ISSN: 2576-2842

Research Article

Journal of Gynecology & Reproductive Medicine

# Alarin/FSH Ratio Might Be A New Biological Marker in Polycystic Ovary Syndrome Women with Normal and Women with Obese

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**Submitted**: 19 Oct 2019; **Accepted**: 24 Oct 2019; **Published**: 30 Oct 2019

## Abstract

*Neuropeptides coordinate and regulate physiological processes in all animals. Alarin is a 25 amino acid neuropeptide which promotes the secretion of luteinizing hormone (LH). It has been known that serum luteinizing hormone levels are increased in women with polycystic ovary syndrome. Therefore, purpose of this was to examine the association of circulating gonadotropin secretions, and alarin with women with polycystic ovary syndrome, and to compare these findings with those of control subjects in an effort to better understand the pathophysiology of PCOS. 28 participants with a diagnosis of PCOS with normal weight and 28 participants with a diagnosis of PCOS with obese and 28 control group participants were included in this case-control study. Hormone profiles of the participants (alarin, insulin, estradiol (E2), follicle-stimulating hormone (FSH), luteinizing hormone (LH), dehydroepiandrosterone sulfate (DHEA-SO$_4$), lipid profiles total testosterone, low-density lipoprotein (LDL), high-density lipoprotein (HDL), triglyceride, cholesterol) and fasting blood sugar (FBS) values were measured. Results: Serum androgens were elevated in the PCOS. Blood LH was also elevated ($P < 0.05$) but was higher in PCOS than Control. Patients with PCOS had an increased alarin compared with controls. LH/FSH ratio and Alarin /FSH ratio were greater than 2.1, 2.4, respectively. The blood alarin levels were significantly correlated with the serum LH levels ($r=0.492$, $p=0.002$) and the LH/FSH ratios ($r=0.450$, $p<0.001$) and Alarin/ FSH ratios. The FSH/LH and alarin /FSH ratio were elevated in the PCOS. Based on these results, the FSH/LH and Alarin /FSH ratio appears to be a useful marker of PCOS.*

## Introduction

Polycystic ovary syndrome (PCOS) is a hormonal disorder that affects up to 5% to 10% of women during their childbearing years (ages 15 to 44) [1]. PCOS affects a woman's ovulation and ovaries, the reproductive organs that produce androgens, estrogen and progesterone hormones that regulate the menstrual cycle. The presence of two of three of the following criteria is currently sufficient to diagnose PCOS: oligo or anovulation, clinical (like acne, male-pattern balding, or extra hair growth on face, chin, or body), and/or biochemical signs of higher levels of androgen (male hormones), and/or cysts in ovaries as demonstrated in an ultrasound exam after exclusion of other etiologies. Ultrasound criteria consist of the presence of 12 or more follicles in either ovary measuring 2-9 mm in diameter and/or elevated of ovarian volume (>10 cm$^3$) [2, 3].

Inappropriate gonadotropin secretion, including elevated luteinizing hormone (LH) concentrations, low-normal follicle-stimulating hormone (FSH) levels, and elevated LH/FSH ratios are clinically meaningful in women with PCOS [4]. It has been also shown that alarin hormone has a link between metabolism and reproduction [5]. For example, the central nervous system infusions of alarin stimulate the secretion of both gonadotropin-releasing hormone and LH in female rats and induces sexual behavior in males [6]. Recent studies showed that the intracerebroventricular (ICV) and peripheral injection of alarin leads to elevation of food intake and body weight, and alarin stimulated glucose uptake mediated by activation of the Akt pathway in muscle tissues of insulin resistance (IR) rats [7]. IR is a key feature of both obese and lean PCOS. IR presents in 70-95% of women with obese PCOS and 30-75% of women with lean PCOS [8, 9]. Too much insulin is an underlying physiological driver of PCOS [8].

Based on above information and to the best of our knowledge, there is no study investigating the relationship between gonadotropin secretions and alarin levels in women with obese and PCOS women with lean PCOS. Therefore, the aim of the present study was to

investigate the relationship between serum gonadotropin secretions and alarin levels in women with obese (class I) and women with normal weight PCOS and control.

## Materials and Methods

This study was approved by the Institutional Review Board of Kafkas University Hospital (dated, 04.03.2019, issue no: 80576354-050-99). All patients were informed about the study, and their informed consent was obtained. Participants aged 18-36 years who were admitted to the Obstetrics and Gynecology Department of Fırat University and Kafkas University Hospital for irregular menstruation and were diagnosed with PCOS were included in the study. 28 women with obese PCOS and 28 women with lean PCOS and equal number as controls (28 women) were enrolled. Diagnosis of PCOS were performed by using the 2003 Rotterdam criteria (2 out of 3) as follows: 1) clinical and/or biochemical signs of hyperandrogenism 2) oligo-anovulation (menstrual cycle of >35 days); 3) PCOS as identified by ultrasonography, after the exclusion of other etiologies including congenital adrenal hyperplasia, androgen-secreting tumors, and Cushing's syndrome [2]. The body mass index (BMI) was calculated by dividing a person's weight in kilograms by the square of height in meters. Formation of the groups in this study was based on the Canadian Guidelines for Body Weight Classification in Adults, 2003 (normal weight: 18.5–24.9, and obese (class I: 30.0-34.9) [10,11]. Clinical hyperandrogenism was described by the presence of hirsutism (Ferriman-Gallwey score over 8) [12], and biochemical hyperandrogenism was described as an increased androgen concentration Chae et al. [13].

Patients who were previously had a history of thyroid disease or hyperprolactinemia, history of previous ovarian surgery, patients with type 1 and 2 diabetes, physical activity in the sedentary range, lipid-lowering drugs, glucocorticoids, or other hormonal drugs, history of serious disease, significant history of alcohol and tobacco abuse and use oral contraceptives were excluded from the present study. The participants in the control group were voluntary individuals without any medically known disease who were admitted to our hospital for annual routine check-up. Following the overnight fasting on the second day of the menstruation cycle, 8 mL of venous blood was taken from the study participants into biochemistry tubes with and without aprotinin as described previously. The samples were centrifuged at 4000 rpm. The serum/plasma obtained was stored at -80°C until the study. Blood estradiol (E2) (pg/mL), FSH (mIU/mL), LH (mIU/mL), dehydroepiandrosterone sulfate (DHEA-SO4) (µg/dL), total testosterone (nmol/L), prolactin, TSH, low-density lipoprotein (LDL) (mg/dL), high-density lipoprotein (HDL) (mg/dL), triglyceride (TG) (mg/dL), total cholesterol (TC) (mg/dL), fasting blood glucose (FBS) (mg/dL), insulin (lIU/mL) and alarin were analyzed.

## Alarin (galanin-Like peptide) Measurements

Alarin (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) value of the alarin (galanin-Like peptide) kit was <10%, the Inter-Assay (CV) values were <12%. The measuring range (standard curve range) of the alarin kit was 0,25ng/mL-70ng/mL, and the minimum measurable level was 0.214 ng/mL ng/mL. While the automatic washer Bio-Tek ELX50 (BioTek Instruments, USA) was used for plate washing, the ChroMate, Microplate Reader P4300 instrument (Awareness Technology Instruments, ABD) was used in absorbance readings.

## Statistical Analysis

All statistical analyses were performed using SPSS ver.22.0 (SPSS Inc., Chicago, IL, USA). All data were expressed as mean±SD. The multiple linear regression analysis was performed to compare the effects of BMI, reproductive hormones, HOMA-IR and alarin. *P-values* less than 0.05 were considered significant for all the analyses.

## Results

The clinical and hormonal characteristics of participants are shown in Table 1. No difference was found between the two PCOS groups in terms of age, hirsutism scores, but there is significantly differences in term of BMI (Table 1) compared with control. Likewise, there was also no statistically significant difference between FBG, LDL-C, HDL-C, TG, DHEA-SO4 levels and the Ferriman-Gallwey score between the two PCOS groups. Testosterone and LH, insulin levels were significantly higher in women with normal and obese PCOS. Alarin levels of women with normal weight and obese PCOS were significantly higher compared to the values of the control group. The correlation analyses were also performed between alarin and other various variables, and these correlations were presented in Table 2.

**Table 1: The Comparison of Demographic and Some Laboratory Variables in Polycystic Ovary Syndrome Women with Normo-Women with Obese and Control**

| Variables | Control (n: 28) | PCOS (normal weight; n:28) | PCOS (obese; n:28) |
|---|---|---|---|
| Age (years) | 29.1±3.2 | 27.8±2.9 | 28.72±2.4 |
| BMI (kg/m$^2$) | 23.66 ±4.1 | 22.52 ±4.3 | 32.4 ±5.2* |
| Ferriman-Gallwey score | 7.2±1.4 | 8.3±1.5 | 9.4±1.8* |
| FBS (mg/dL) | 96.26±8.4 | 114.8±9.2 | 123.3±10.2 |
| DHEA-SO$_4$ (µg/dL) | 136.1±34.8 | 144.7±41.3 | 172.1±29.8* |
| Testosterone (ng/mL) | 0.39±0.22 | 0.61±0.32 | 0.84±0.41* |
| E2 (pg/mL) | 72.9±31.3 | 94.3±22.4 | 103.3±34.1* |
| FSH (mIU/mL) | 6.99±1.2 | 7.08±1.3 | 7.62±1.72 |
| LH (mIU/mL) | 6.4±1.22 | 14.9±1.23 | 17.4±1.3 |
| Alarin (ng/mL) | 6.52 ± 0.44 | 15.87 ± 0.59 | 18.6± 1.1* |
| Insulin (µIU/mL) | 12.62±4.71 | 16.71±5.12 | 17.98±5.68* |
| HOMA-IR | 2.77±0.12 | 3.94±0.15 | 4.36±0.62* |
| TC (mg/dL) | 176.09±18.4 | 192.01±22.6 | 212.08±29.8* |
| TG (mg/dL) | 122.5±48.4 | 131.4±52.2 | 163.4±58.2 |
| LDL-C (mg/dL) | 118.32±16.4 | 116.39±17.2 | 136.42±18.1 |
| HDL-C (mg/dL) | 47.17±6.12 | 48.28±7.02 | 38.97±6.14 |
| LH/FSH | 0.9 | 2.1 | 2.3 |
| Alarin/FSH | 0.93 | 2.26 | 2.44 |

**Table 2: The Relation between Alarin, Demographic and Some Laboratory Variables**

| Parameters | Control | | PCOS-normal weight | | PCOS-obese | |
|---|---|---|---|---|---|---|
| | *r* Value | *p* Value | *r* Value | *p* Value | *r* Value | *p* Value |
| Age | 0.062 | 0.78 | 0.074 | 0.52 | 0.081 | 0.56 |
| BMI | 0.42 | 0.51 | 0.37 | 0.42 | 0. 58 | 0.05 |
| Ferriman-Gallwey score | 0.067 | 0.56 | 0. 25 | 0.512 | 0.851 | 0.002 |
| FBS | 0.167 | 0.36 | 0.183 | 0.09 | 0.48 | 0.05 |
| DHEA-$SO_4$ | 0.193 | 0.157 | 0.312 | 0.11 | 0.42 | 0.05 |
| Testosterone | 0.06 | 0.72 | 0.162 | 0.7 | 0.326 | 0.06 |
| E2 | 0.172 | 0.311 | 0.402 | 0.5 | 0.428 | 0.571 |
| FSH | 0.058 | 0.882 | 0.048 | 0.47 | 0.0151 | 0.766 |
| LH | 0.072 | 0.712 | 0.532 | 0.02 | 0.67 | 0.01 |
| Insulin | 0.051 | 0.562 | 0.238 | 0.15 | 0.562 | 0.047 |
| HOMA-IR | 0.077 | 0.711 | 0.316 | 0.01 | 0.553 | 0.027 |

**BMI:** Body mass index; **DHEA-$SO_4$:** Dehydroepiandrosterone sulfate; **E2:** Estradiol; **FBS:** Fasting blood sugar; **FSH:** Follicle-stimulating hormone; **HOMA-IR:** Homeostasis model assessment of insulin resistance index; **LH:** Luteinizing

## Discussion

In this study we first time reported that circulating alarin concentrations were significantly higher in both women with normal weight PCOS and women with obese PCOS subjects than in healthy individuals, whereas in women with obese PCOS patients, circulating alarin levels were higher than in normal weight PCOS subjects, suggesting a progressive increase of circulating alarin concentrations normal weight to obese situation. Alarin is found to ameliorate insulin resistance and insulin levels and blood glucose *in vivo* [14,15]. Also, circulating alarin is relative to the regulation of energy balance and blood glucose levels [15,16]. It is possible that metabolic disorder (PCOS) may cause the resistance of alarin actions, like insulin, leading to the increase of alarin secretion and release. Furthermore, the increment of circulating alarin in subjects with normal weight PCOS and women with obese PCOS subjects might be a compensatory up-regulation *in vivo* for counteracting the metabolic stress produced by obesity. Our alarin results are consistent with previous prediabetes and type 2 diabetes subjects studies [17].

Our results also indicated that alarin was positively associated with the parameters of IR (HOMA-IR) and glucose metabolism. Therefore, it might be possible that elevating alarin concentration in women normal weight PCOS and women with obese PCOS subjects might be related to a defensive response, which may represent ability for adaptation to IR or increased blood glucose levels.

In our study blood LH concentrations were also increased in women normal weight PCOS and women with obese PCOS subjects when compared with control group. Increased LH concentrations in women normal weight PCOS and women with obese PCOS subjects might be associated with increased alarin concentration. Since it has been reported that alarin promotes the secretion of luteinizing hormone (LH) [18]. Also, in this study we found that Alarin/FSH and LH/FSH ratios were elevated in women normal weight PCOS and women with obese PCOS subjects, which is consistent with the results of previous LH/FSH ratios. It has been previously revealed that abnormal secretion of LH and LH/FSH ratios are associated with the continuation of the anovulatory state in PCOS subjects [19]. Increased Alarin/FSH or LH/FSH ratios might be related with increased alarin concentrations. Beside the LH/FSH ratio in the evaluation of PCOS, the alarin/FSH ratio is more valuable diagnostic tool for the evaluation of women with PCOS and oligo menorrhea or anovulation in the future.

The present study has limitations. One of the limitations of this study had to do with the small number of participants. This study was a preliminary study, and we did not investigate ultra-sonographic parameters including ovarian stromal echogenicity and ovarian arterial blood flow indices.

## Conclusion

In our study, the alarin, LH level and the LH/FSH ratio alarin/FSH were significantly correlated with women with PCOS, which is consistent with the results of a previous study [20]. Based on above results it has been expected that the alarin/FSH ratio might be also a valuable diagnostic tool for the evaluation of women with PCOS [21]. Alarin/FSH ratios are more useful for diagnosing of PCOS than the LH/FSH ratio in women with PCOS.

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