Research Article

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Alarin: A Novel Circulating Peptide Hormone Linked to Luteinizing Hormone and Hiperandrogenism in Polycystic Ovary Syndrome

Merve Bicer¹, Murat Alan², Pinar Alarslan³, Aslı Guler⁴, Gokcenunal Kocabas³, Cetin Imamoglu⁵, Murat Aksit⁶, Giray Bozkaya⁷, Ahmet Cagdas Acara⁸, Ali Baloglu⁹, Behnaz Aslanipour¹⁰ and Mehmet Calan^{3*}

¹Department of Obstetrics and Gynecology, Medical Park Hospital, 35575, Izmir, Turkey

²Department of Obstetrics and Gynecology, Izmir Tepecik Training and Research Hospital, 35120 Tepecik, Izmir, Turkey

³Division of Endocrinology and Metabolism, Department of Internal Medicine, Izmir Bozyaka Training and Research Hospital, 35170 Bozyaka, Izmir, Turkey

⁴Department of Family Physician, Izmir Bozyaka Training and Research Hospital, 35170 Bozyaka, Izmir, Turkey

⁵Department of Radiology, Izmir Bozyaka Training and Research Hospital, 35170 Bozyaka, Izmir, Turkey

⁶Department of Biochemistry and Clinical Biochemistry, Izmir Tepecik Training and Research Hospital, 35120 Tepecik, Izmir, Turkey

⁷Department of Biochemistry and Clinical Biochemistry, Izmir Bozyaka Training and Research Hospital, 35170 Bozyaka, Izmir, Turkey

⁸Department of Emergency Medicine, Izmir Bozyaka Training and Research Hospital, 35170 Bozyaka, Izmir, Turkey

⁹Department of Obstetrics and Gynecology, Private Gynecology Clinic 35172 Alsancak, Izmir, Turkey

¹⁰Department of Bioengineering, Faculty of Engineering, EgeUniversity, Izmir, Turkey

***Correspondence:** Mehmet Calan, Division of Endocrinology and Metabolism, Department of Internal Medicine, Izmir Bozyaka Training and Research Hospital, 35170 Bozyaka, Izmir, Turkey, E-mail: drmehmetcalan@gmail.com

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Abstract

Alarin is a newly identified peptide hormone. It is implicated that alarin plays roles in regulation of energy metabolism and hypothalamo-pituitary-gonadal axis. Polycystic Ovary Syndrome (PCOS) is a common reproductive and metabolic disease in women during reproductive ages. Over-secretion of Luteinizing Hormone (LH) causes hyperandrogenism in women with PCOS. The purpose of the study was to determine the comparison of alarin levels in women with or without PCOS as well as to investigate the relationship between alarin and LH. Eighty-four women with PCOS and 84 age- and BMI- matched controls were recruited in this cross-sectional study. Circulating alarin levels were assessed via ELISA method. The hormonal and metabolic parameters of the recruited subjects were also determined. The circulating levels of alarin in PCOS subjects were higher than controls (6.11 ± 1.91 vs. 3.93 ± 1.60 ng/ml, $P < 0.001$). Alarin showed a positive correlation with insulin resistance marker, BMI, LH and androgens. Moreover, alarin levels were elevated in women with PCOS having insulin resistance compared to those PCOS women without insulin resistance. In both control and PCOS groups, overweight subjects showed an elevation in circulating levels of alarin with respect to normal weight subjects. In the present study, Alarin level with the highest tertile dosage comparing to alarin level with the lowest tertile dosage could highly increase the probability of PCOS risk prevalence in women. Elevated alarin levels in women with PCOS were associated with not only LH and metabolic parameters but also high probability of

having PCOS risk independently.

Keywords: Polycystic ovary syndrome, Alarin, Luteinizing hormone, Androgens

Abbreviation: BMI: Body Mass Index; CI: Confidence Interval; cIMT: Carotid Intima Media Thickness; CV: Coefficient of Variability; DBP: Diastolic Blood Pressure DHEA-S: Dehydroepiandrosterone Sulfate; FAI: Free Androgen Index; FBG: Fasting Blood Glucose; FG: Ferriman-Gallwey; GLP-1: Glucagon like peptide-1; HDL-C: High Density Lipoprotein Cholesterol; HOMA-IR: Homeostasis Model Assessment of Insulin Resistance; hs-CRP: High sensitivity; C-Reactive Protein; INSL-5: Insulin-like peptide 5; LDL-C: Low Density Lipoprotein Cholesterol; OGTT: Oral glucose tolerance test; OR: Odds Ratio; PCOS: Polycystic Ovary Syndrome; SBP: Systolic Blood Pressure; VIF: Variance inflation factor

Introduction

Alarin is a newly identified peptide hormone which is a member of the galanin family neuropeptides. Alarin, which was firstly found in gangliocytes in neuroblastic tumors of human, is expressed in central nervous system and periphery tissue such as vascular smooth muscle cells and thymus [1-3]. *In vivo* studies implicated that alarin plays different roles in glucose metabolism, hypothalamo-pituitary-gonadal axis, food intake and vasoconstriction [1],[4-9]. The relationship between alarin and luteinizing hormone (LH) was firstly defined in castrated male rats [4]. The study suggested that there was an increase in levels of LH during treatment of castrated rats with Alarin albeit there was no similar effect on intact rats. In the following studies, alarin injection showed an elevation of LH secretion with a gonadotropin-releasing hormone (GnRH) related manner in intact male rats. Besides, orexigenic property of alarin was defined and administration of alarin induced food intake in rats as well as increased body weight of the rats [5-7]. In another preclinical study, Alarin showed an influence on the regulation of glucose metabolism as well. In the same study, treatment of alarin in type 2 diabetic rats represented an effectively positive response of alarin over insulin sensitivity as well as glucose uptake via increasing glucose transporter receptor 4 (GLUT-4) and activating Akt signal pathway [7,9].

Polycystic ovary syndrome (PCOS) is a common reproductive and metabolic disease in women during reproductive ages. Typical features of PCOS are defined as ovulatory dysfunction, clinical and/or laboratory hyperandrogenism and polycystic ovaries. Although both genetic and environmental factors are effective in development of the disorder, the pathophysiology of PCOS has not yet been clearly described [10-12]. Induction of insulin resistance and alteration of gonadotropin-releasing hormone secretion have been thought to have central roles in the pathophysiology of PCOS. It is revealed that both LH pulse frequency and its amplitude are increased in PCOS subjects. The negative feedback effect

of androgens over LH secretion is impaired. Moreover, metabolic abnormalities such as insulin resistance, glucose metabolism abnormality and obesity are also commonly appeared in women with PCOS. Apart from this, interaction of insulin resistance with LH contributes to development of hyperandrogenism in PCOS subjects [10,13].

We aimed to focus on whether alarin levels are differently secreted in subjects with PCOS when compared with controls due to the presence of a possible link between alarin and insulin resistance and LH secretion. Furthermore, we tried to explore whether Alarin is associated with both hormonal and metabolic parameters and carotid intima media thickness (cIMT).

Materials and Methods

Ethics statement

The approval for the current study was taken from ethics committee of Bozyaka Hospital. After receiving the written agreement, all the subjects were involved in the research and the principles Declaration of Helsinki (revised in 2008) were carefully abided during the study.

Subjects and study design

This study was planned to be a case control study. Two altered groups including 84 subjects with PCOS and 84 age- and body mass index (BMI)-matched subjects with standard menstrual cycles were selected among the series of women who were 18-45 years old for both groups. The trial lasted from November 2015 to November 2016 in Endocrinology Department, Training and Research Hospital in Izmir, Turkey. For the physical examination and detailed history test, a single expert scanned the subjects. Apart from this, the enrolled subjects were also verified for a standard 75 g 2-h oral glucose tolerance test (OGTT). We selected the subjects who did not consume any alcohol and tobacco.

PCOS group

The PCOS group included the subjects considering

the Rotterdam consensus criteria. They followed three following criteria (after excluding other causes of hyperandrogenism and ovulatory dysfunction) to achieve a suitable homogeneity; 1- Diagnosis of oligo- and/or anovulation, 2- Diagnosis of clinical and/or biochemical signs of hyperandrogenism - Using the Ferriman-Gallwey [FG] for hirsutism determination [14] and 3- The occurrence of ≥ 12 follicles with the size of 2-9 mm in diameter or an ovarian volume of > 10 ml (without a cyst or dominant follicle in either ovary) for recognition of typical ultrasonographic signs of polycystic ovaries (with one ovary being sufficient for diagnosis) [15].

The subjects with FG score ≥ 8 were determined as hirsute. Biochemical hyperandrogenism was distinctly diagnosed in the case of having testosterone (normal range: 0.52-2.42 nmol/l), and/or dehydroepiandrosterone sulfate (DHEA-S) (normal range: 10-248 $\mu\text{g/dl}$), and/or free androgen index (FAI) $\geq 5\%$ [16] of serum concentrations level upper the reference interval limitation.

Control group

The test subjects were selected among either the women who visited the gynecology or endocrinology clinic for routine examination or the volunteer hospital employees as all of them had normal menstrual cycle. None of them showed disorders such as concomitant health problems or signs of hirsutism, acne or hyperandrogenism.

Exclusion criteria

The subjects without needed criteria such as having various reasons of irregular menstrual cycles and/or androgen overplus (i.e. Hyperprolactinemia, cushing's syndrome, congenital adrenal hyperplasia, or other diseases of the adrenal gland, thyroid disorders, galactorrhea, breastfeeding and pregnancy), reduced glucose tolerance or type 1/type 2 diabetes, hypertension, familial hyperlipidemia, liver/renal disorders, or congestive heart failure, having a coronary artery disease history, gestational diabetes mellitus, malignancy or acute infection (within the preceding 14 days) and also existence of any chronic inflammatory and autoimmune disease, hormonal contraception and/or anti-androgen treatment (within the preceding 6 months) were excluded from the test. Additionally, taking some medications for treatment of dyslipidemia, hypertension, hyperglycemia, insulin resistance or obesity was an exclusion criterion of this study.

Anthropometric evaluation

Some specific standards including anthropometric measurements, comprising age, weight(kilogram), height (centimeter) and waist circumference (centimeter) of the subjects were measured while they were barefoot and in casual clothes. Lower rib margin and the iliac crest at the end of a gentle expiration' midway was measured for evaluation of waist circumference. As the subjects were in sitting position, their blood pressure was measured after a 15 minute resting period. The following formula was used to calculate the BMI value. BMI: weight (kg)/square meter of height (m^2).

Biochemical evaluation

Venous blood samples were obtained from the antecubital veins belonging to the test subjects in the early follicular phase of menstrual bleeding (3rd to 5th days) in the morning (between 08:00-09:00) almost after both onset of early spontaneous or progesterone-induced menses and at least 10 hour fasting period. For making the blood samples in clotted form, they were centrifuged for 15 minutes at $2000 \times g$ and then were kept in room temperature for 30 minutes. The obtained serum samples were then kept in aliquots at -80°C for alarin analysis. The measurement of all following criteria including fasting blood glucose (FBG), serum insulin, glycated hemoglobin A1_c (HbA1_c), total cholesterol, low and high density lipoprotein cholesterol (LDL-C and HDL-C), DHEA-S, total testosterone, LH, triglycerides, sex hormone binding-globulin (SHBG), estradiol (E_2), high-sensitivity C-reactive protein (hs-CRP), follicle stimulating hormone (FSH), 2-h plasma glucose and Alarin levels was completed. Dedicated kits (Beckman Coulter Inc, CA, USA) belonging to an auto-analyzer (Olympus AU 2700 Beckman Coulter Inc, CA, USA) were used for determination of levels of different criteria such as FBG and 2-h plasma glucose, total cholesterol, serum hs-CRP, triglyceride and total HDL-C in the serum of study cases. The following formula named Friedewald was considered for calculation of LDL-C as $\text{LDL-C} = \text{total cholesterol} - (\text{HDL-C} + \text{Triglyceride}/5)$. Chemiluminescent Microparticle Immunoassay (CMIA) along with its dedicated kits (Beckman Coulter Inc, CA, USA) and an auto-analyzer named UniCel DxI 800, Beckman Coulter Inc, CA, USA were used for measuring serum insulin levels, High performance liquid chromatography (Variant II Turbo, Bio-Rad, CA, USA) was performed for measuring the levels of HbA1_c. The additional standards containing LH, FSH, E_2 , DHEA-S, total testosterone and SHBG levels were measured by CMIA (UniCel DXI 800, Beckman

Coulter Inc., CA, USA). The following formula was used to calculate the amount of FAI as FAI: (total testosterone / SHBG) $\times$ 100. Each test participant was enrolled in insulin resistance utilizing homeostasis model assessment of insulin resistance: HOMA-IR = fasting insulin (μ U/ml) $\times$ fasting glucose (mg/dL) / 405 [17].

cIMT measurement

For the measurement of cIMT criterion, a previously described method was considered. High-resolution ultrasound (Toshiba Aplio 300, Toshiba Medical Systems Corporation, Tokyo, Japan) was done by a single expert doctor [18].

Measurement of circulating alarin by ELISA

Commercially available human ELISA kits, with Catalog number CSB-EL009222HU, obtained from Cusabio Biotech, Hubei, China was used to measure serum alarin levels in duplicate in accordance with the instructions of manufactures. The percentages < 6% and < 8% were the rates for the intra-assay coefficient of variability (CV) and the inter-assay CV respectively. A circulating level between 0.156 and 10 ng/ml was distinguished for alarin.

Statistical analysis

Power analysis: G Power 3.0.10 G for Windows (Heinrich-Heine-Universität Düsseldorf, Düsseldorf, Germany) was used to determine the population's size in the ongoing study [19]. The values of 0.05 for α and 0.90 for study power were calculated for each group (74 subjects). All data were analyzed using the Statistical Package of the Social Sciences software version 18.0 (SPSS Inc. Chicago, IL, USA). Kolmogorov-Smirnov test was considered to discover the variables' distribution in each group. According to the results of this test, numeric variables were distributed normally. All data were shown as mean with standard deviation (SD). The pair's comparison in demographic and laboratory characteristics of both test groups was calculated by t-test. Subjects were separated into four different subgroups according to their BMI (<25 kg/m² and $\geq$ 25 kg/m²) and PCOS status. In each group, circulating alarin levels were analyzed using t-test. PCOS group was divided into two groups according to having or not having insulin resistance (HOMA-IR > 2.71 and HOMA-IR $\leq$ 2.71) [20]. We used t-test to compare circulating alarin levels in subgroups (having and not having insulin resistance) as well. The relation of alarin with other variables was determined using Pearson's correlation coefficients. Moreover, a multiple regression model was considered to elucidate the independent link

between alarin and metabolic or hormonal parameters. Multicollinearity was calculated for independent variables; the variables were accepted as a collinear. Variables with the variance inflation factor (VIF) surpassed 2.5 were not taken into the model. Furthermore, odds ratio (OR) was calculated via multivariate logistic regression analysis to show the link between alarin levels (tertile) and PCOS development risk. The following potential confounders including age, BMI, HOMA-IR and FAI were added into the model for adjustment. Compatibility of the model was tested using Hosmer and Lemeshow test ($P > 0.05$). The level of 95% for confidence interval (CI) was used. A two sided P value < 0.05 was considered as a statistically significant.

Results

Clinical and laboratory characteristics of the study population

Clinical and laboratory parameters of the subjects are given in table 1.

The circulating levels of alarin in PCOS subjects were higher than controls (6.11 ± 1.91 vs. 3.93 ± 1.60 ng/ml, $P < 0.001$) (Figure 1A). Furthermore, serum insulin levels, FBG and HOMA-IR were found to be elevated in PCOS group. The circulating LH levels were increased in PCOS subjects significantly. Additionally, the level of androgens including total testosterone, FAI and DHEA-SO₄ were notably increased in subjects with PCOS while SHBG was declined. Moreover, a thicker cIMT was observed in subjects with PCOS in comparison to controls. Apart from this, circulating hs-CRP levels were also higher in PCOS subjects. We also compared alarin levels between women with PCOS having insulin resistance and women with PCOS not having insulin resistance. A number of 58 subjects out of 84 (total PCOS subjects) were shown to be insulin resistant. The circulating alarin levels displayed an elevation in PCOS subjects with insulin resistance with respect to PCOS subjects without insulin resistance (6.42 ± 1.95 vs. 5.43 ± 1.66 ng/ml, $P = 0.029^*$) (Figure 1B). Consequently, we divided PCOS and control groups referring to their BMI (< 25 kg/m² and $\geq$ 25 kg/m²) from which forty-three participants from PCOS group and forty-one subjects from control group were overweight. There was no significant difference between the groups in the case of being overweight ($P = 0.877$). The mean values of circulating alarin levels were found to be remarkably elevated in overweight subjects in each group with respect to normal weight subjects (6.53 ± 1.83 vs. 5.67 ± 1.91 ng/ml, $P = 0.038^*$, PCOS group; 4.30 ± 1.57

Table 1: Comparison of the demographic and laboratory characteristics of the subjects.

Variables	PCOS	Controls	Pa
	n=84	n=84	
Age, years	30.31 ± 7.10	30.66 ± 7.06	0.345
BMI, kg/m ²	26.33 ± 4.42	26.58 ± 4.56	0.243
Waistcircumference, cm	93.40 ± 12.23	93.09 ± 14.26	0.883
SBP, mmHg	107.88 ± 12.58	107.72 ± 11.40	0.931
DBP, mmHg	74.49 ± 6.49	73.36 ± 5.63	0.232
Ferriman-Gallweyscore	14.50 ± 2.87	4.16 ± 1.22	<0.001*
FBG, mg/dl	84.31 ± 8.21	81.79 ± 6.04	0.025*
2-h OGTT, mg/dl	123.27 ± 12.78	120.40 ± 11.75	0.13
A1c, %	5.29 ± 0.18	5.26 ± 0.19	0.302
Insulin, µIU/ml	17.73 ± 6.47	11.20 ± 4.57	<0.001*
HOMA-IR	3.71 ± 1.43	2.25 ± 0.90	<0.001*
Total cholesterol, mg/dl	208.59 ± 33.55	205.10 ± 44.72	0.568
LDL-C, mg/dl	137.90 ± 28.25	133.29 ± 28.11	0.291
HDL-C, mg/dl	41.78 ± 9.39	49.51 ± 11.03	<0.001*
Triglycerides, mg/dl	145.54 ± 33.09	111.43 ± 30.67	<0.001*
Hs-CRP, mg/l	1.24 ± 0.55	0.68 ± 0.21	<0.001*
FSH, mIU/ml	6.96 ± 1.78	7.42 ± 1.85	0.103
LH, mIU/ml	13.68 ± 4.35	8.59 ± 2.91	<0.001*
Estradiol, pg/ml	51.26 ± 12.05	49.54 ± 8.14	0.281
Total-testosterone, nmol/l	2.92 ± 0.41	1.71 ± 0.35	<0.001*
SHBG, nmol/l	38.06 ± 11.50	69.27 ± 14.96	<0.001*
FAI, %	8.12 ± 1.67	2.49 ± 0.11	<0.001*
DHEA-SO ₄ , µg/dl	191.09 ± 75.17	155.33 ± 39.63	<0.001*
cIMT, mm	1.01 ± 0.44	0.61 ± 0.30	<0.001*

vs. 3.58 ± 1.57 ng/ml, $P = 0.040^*$, control group) (Figure 1C).

Figure 1A: Circulating alarin levels in the control and PCOS groups.

Figure 1B: Circulating alarin levels in PCOS women with and without insulin resistance (HOMA-IR > 2.71 insulin resistant; HOMA-IR ≤ 2.71 normal).

Figure 1C: Circulating alarin levels according to their BMI levels (normal-weight: BMI < 25 kg/m²; overweight/obese: BMI ≥ 25 kg/m²). A P value of <0.05 was considered significant (*).

Correlation of alarin with demographic and metabolic parameters

We next investigated the link between alarin and demographic or metabolic-hormonal parameters in both groups using Pearson's correlation analysis. The correlation coefficients are given in table 2.

Alarin levels showed a notably positive correlation with LH levels in both groups (Figure 2A and 2B). Moreover, alarin levels were correlated with FAI and DHEA-SO₄. Additionally, we found a positive correlation between alarin and BMI, insulin, FBG as well as HOMA-IR on the contrary, there was no correlation between alarin and 2-h OGTT, A1c. Furthermore, we could not observe a correlation between alarin and blood pressure, lipid profiles as well as hs-CRP and cIMT.

Figure 2: Scatter plots showing the correlation circulating alarin levels and LH in PCOS group (Figure 2A) and in control group (Figure 2B).

Table 2: Correlation coefficient between alarin levels and clinical parameters.

	Alarin			
	PCOS		Control	
	r	P	r	P
Age	0.128	0.153	0.102	0.104
BMI	0.314	0.021*	0.296	0.024*
Waist circumference	0.368	0.016*	0.304	0.019*
Insulin	0.224	0.031*	0.117	0.037*
FBG	0.198	0.039*	0.109	0.042*
2-h OGTT	0.148	0.368	0.156	0.141
HOMA-IR	0.211	0.033*	0.113	0.040*
A1c	0.135	0.169	0.257	0.374
FSH	0.173	0.217	0.105	0.129
LH	0.489	<0.001*	0.204	0.005*
FAI	0.378	<0.001*	0.193	0.009*
DHEA-S	0.259	0.027*	0.113	0.039*
Hs-CRP	0.201	0.237	0.149	0.314
cIMT	0.092	0.185	0.102	0.216
Ovarian follicular number	0.159	0.216	-	-
Total cholesterol	0.094	0.185	0.106	0.169
LDL-C	0.181	0.103	0.115	0.179
HDL-C	-0.145	0.274	0.014	0.105
Triglycerides	0.195	0.242	0.122	0.131

Multivariate regression analysis

A multiple linear regression was used to show the independent relationship between alarin and LH, HOMA-IR as well as FAI and DHEA-S. The following parameters such as PCOS status and lipid parameters were added to regression model to adjust the effect of these parameters (Table 3). Regression analysis revealed that alarin levels were independently associated with LH, HOMA-IR and FAI.

Multivariate binary logistic regression analysis

The independent association between alarin levels (tertile) and developing the risk of PCOS was analyzed using binary logistic regression analysis. The model was adjusted with potential confounders including age, BMI, HOMA-IR and FAI (Figure 3).

Figure 3: Association of alarin with PCOS in adjusted models. Multivariate adjusted OR for having PCOS according to the tertiles of alarin (reference, the lowest tertile). Model adjusted for age, BMI, HOMA-IR, FAI, LH and hs-CRP. OR: Odds Ratio; CI: Confidence Interval. A *P* value of <0.05 was considered significant (*).

The logistic regression analysis revealed that the subjects in the highest tertile of alarin showed significantly higher odds of having PCOS risk in comparison with the subjects in the lowest tertile (OR = 2.31, 95 % CI = 1.88 – 2.83, *P* = 0.009). Subjects in both second and the lowest Alarin tertile did not show any difference in having PCOS risk (OR = 1.32, 95 % CI = 0.86 – 2.02, *P* = 0.126).

Table 3: Multiple linear regression analysis: independent factors associated with circulating alarin levels in all study population (R2=0.521).

	β	95% CI		P
	Lower	Upper		
Independent factors				
Age	0.152	-0.924	1.228	0.148
BMI	0.967	0.213	1.721	0.017*
HOMA-IR	0.821	0.117	1.525	0.021*
LH	1.164	0.472	1.856	0.005*
FAI	0.789	0.301	1.277	0.031*
DHEA-S	0.093	-0.128	0.058	0.157

Discussion

Although PCOS is a common disorder, the etiopathogenesis of the disease is not fully understood. LH is responsible for synthesis of androgen from theca cells in ovaries. As the level of LH increases relatively to FSH, androgen is highly synthesized from the ovaries. Both amplitude and secretion of LH appear to be remarkably stimulated in subjects with PCOS with respect to those

women without PCOS. These pathways have been thought to have crucial roles in PCOS pathophysiology [10]. Alarin which is a novel peptide hormone is inferred to have the ability of increasing LH secretion in male rats [4,5]. Data shows that there has not been any research upon investigation of an interaction between LH and alarin in women with PCOS. In the current study, we investigated alarin levels in women with PCOS and we analyzed the association between alarin levels and hormonal, metabolic parameters. We found that alarin levels were higher in PCOS subjects compared to controls. Furthermore, alarin showed a strongly positive correlation with LH in PCOS group as well as a mildly positive correlation in control subjects. Besides, in this study, logistic regression analysis revealed that increased alarin levels displayed high possibility of having PCOS risk independently.

Alarin, known as a newly identified galanin family member, is a peptide which has a strong potential in stimulating of LH secretion in both mice and rats [2-6]. Van Der Kolk et al. suggested that alarin injection in castrated male rats caused the presence of a higher level of LH while there was no induction in intact male rats [4]. In another study, Boughton et al. [5] reported that alarin stimulated the LH secretion via Gonadotropin-Releasing-Hormone (GnRH) in intact male rats as well. Similar results have been reported in male mice by Fraley et al. [6] and the results suggested that following injection of alarin caused a dependent effect of LH induction via GnRH signaling. In the present study, the interaction between alarin and LH was elucidated in both PCOS and control groups. A strongly positive correlation of alarin and LH was found in PCOS group particularly. Alarin levels also correlated with FAI and DHEA-S. The relationship between alarin and LH via clarifying other potential confounders' effects was also analyzed. According to the results of multiple linear regression analyses; there was an interaction between alarin and LH independently. Additionally, regression analyses revealed a relationship between alarin and FAI independently whereas the link between alarin and DHEA-S was disappeared. Moreover, logistic regression analysis showed that elevated levels of alarin could increase the risk of PCOS. This data gives the fact that alarin may play a role in the pathogenesis of PCOS.

Growing data supported that there is a link between alarin and regulation of metabolism such as food intake, body weight and glucose metabolism. In a study by Fraley et al. [6] alarin was shown to be a potential neuromediator

of food intake in mice. In a parallel study upon rats, alarin displayed increased food intake and body weight [4]. In another study, the effect of alarin over insulin resistance was investigated in type 2 diabetic rats. The results showed that alarin treatment could significantly increase glucose uptake through induction of GLUT4 content. Additionally, they showed that alarin treatment notably increased body weight of test animals [7]. Another preclinical study displayed that alarin increased glucose uptake via activating Akt signal pathway in type 2 diabetic rats as well [9]. It is indicated that insulin resistance plays a crucial role in development of PCOS. Although up to 70% of women with PCOS have insulin resistance, underlying its pathophysiologic defects is not clearly understood yet [10,13]. In the present study, we found that insulin resistance marker-HOMA-IR was elevated in women with PCOS. We also found an association between alarin and BMI or HOMA-IR. This association may explain that increased alarin levels in women with PCOS may overcome insulin resistance as a compensatory. In the current study, linear regression analysis results showed that there was an independent link between alarin and LH apart from insulin resistance.

Additionally, PCOS subjects have increased cardiovascular risk including hypertension, lipid metabolism disorders and atherosclerosis [21,22]. In our study, we evaluated cardiovascular risk factor of all subjects via measuring cIMT, a good predictor of cardiovascular risk [23-25]. We found that cIMT was higher in women with PCOS compared to controls. Moreover, PCOS subjects had lipid metabolism disturbance such as triglycerides elevation and HDL-C reduction. We could not observe any correlation between alarin and lipid profiles, cIMT. Moreover, Dewailly et al. [1] reported the vasoactive properties of alarin, the results of their study suggested that alarin was a potent and dose-dependent vasoconstrictive peptide in cutaneous dorsal microvasculature. In our study, we could not find any correlation between alarin levels and systemic blood pressure.

There are some limitations to the current study. Although our sample size is relatively large, the cross-sectional design of the study cannot prove causality. Besides, we could not measure level of alarin during cyclic period in control women. Another limitation of the study is the less invasive HOMA-IR method, which was used to evaluate insulin resistance although it is inferior to the clamp technique.

Conclusion

The results of this study demonstrate that the elevated alarin levels are associated with increased odds of having PCOS. An additional novel finding of current study suggests that alarin is also strongly associated with LH. These results suggest that increased alarin levels may play a possible role in the development of PCOS via stimulating of LH.

Compliance with ethical standards

The subjects gave their oral and written informed consent before their inclusion in the study. The study adhered strictly to the principles of the Declaration of Helsinki as revised in 2008.

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Conflict of interest

We declare that we have no conflict of interest.

Author contributions

M.C., A.G., M.B., M.A., P.A., G.U.K. participated in study design and G.B., M.A. performed ELISA. M.B., M.C., A.G., M.A., A.B., A.M.I., A.C.A., B.A. and C.I. participated in study design, analyzed the data, wrote, reviewed and edited the manuscript. M.B., A.G., M.C., M.A., G.U.K. and P.A. provided serum samples and contributed to discussions of data interpretation. C.I. measured carotid intima media thickness of the participants. All authors reviewed and edited the manuscript. M.C. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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