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Kisspeptin And Galanin-like Peptide (GALP) Levels In Women With Polycystic Ovary Syndrome

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Abstract: ***The aim of the study was to compare kisspeptin and GALP levels between women with and without PCOS and to analyze the correlation between kisspeptin, GALP and PCOS-related hormonal and metabolic disturbances.***
Patients and methods: The study included 87 PCOS women, and a control group of 42 healthy women with a regular menstrual pattern and no signs of hyperandrogenisms. Circulating levels of luteinizing hormone (LH), follicle-stimulating hormone (FSH), testosterone (T), sex-hormone binding globulin (SHBG), thyroid stimulating hormone (TSH), prolactin, fasting insulin, kisspeptin and GALP were measured.
Results: Kisspeptin (P<0.05) and GALP (P>0.05) levels were significantly higher in the PCOS women than in the controls. In the normal weight PCOS women kisspeptin correlated positively with GALP (P<0.01), waist circumference (WC) (P<0.05) and free androgen index (FAI) (P<0.05) and negatively with fasting glucose (P<0.05). In this group GALP correlated positively with WC (P<0.05) and kisspeptin (P<0.01). In the overweight PCOS women kisspeptin correlated positively with insulin (P<0.05), T (P<0.05), SHBG (P<0.01) and FAI (P<0.001). In this group there was a significant positive correlation between GALP and LH (P<0.05), LH/FSH ratio (P<0.05), T (P<0.01) and FAI (P<0.01).
Conclusions: Our results indicate that GALP and kisspeptin are increased in women with PCOS and positively associated with hyperandrogenism accompanying this condition. GALP and kisspeptin probably are involved in the interaction between reproduction and metabolic state, affecting the hypothalamic-pituitary-gonadal axis.

Keywords: Polycystic Ovary Syndrome, Kisspeptin, GALP

1. Introduction

Polycystic ovary syndrome (PCOS) is the most common cause of female infertility. The frequency depends on the criteria used for diagnosis, the so called Rotterdam criteria – up to 15%, most often cited [1]. Women with PCOS commonly display neuroendocrine dysfunction [2] with deregulated gonadotropin secretion, which might be indicative of disrupted gonadotropin releasing hormone (GnRH) secretory activity. It is assumed that neural elements over GnRH secretory neurons, such as kisspeptin and galanin-like peptide (GALP), play a significant role in the development of PCOS.

Kisspeptin is a 54-amino-acid peptide, which influences the control of the gonadotropic axis in various ways. Loss of Kiss-1 gene function is reported to be associated with hypogonadotropic hypogonadism [3,4], while exogenous administration of kisspeptin activates the gonadotrophin axis before puberty in rodents, sheep and non-human primates [5]. In recent years, kisspeptin has been revealed as the most powerful enducer of GnRH-mediated LH response [6]. Sex steroids and other metabolic signals also modulate Kiss1 neurons expression, thus regulating the reproductive axis.

GALP is a 60-amino-acid neuropeptide that was isolated as an endogenous ligand capable of binding to and activating galanin receptor 1 and 2 in vitro [7]. GALP-immunoreactive fibers are mainly found in the nucleus arcuatus (ARC) and the posterior pituitary [8]. GALP-positive neurons in the ARC are projected to several brain regions where they seem to come into contact with multiple neuromodulators. The latter appear to be involved in the regulation of reproduction and energy homeostasis. GALP is thought to stimulate GnRH-mediated LH response since GALP administration in the central nervous system increases LH secretion [9]. Intracerebral administration of GALP stimulates Kiss1 gene expression in the ARC [10]. The available data indicates a functional link between GALP and kisspeptins.

These observations indicate that both GALP and kisspeptin are molecular signals that couple metabolism to the neuroendocrine reproductive system and, thus, regulate reproductive activity as a function of the energy state.

Given the complex relationship between kisspeptin and GALP and the hypothalamic-pituitary-gonadal axis, the present study ***aimed*** to compare kisspeptin and GALP levels between women with and without PCOS and to analyze the correlation between kisspeptin, GALP and PCOS-related hormonal and metabolic disturbances.

2. Patients and Methods

The study was conducted at the Clinic of Endocrinology and Metabolic Diseases, University Hospital "St. George", Faculty of Medicine, Medical University Plovdiv, Bulgaria. The Ethics Committee of Medical University - Plovdiv approved this study, and signed informed consent was obtained from each of the subjects before recruitment.

Subjects

The study population included 87 women diagnosed with PCOS, and 42 women with a regular menstrual pattern and no signs of hyperandrogenism, used as a control group.

The diagnosis of PCOS was made according to the Rotterdam criteria, in the presence of at least two of the following: oligomenorrhea and/or anovulation; biochemical and/or clinical

hyperandrogenism; and ultrasound appearance of polycystic ovaries (multiple cysts >12 in number of 2–9 mm size) [1]. Other common causes of hyperandrogenism or menstrual disorders were excluded.

Over the three months preceding the study no subject had been on hormonal contraceptives or other medications which could affect lipid and carbohydrate metabolism.

Hormonal and Biochemical Measurements and Calculations.

At their initial visits, all subjects underwent medical evaluation and demographic data was recorded. A detailed clinical history was taken for all participants. Hirsutism was evaluated according to the modified Ferriman–Gallwey score [11]. The women were further divided into two groups, based on BMI: overweight or obese (BMI≥25) and normal weight (BMI<25).

Blood samples were collected in the early follicular phase of a spontaneous menstrual cycle or until day 7 after progesterone induced bleeding in the PCOS group. Blood was drawn between 7:30 and 8:00 AM, after at least a 10-hour overnight fast. Glucose and hormone levels were determined immediately after centrifugation. For the determination of GALP and Kisspeptin levels, serum samples were apportioned into 0.5 ml aliquots and stored at –20°C until processing.

Fasting serum glucose was measured by a commercially available Konelab 60i, Thermo Electron Corporation (Finland) chemistry auto analyzer. Routine hormonal analysis of serum LH, FSH, thyroid stimulating hormone (TSH), prolactin (PRL), insulin, total testosterone (T) and sex hormone binding globulin (SHBG) were done by chemiluminescence methods using Access 2 Immunoassay System, Beckman Coulter, Inc., USA. GALP (Cusabio, China) and Kisspeptin (Cusabio, China) levels were determined by commercially available enzyme-linked immunosorbent assays (ELISA) according to the manufacturers' instructions. The minimum detectable concentration for GALP was 0.039 ng/mL and for Kisspeptin was 0.078 ng/mL. The intra- and inter-assay CVs for both kits were <8% and <10%.

The BMI, the free androgen index (FAI) and the homeostatic model assessment–insulin resistance (HOMA-IR) were calculated according to the well-known formulas [12,13].

Statistical analysis

Data analysis was performed by using IBM SPSS Statistics for Windows, version 17.0. Values are presented as mean±standard error for mean (SEM). Whether the distributions of metric discrete and continuous variables were normal or not was determined by the Kolmogorov–Smirnov test. The Levene test was applied for determining the homogeneity of variances. The mean differences between groups were compared by Student's t test, otherwise the Mann–Whitney U test was applied for comparisons of the median values. Degrees of association between continuous variables were calculated by Spearman's correlation coefficient. The level of statistical significance was set at 5%.

3. Results

Basic demographic, clinical, biochemical and hormonal characteristics of the women studied are summarized in Table 1.

No difference in terms of age, BMI and WC (waist circumference) was observed between the two groups (p=0.055; p=0.72 and p=0.27). LH levels and the LH/FSH ratio were significantly higher in the PCOS group compared to controls (p=0.001 and p<0.001), but no significant difference was found in FSH (p=0.41), PRL (p=0.16) or TSH (p=0.19) levels.

Table 1. Basic demographic, clinical, biochemical and hormonal characteristics of the women studied.

	PCOS (n=87)	Controls (n=42)	P
Age y	24.99±0.49	26.65±0.71	NS
BMI kg/m^2	25.59±0.65	24.88±0.89	NS
WC sm	86.95±1.77	82.45±3.24	NS
Fasting Glucose mmol/L	5.24±0.08	4.99±0.08	NS
Fasting Insulin μIU/mL	7.62±0.49	6.19±0.53	NS
HOMA-IR	1.82±0.13	1.39±0.20	0.018
LH IU/L	7.68±0.58	5.09±0.43	0.001
FSH mIU/L	6.55±0.19	7.09±0.34	NS
LH/FSH ratio	1.22±0.09	0.76±0.07	<0.001
T ng/mL	0.73±0.03	0.51±0.04	<0.001
SHBG nmol/L	40.65±3.01	57.86±6.64	0.010
FAI	8.83±0.88	4.69±0.93	0.002
TSH mIU/L	2.06±0.11	2.41±0.21	NS
PRL mIU/L	320.58±17.68	298.07±26.74	NS
GALP ng/mL	1.68±0.35	0.54±0.24	0.008
Kisspeptin ng/mL	0.23±0.20	0.16±0.01	0.021

Women with PCOS also had significantly higher HOMA-IR values (p=0.018), but no significant differences were observed in glucose (p=0.09) and insulin levels (p=0.07). T and FAI score (p<0.001 and p=0.002, respectively) were significantly higher, whereas SHBG levels were lower in the PCOS group (p=0.010). Kisspeptin and GALP levels were also higher in women with PCOS than controls (p=0.021 and p<0.001).

To find if overweight or obesity affects GALP and kisspeptin secretion we further divided the women into two subgroups based on BMI – normal weight women and overweight or obese ones (Table 2).

In the normal-weight group (Table 2) we found no significant differences in age (p=0.07), BMI (p=0.23) and WC (p=0.07). LH levels and the LH/FSH ratio were significantly higher in the PCOS group compared to controls (p=0.002 and p=0.001), but no significant difference was found in FSH (p=0.35), PRL (p=0.80) or TSH (p=0.18) levels between the two subgroups. Women with PCOS also had significantly higher glucose levels (p=0.027), but no significant differences were observed in insulin levels (p=0.57) and HOMA-IR values (p=0.44). T and FAI score (p=0.035 and p=0.012) were significantly higher, whereas SHBG levels were lower (p=0.039) in the PCOS subgroup. In the group with BMI<25, GALP levels continued to be higher (p=0.019), but there was no significance in kisspeptin levels (p=0.11), although they were higher in the PCOS group.

In the group with BMI≥25 (Table 2) no difference in terms of age, BMI and WC was observed between the two subgroups (p=0.30; p=0.37 and p=0.38). No significant difference was found in LH, FSH levels, LH/FSH ratio, PRL and TSH levels

between PCOS and control group. Women with PCOS also had significantly higher fasting insulin levels (p=0.005) and HOMA-IR values (p=0.001), but no significant difference was observed in fasting glucose levels (p=0.27). T and FAI score (p<0.001 and p=0.015) were higher, whereas no difference in SHBG levels was found (p=0.06) in the PCOS subgroup compared to controls. Kisspeptin and GALP levels were also higher in PCOS patients but they did not reach significance.

Table 2. Demographic, clinical, biochemical and hormonal characteristics of women with PCOS and controls regarding BMI.

	PCOS BMI≥25 (n=40)	**PCOS BMI<25 (n=47)**	**Controls BMI≥25 (n=20)**	**Controls BMI<25 (n=22)**
Age y	26.05±0.77	24.13±0.60	27.50±1.21	25.95±0.82
BMI kg/m^2	30.97±0.73	21.00±0.31$^{\#\#\#}$	29.89±0.84	20.31±0.50***
WC sm	99.13±2.02	75.64±1.35$^{\#\#\#}$	96.17±2.38	69.46±4.06***
Fasting Glucose mmol/L	5.34±0.14	5.14±0.08^	5.10±0.13	4.86±0.09
Fasting Insulin μIU/mL	10.23±0.81$^{\uparrow\uparrow}$	5.33±0.29$^{\#\#\#}$	7.09±0.68	4.95±0.74
HOMA-IR	2.52±0.23$^{\uparrow\uparrow}$	1.21±0.06$^{\#\#\#}$	1.59±0.15	1.09±0.17
LH IU/L	7.64±0.97	7.72±0.71^^	5.27±0.69	4.88±0.51
FSH mIU/L	6.34±0.23	6.72±0.28	6.94±0.45	7.26±0.54
LH/FSH ratio	1.23±0.15	1.22±0.13^^	0.81±0.12	0.70±0.06
T ng/mL	0.79±0.04$^{\uparrow\uparrow\uparrow}$	0.66±0.31^$^{\#\#}$	0.49±0.06	0.53±0.04
SHBG nmol/L	27.42±3.99	51.02±3.56^$^{\#\#\#}$	41.01±4.78	76.4±10.25**
FAI	13.51±1.53$^{\uparrow}$	5.15±0.43^$^{\#\#\#}$	6.39±1.58	2.78±0.39
TSH mIU/L	2.19±0.17	1.96±0.14	2.46±0.31	2.36±0.28
PRL mIU/L	315.74±28.0	324.95±22.4	261.77±24.7	337.16±47.5
GALP ng/mL	2.00±0.56	1.41±0.44^	0.77±0.49	0.32±0.07
Kisspeptin ng/mL	0.28±0.06	0.20±0.03	0.32±0.14	0.16±0.01

$^{\#}$ Significant difference between overweight and lean in PCOS group: $^{\#}$ p<0.05; $^{\#\#}$ p<0.01; $^{\#\#\#}$ p<0.001.
* Significant difference between overweight and lean in control group: * p<0.05; ** p<0.01; *** p<0.001.
↑ Significant difference between PCOS and controls with BMI ≥ 25: $^{\uparrow}$ p<0.05; $^{\uparrow\uparrow}$ p<0.01; $^{\uparrow\uparrow\uparrow}$ p<0.001.
∧ Significant difference between PCOS and controls with BMI < 25: ^ p<0.05; ^^ p<0.01; ^^^ p<0.001.

When we compared the two PCOS subgroups (Table 2), we found no age difference (p=0.16), but BMI (p<0.001) and WC (p<0.001) were higher in the overweight subgroup. No significant difference was found in LH, FSH levels, LH/FSH ratio, PRL and TSH levels between them. Women with PCOS and BMI≥25 also had significantly higher fasting insulin levels (p<0.001) and HOMA-IR values (p<0.001), but no significant difference was observed in fasting glucose levels (p=0.21). T and FAI score (p=0.005 and p<0.001, respectively) were significantly higher, whereas SHBG levels were lower (p<0.001) in the overweight PCOS subgroup. Despite GALP and kisspeptin levels being higher in that subgroup, they did not reach significance (p=0.41 and p=0.26).

In the normal-weight PCOS women GALP levels were positively correlated only with kisspeptin (r=0.656; p<0.01) and WC (r=0.355; p=0.021). Kisspeptin levels were positively correlated with WC (r=0.486, p<0.05), FAI (r=0.456, p<0.05) and GALP (r=0.656; p<0.01) and negatively correlated with fasting glucose (r=-0.494; p<0.05) (Table 3).

In the overweight PCOS subgroup we found a significant positive correlation between GALP and LH (r=0.361; p=0.026), LH/FSH ratio (r=0.345; p=0.034), T (r=0.486; p=0.002) and FAI (r=0.510; p=0.006). Kisspeptin levels were positively correlated with age (r=0.498, p<0.05), fasting insulin (r=0.510; p<0.05), HOMA-IR (r=0.483; p<0.05), T (r=0.512; p<0.05), SHBG (r=0.660; p<0.01) and FAI (r=0.751; p<0.001). No correlation was noted between kisspeptin and GALP levels in the overweight PCOS group (Table 3).

Table 3. Significant correlations between GALP and kisspeptin in women with PCOS.

	PCOS BMI < 25		**PCOS BMI ≥ 25**	
	Kisspeptin ng/mL	GALP ng/mL	Kisspeptin ng/mL	GALP ng/mL
	r			
Age y			0.498*	
WC sm	0.486*	0.355*		
Fasting Glucose mmol/L	-0.494*			
Fasting Insulin μIU/mL			0.510*	
HOMA-IR			0.483*	
LH (IU/L)				0.361*
LH/FSH ratio				0.345*
T (ng/mL)			0.512*	0.486**
SHBG nmol/L			0.660**	
FAI	0.456*		0.751***	0.510**
GALP ng/mL	0.656**			
Kisspeptin (ng/mL)		0.656**		

* Significant difference: * p<0.05; ** p<0.01; *** p<0.001.

4. Discussion

During the last few years GALP and kisspeptin have proved themselves to be affecting the reproduction and the regulation of food intake [14,15]. As they have been noticed to regulate the GnRH-madiated LH secretion [5,16] and having in mind that PCOS is a condition associated with disordered hypothalamic-pituitary-gonadal axis, the present study was designed to analyze and search for the possible role of kisspeptin and GALP in the pathogenesis of PCOS.

The data, that we have regarding changes in kisspeptin and GALP levels and its relation to metabolic and hormonal disturbances in PCOS, is little but controversial.

Since GALP was found to be a significant factor in initiating GnRH secretion and for regulating GnRH induced LH secretion, it is reasonable to find higher GALP levels in patients with PCOS. The present study shows statistically higher GALP and LH levels in patients with PCOS compared to controls.

In their study Panidis et al. indicated that obese and overweight women with PCOS had significantly lower kisspeptin levels compared with controls [17]. However, our results are not in accordance with this. Our findings are in agreement with two other works that reported increased kisspeptin levels in women with PCOS [18,19].

Women with PCOS usually show impaired gonadotrophin secretion with higher LH pulsatility and perturbed LH/FSH ratios. The chronic exposure to this endogenous LH excess can develop into stromal and thecal hyperplasia which results in ovarian hyperandrogenism [2]. Higher GALP and kisspeptin levels in women with PCOS may cause an increase in LH pulsatility which in turn leads to a persistent rise in androgen levels. Even though with no correlation in the whole PCOS group LH, GALP and kisspeptin levels were increased in the PCOS patients.

GALP has been noticed to respond to body energy status and insulin levels, and that it may be suppressed by conditions of energy insufficiency [20]. When we analyzed GALP levels according to the BMI of healthy women and those with PCOS, we found that GALP levels were increased in both obese and non-obese women with PCOS compared with controls, although in the overweight group they did not reach significance. Also we found no important differences between GALP and LH levels of obese and normal-weight patients with PCOS. GALP levels showed a strong positive correlation with LH, LH/FSH ratio, T and FAI in overweight and obese patients with PCOS, in contrast to the non-obese ones, where there was no significant dependence.

Potential factors involved in the different GALP and kisspeptin secretion between normal-weight and obese PCOS women may be insulin and/or different neurotransmitters. The regulation of mammalian energy balance and reproduction is strongly affected by the ARC, in which most GALP-containing cell bodies are located. Several populations of neurons in this nucleus are targets for the satiety hormones from adipose tissue. As our study failed to show a correlation between GALP levels and other metabolic parameters of obesity and insulin resistance, we assume that increased GALP levels in PCOS may be linked to other characteristics of the syndrome.

Our results, however, showed a positive correlation between kisspeptin and insulin levels and HOMA-IR in the overweight PCOS group and a positive correlation with waist circumference in the lean one. So increased kisspeptin levels in PCOS could be linked to insulin resistance and the secondary hyperinsulinemia. Obesity and other factors related to adipose tissue acting on the hypothalamic level, may modulate kisspeptin secretion in the obese state and probably are the major pathogenic contributors to this condition.

Hyperinsulinemia acts synergistically with LH to increase ovarian androgen production. Our data supports this, showing a strong positive correlation of kisspeptin levels with T, SHBG and FAI in overweight patients with PCOS and with FAI in normal-weight ones - indicators that are in direct association with LH and could be accepted as an indirect evidence of kisspeptin effect on LH.

An interesting finding of our study is the association between the levels of kisspeptin and GALP in the non-obese PCOS group. There are data in the literature that suggest a functional relationship between GALP and kisspeptin systems [10]. Our findings showed that women with PCOS have increased serum kisspeptin and GALP levels. Kisspeptin levels were positively correlated with GALP levels and act concomitantly in developing this condition in the non-obese women. We also showed that kisspeptin might be involved in insulin resistance among patients with PCOS. Still, the positive correlation between kisspeptin levels with FAI and SHBG in the overweight PCOS patients may be reflective of the hyperandrogenism and insulin resistance of this group.

A limitation of this study is the relatively small size of the patients and the control group. Additionally, due to the complexity and heterogeneity of the disease itself and the various mechanisms of action of the peptides, many confounding factors could have affected the correlations and interpretation of our results.

5. Conclusions

Our results indicate that GALP and kisspeptin are increased in women with PCOS and positively associated with hyperandrogenism accompanying this condition. GALP and kisspeptin probably are involved in the interaction between reproduction and metabolic state, affecting the hypothalamic-pituitary-gonadal axis. However, their mechanism for regulating gonadotropin secretion still remains unknown. Further characterization of the pathophysiologic association between GALP and kisspeptin signaling and PCOS is needed.

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Disclosure Statement

No potential conflicts of interest were disclosed.

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1991 - Specialty Internal Medicine, Higher Medical Institute, Medical University - Sofia;
1993 - Specialty Endocrinology and Metabolic diseases, Higher Medical Institute, Medical University - Sofia;
2005 - Academic degree Associate Professor, Medical University – Sofia;
2011 - Master degree in Public Health and Health Management, Medical University – Sofia; WOAR Journals Page 18 2013 - Academic degree Professor, Medical University – Plovdiv;
Relevant Previous Positions:
1987 -2005 - Senior Research Fellow, Assistant Professor, Clinical Centre of Endocrinology and Gerontology, ″Acad. Iv. Penchev″ University Hospital, Medical University – Sofia
1986 -1987 – Intern, Department of Internal Medicine with intensive sector, Primary Regional Hospital – Byala Slatina; "Pioner" and "9th Septemvri" Factories, Byala Slatina, Municipality of Montana, Bulgaria.
Relevant Job Related Training:
1993 - Specialization (IDF scholarship), Clinic of Endocrinology and Diabetes, Medical School, Department Medicine, University New Castle, England;
1997 - Course "Practical Diabetology", Steno Diabetes Centre, Gentofte and Copenhagen, Denmark; 1999 - Specialization, Clinic of Arterial Hypertension, "Brousse" Hospital, Paris, France;
2009 - Training course for treatment of diabetic foot, Diabetic Foot Clinic, King's College Hospital, London;
2012 - Qualification course in the field of diabetes, University "St. Andrew", United Kingdom.
Other Activities:
Pertinent to Professional Qualifications Project Manager in 4 and collaborator in 6 Projects of Ministry of Education and Science;
Project Manager in 12 Projects of Medical University -Sofia and Medical University -Plovdiv, Bulgaria Collaborator in MEDUCATOR - an International Project of Medical University - Plovdiv – E -learning in medicine Supervisor of 8 PhD students, consultant of 4 PhD students, Supervisor of 15 specializations in Endocrinology;
Editor -in -Chief of "ENDOCRINOLOGIA" Journal - printed edition of the Bulgarian Society of Endocrinology
Membership in scientific societies: Bulgarian Society of Endocrinology; Management Board Bulgarian Institute of Metabolic Syndrome; Management Board Bulgarian Association of Endocrine Hypertension /Alliance/; Management Board European Society of Endocrinology; European Association of Gynecological Endocrinology; European Association for the Study of Diabetes and Diabetes Education Study Group; European Thyroid Association
Scientific interests and publications in the field of: PCOS, reproductive disorders and hyperandrogenic states in women; insulin resistance, metabolic syndrome, obesity, adipocytokines and appetite regulator hormones, diabetes mellitus; Cushing's syndrome; endocrine hypertension; pituitary tumours; osteoporosis.