243 and p=0.040, respectively), as shown in Table 2. However, we did not observe any correlation between granulin and all study parameters in women with DOR (Table 2).

Discussion

Our results showed that serum granulin levels did not differ between women with NOR and DOR. Only a few studies investigated the role of granulin on reproduction in the known literature. Several studies reported that granulin might provide various reproductive functions [16,17]. Suzuki et al. have suggested that granulin is an oocyte-secreted growth factor in late phases of maturation, and may act as an autocrine stimulator for maturation of oocytes [16]. The authors also emphasized the role of growth differentiation factor-9, a highly specific oocytic molecule modulating growth and differentiation of the early ovarian follicles [18].

Granulin has a number of characteristics that play role in other reproduction processes. In an experimental study on monkeys, Vendola et al. reported that granulin may cause excessive early follicular growth, similar to androgens [19]. In fact, granulin and cystein-rich growth factors have been reported to contribute early ovarian follicle development in ovary [20,21]. Moreover, it was suggested that granulin might play a part in interaction of oocyte and sperm, since acrosome of spermatozoa contains progranulin [17].

In our study, although the granulin levels did not show any correlation with the age of the patients, it was considered that it might be involved in senescence, similar to what happens in plants and other animals, probably independent of aging. Granulin levels gradually decrease with aging in plants. The granulin levels of POF patients are lower than the healthy individuals [22]. Those findings and the results of Suzuki et al. showing oocyte-specific expression of the granulin precursor, Progranulin, made us to suggest that the level of Granulin may be a marker for senescence of the human ovary, rather than senescence of human life [16].

In a study by Ersoy et al. it was reported that granulin levels were significantly lower in the patients with premature ovarian failure (POF) compared to the control group. However, their study group was not identical with ours. The authors suggested that granulin could be a candidate biomolecule that could have a potential for providing understanding of POF pathogenesis [23].

Our study has some limitations. Firstly, the sample size is small. Therefore, nonsignificant associations of granulin with some variables could have been found statistically significant if the sample size was larger. Secondly, our study included infertile women, not a normal women population.

In conclusion, we did not find any relationship of serum granulin level with DOR. Although the granulin level is correlated with anthropometric measurements including BMI, WC and HC in women with NOR, those correlations do not exist in women with DOR. Further prospective larger studies are needed to investigate the role of granulin on ovarian reserve and reproductive functions.

Declarations

Ethics approval and consent to participate: This prospective cross-sectional study was approved by the Institutional Review Board of Ankara Numune Education and Research Hospital (E-15-563).

Consent for publication

All participants provided their written informed consents prior to the study.

Availability of data and material

All data generated or analyzed during this study are included in this published article.

Competing interests

The authors declare that they have no competing interests.

Funding

This research was supported by the Scientific Research Unit of Hitit University, Corum, Turkey (TIP19001.14.005).

Authors' contribution

Umit Gorkem designed and performed the study. F Kerim Kucukler analyzed and interpreted the patient data. Cihan Togrul performed the statistical analysis. Nafiye Yılmaz supervised the study. All authors read and approved the final manuscript.

Acknowledgements

The authors acknowledge the staff of Scientific Research Unit of Hitit University, Corum, Turkey.

References

1. Menken J, Trussell J, Larsen U (1986) Age and infertility. Science. 233: 1389-1394.
2. Templeton A, Morris JK, Parslow W (1996) Factors that affect outcome of *in-vitro* fertilisation treatment. Lancet. 348: 1402-1406.
3. Trout SW, Seifer DB (2000) Do women with unexplained recurrent pregnancy loss have higher day 3 serum FSH and estradiol values?. Fertil Steril. 74: 335-337.
4. Freeman SB, Yang Q, Allran K, Taft LF, Sherman SL, et al. (2000) Women with a reduced ovarian complement may have an increased risk for a child with Down syndrome. Am J Hum Genet. 66: 1680-1603.
5. Keay SD, Liversedge NH, Mathur RS, Jenkins JM (1997) Assisted conception following poor ovarian response to gonadotrophin stimulation. Br J Obstet Gynaecol. 104: 521-527.
6. Patrizio P, Vaiarelli A, Levi Setti PE, Tobler KJ, Shoham G, et al. (2015) How to define, diagnose and treat poor responders? Responses from a worldwide survey of IVF clinics. Reprod Biomed Online. 30: 581-592.
7. Tolkatchev D, Malik S, Vinogradova A, Wang P, Chen Z, et al. (2008) Structure dissection of human progranulin identifies well-folded granulin/epithelin modules with unique functional activities. Protein Sci. 17: 711-724.
8. Le Ber I, Camuzat A, Hannequin D, Pasquier F, Guedj E, et al. (2008) French research network on FTD/FTD-MND: phenotype variability in progranulin mutation carriers: a clinical, neuropsychological, imaging and genetic study. Brain. 131: 732-746.
9. Gijselinck I, Van der Zee J, Engelborghs S, Goossens D, Peeters K, et al. (2008) Progranulin locus deletion in frontotemporal dementia. Hum Mutat. 29: 53-58.
10. Eriksen JL, Mackenzie IR (2008) Progranulin: normal function and role in neurodegeneration. J Neurochem. 104: 287-297.
11. Tangkeangsirisin W, Hayashi J, Serrero G (2004) PC cell-derived growth factor mediates tamoxifen resistance and promotes tumor growth of human breast cancer cells. Cancer Res. 64: 1737-1743.
12. Serrero G (2003) Autocrine growth factor revisited: PC-cell-derived growth factor (progranulin), a critical player in breast cancer tumorigenesis. Biochem Biophys Res Biochem Biophys Res Commun. 308: 409-413.
13. He Z, Ong CH, Halper J, Bateman (2003) A Progranulin is a mediator of the wound response. Nat Med. 9: 225-229.
14. He Z, Bateman A (2003) Progranulin (granulin-epithelin precursor, PC-cell derived growth factor, acrogranin) mediates tissue repair and tumorigenesis. J Mol Med (Berl). 81: 600-612.
15. Ferraretti AP, La Marca A, Fauser BC, Tarlatzis B, Nargund G, et al. (2011) ESHRE working group on poor ovarian response definition. ESHRE consensus on the definition of 'poor response' to ovarian stimulation for in vitro fertilization: the Bologna criteria. Hum Reprod. 26: 1616-1624.
16. 16: Suzuki M, Matsumuro M, Hirabayashi K, Ogawara M, Takahashi M, et al. (2000) Oocyte-specific expression of granulin precursor (acrogranin) in rat ovary. J Reprod Dev. 46: 271-277.
17. Baba T, Hoff HB, Nemoto H, Lee H, Orth J, et al. (1993) Acrogranin, an acrosomal cysteine-rich glycoprotein, is the precursor of the growth-modulating peptides, granulins, and epithelins, and is expressed in somatic as well as male germ cells. Mol Reprod Dev. 34: 233-243.
18. McGrath SA, Esquela AF, Lee SJ (1995) Oocyte-specific expression of growth/differentiation factor-9. Mol Endocrinol. 9: 131-136.
19. Vendola KA, Zhou J, Adesanya OO, Weil SJ, Bondy CA, et al. (1998) Androgens stimulate early stages of follicular growth in the primate ovary. J Clin Invest. 101: 2622-2629.
20. Wang J, Van Damme P, Cruchaga C, Gitcho MA, Vidal JM, et al. (2010) Pathogenic cysteine mutations affect progranulin function and production of mature granulins. J Neurochem. 112: 1305-1315.
21. Hsueh AJ, Kawamura K, Cheng Y, Fauser BC (2015) Intraovarian control of early folliculogenesis. Endocr Rev. 36: 1-24.
22. Yamada K, Matsushima R, Nishimura M, Hara-Nishimura I (2001) A slow maturation of a cysteine protease with a granulin domain in the vacuoles of senescing Arabidopsis leaves. Plant Physiol. 127: 1626-1634.
23. Ersoy AO, Oztas E, Ersoy E, Ozler S, Ergin M, et al. (2015) Granulin levels in patients with idiopathic premature ovarian failure. Eur J Obstet Gynecol Reprod Biol. 193: 108-110.

Citation: Gorkem U, Kucukler FK, Togrul C, Kocak O, Arslan E (2018) Are Serum Granulin Levels Associated with Diminished Ovarian Reserve Status? J Obstet Gynecol Womens Health Vol: 1, Issu: 1 (07-10).