Association of Circulating Spexin Levels with Metabolic and Hormonal Disturbances in Polycystic Ovary Syndrome Women and Normal Controls

Maliha Shameem[1], Taseer Ahmed Khan[2], Kauser Abbas Saldera[3], Hina Rashid[4], Ufaque Batool K. Samo[5]

Abstract

Objective: To evaluate and compare theserum Spexin levels in women with and without polycystic ovary syndrome (PCOS) and to find if there is any association of Spexin levels with metabolic and hormonal parameters among PCOS women.

Methods: The case-control study was conducted from August 2021 till September 2022 in the Physiology Department at Basic Medical Sciences Institute (BMSI), Jinnah Postgraduate Medical Center, Karachi and Infertility Clinic, Jinnah Postgraduate Medical Center, Karachi. The present study investigated 160 subjects in reproductive age group of 15-45 years with 80 cases of PCOS diagnosed according to Rotterdam criteria and 80 controls without PCOS. The sample size was calculated using the Online Open Epi sample Size Calculator. IBM-SPSS version 20 was used to both store and analyze the data.

Results: The levels of serum Spexin were found to be lower in patients with polycystic ovary syndrome 1.85 ± 56ng/ml, compared to the levels of 2.65 ± 50ng/ml in the healthy group ($p<0.001$). The metabolic markers such as BMI, fasting blood glucose, serum Insulin, Homeostatic Model Assessment for Insulin Resistance, Cholesterol and Triglycerides were found to be elevated in polycystic ovary syndrome patients and were found to have a negative correlation with Spexin levels. High density lipoprotein was lower in polycystic ovary syndrome patients when compared to healthy controls, and indicated a positive link with Spexin. Also subjects with polycystic ovary syndrome had elevated levels of Luteinizing Hormone and Testosterone with Luteinizing Hormone negatively connected with Spexin.

Conclusion: Decreased Spexin level as compared to healthy peers were inversely associated with unfavoruable metabolic and hormonal profiles in PCOS subjects, suggesting the inter-related roles of Spexin in the different metabolic and endocrine pathways of PCOS.

Key Words: PCOS, Spexin, Insulin Resistance, Hormonal imbalance.

IRB: Approved by institutional review Board of jinnah postgraduate medical centre. Ref # F-2-81/2021 GENL/37775/JPMC. dated 3rd February 2021.

Citation: Shameem M, Khan TA, Saldera KA, Rashid H, Samo UBK. Association of Circulating Spexin Levels with Metabolic and Hormonal Disturbances in Polycystic Ovary Syndrome Women and Normal Controls [Online]. Annals ASH & KMDC.

(ASH & KMDC 26(4):179-184;2023

Introduction

Spexin a unique endogenous peptide hormone discovered relatively recently (Neuropeptide Q) encoded by the C12ORF39 gene[1]. Spexin gene is significantly expressed in various tissues like pancreas, liver, visceral fat, adrenal glands, ovaries, testes and thyroid. It is implied that spexin regulates the secretion of Insulin in pancreatic tissue[2]. Spexin has been found to show link with decreased food intake thereby suppressing food intake, body weight, satiety as well as effecting nutritional behavior in animals[3]. As an adipokine, Spexin also contributes significantly in controlling fat metabolism. In addition, lower Spexin levels were found in obese subjects when compared to normal weight peers. Spexin also inhibited the uptake of long chain fatty acids, which helped in weight loss in diet-induced obesity animals[4]. Spexin along with energy metabolism mediator, also plays an important role in regulating reproductive axis. Research shows that Spexin inhibits luteinizing hormone secretion thus effects reproduction[5].

[1,3-5] Department of Physiology, Basic Medical Science Institute (BMSI), Jinnah Postgraduate Medical Center, Karachi
[2] Department of Physiology, University of Karachi

Correspondence:
Dr. Maliha Shameem
Department of Physiology, Basic Medical Science Institute (BMSI), Jinnah Postgraduate Medical Center, Karachi
Email: drmalihamohsin@gmail.com
Date of Submission: 22nd October 2023
Date of Acceptance: 29nd October 2023

PCOS is a highly prevalent endocrinopathy with metabolic and reproductive effects seen in reproductive aged women[6]. The characteristic manifestations of PCOS include menstrual dysfunction, polycystic ovaries and signs of hyperandrogenism[7]. Although its pathophysiology remains unclear insulin resistance as well as disruption in androgen secretion and action, relative ratios of gonadotropin and ovulatory dysfunction play an essential role in the development of PCOS[8]. Association found between insulin signaling and PCOS thus links it as a metabolic disorder. Women with PCOS tend to have metabolic abnormalities such as obesity, insulin resistance along with disturbances of glucose and lipid metabolism[9].

Any association of Spexin regarding its relationship with PCOS is still not explored much. We aimed to find the difference in the serum Spexin levels in women with and without PCOS. We also aimed to find any association of Spexin levels with metabolic and reproductive parameters among women with PCOS.

Methodology

This case-control study used non-probability purposive sampling technique A total of n=160 subjects within reproductive age groups of 15-45 years were recruited, 80 women with PCOS (cases) and 80 women without PCOS having regular menstrual cycles (controls). PCOS was diagnosed based on the Rotterdam criteria, which require the presence of at least two of the following: polycystic ovaries observed through ultrasound, oligoovulation/anovulation, biological hyperandrogenism (excessive androgen production), or clinical hyperandrogenism (hirsutism, androgenic alopecia, or acne). Other conditions such as hyperprola-ctinemia, thyroid disorders, diseases of the adre-nals, intake of oral contraceptive pills, and non-classical congenital adrenal hyperplasia were excluded. The sample size for the study was determined using the Online Open Epi sample size calculator, with a power of 80%, a confidence interval of 95%, and a significance level of≥0.05. IBM-SPSS 20 was used to both store and analyze the data. The study obtained ethical permission from the Institutional Review board of JPMC, Karachi. Written informed consent was taken from the study participants.

Qualitative data was represented using pie charts and bar graphs, while the Chi-Square test was employed for the analysis of this data. The physical, metabolic, and hormonal characteristics were measured and their mean and standard deviation were recorded. To compare the quantifiable parameters between the control and case groups, the independent sample t-test was utilized. The correlation between Serum Spexin and BMI, metabolic and hormonal parameters among the cases was evaluated using Pearson's coefficient of correlation (r).

Results

In this study the mean age among healthy controls was 30.48 ± 6.4 years and 30.62 ± 6.11 years among PCOS cases. The mean BMI was 28.05 ± 3.89 kg/m2 among controls and 29.20 ± 3.74 kg/m2 among cases (Table 1).

Table 2 shows that mean fasting blood glucose to be 80.16 ± 8.4 mg/dl among controls and 89.42 ± 14.6 mg/dl among cases. Mean Serum Insulin level found to be 9.11 ± 0.96 µIU/ml among controls and 12.7 ± 1.5 µIU/ml among cases with mean HOMA-IR when calculated was found to be 1.8 ± 0.22 among control group and 2.8 ± 0.63 among cases group. Mean serum cholesterol was 193.49 ± 11.9 mg/dl in control group while 206.86 ± 14.3 mg/dl in cases group. Mean Triglyceride levels were found to be 121.65 ± 7.54 mg/dl in controls and 158.10 ± 19.0 mg/dl in cases. Mean HDL levels were found to be 45.43 ± 5.46 mg/dl among controls and 39.14 ± 4.21 mg/dl among cases. However, mean LDL levels among controls were 124.35 ± 16.04 mg/dl and cases was 126.39 ± 12.3 mg/dl in controls. Results of Independent sample t-test showed statistically significant difference ($p<0.001$) among fasting blood glucose, serum Insulin, HOMA-IR, Cholesterol, triglycerides and H DL levels among both groups. Mean FSH was 6.42 ± 0.60 mIU/ml among controls and 6.23 ± 1.07 mIU/ml among cases. LH levels were 7.7 ± 1.04 mIU/ml among controls and 14.12 ± 2.65 mIU/ml

in cases. Serum Testosterone was 0.38 ± 0.07 ng/ml among controls and 0.45 ± 0.13 ng/ml amo-ng cases. Independent sample t-test showed LH and serum Testosterone levels to be statistically significant among controls and cases (p value of <0.001). Serum Spexin was found to be 2.65 ± 0.50 ng/ml among control group while 1.85 ± 0.56 ng/ml among cases group (p<0.001).

Table 3 shows the correlation of Spexin with metabolic and hormonal parameters using Pearson Correlation analysis. BMI (Kg/m²) was found to show negative correlation with serum Spexin (r = -0.297, p<0.05), fasting blood glucose(FBG) shows negative correlation with Spexin (r = -0.495, p<0.001), serum Insulin shows negative correlation with Spexin (r = -0.408, p<0.001), HOMA-IR shows negative correlation with Spexin (r = -0.601, p<0.001), Cholesterol shows negative correlation with Spexin (r = -0.527, p<0.001), Triglycerides show negative correlation with Spexin (r = -0.535, p<0.001), however, HDL shows positive correlation with Spexin (r = 0.310, p<0.05). Luteinizing Hormone (LH) shows negative correlation with Spexin (r = -0.346, p< 0.05), Testosterone shows negative correlation with Spexin (r = -0.079, p>0.05), however this correlation is not statistically significant.

Table 1. Anthropometric Parameters of Study Participants

Anthropometric Parameters		Groups	
		Non-PCOS Controls (n=80)	PCOS Cases(n=80)
Age Groups (years)	15-25	30 (37.5%)	22 (27.5%)
	26-35	27 (33.7%)	40 (50%)
	36-45	23 (28.7%)	18 (22.5%)
	Mean ± SD	30.48 ± 6.4	30.62 ± 6.11
Weight (Kg)	Mean ± SD	74.05 ± 8.9	75 ± 9.3
Height (m)	Mean ± SD	1.65 ± 0.5	1.6 ± 0.5
BMI (Kg/m²)	Normal	15 (18.7%)	14 (17.5%)
	Overweight	38 (47.5%)	37 (46.2%)
	Obese	27 (33.7%)	29 (36.2%)
	Mean ± SD	28.05 ± 3.89	29.20 ± 3.74

Table 2. Comparison of metabolic and hormonal parameters among study participants

Metabolic and Hormonal Parameters	Groups		p-value
	Non-PCOSControl (n=80)	PCOS Cases(n=80)	
FBG (70-100 mg/dl)	80.16 ± 8.4	89.42 ± 14.6	<0.001*
Serum Insulin (<12.2 µIU/ml)	9.11 ± 0.96	12.7 ± 1.5	<0.001*
HOMA-IR (< 2)	1.8 ± 0.22	2.8 ± 0.63	<0.001*
Cholesterol (<200 mg/dl)	193.49 ± 11.9	206.86 ± 14.3	<0.001*
Triglycerides (<150 mg/dl)	121.65 ± 7.54	158.10 ± 19.0	<0.001*
HDL (>50 mg/dl)	45.43 ± 5.46	39.14 ± 4.21	<0.001*
LDL (<130 mg/dl)	124.35 ± 16.04	126.39 ± 12.3	0.370
FSH (3.85- 8.78 mIU/ml)	6.42 ± 0.60	6.23 ± 1.07	0.172
LH (0.5-10 mIU/ml)	7.7 ± 1.04	14.12 ± 2.65	<0.001*
Testosterone (0.06 - 0.86 ng/ml)	0.38 ± 0.07	0.45 ± 0.13	<0.001*
Serum Spexin (0.01– 4 ng/ml)	2.65 ± 0.50	1.85 ± 0.56	<0.001*

Results are given as mean ± SD.
An Independent samples t-test was used with p value of less than 0.05 considered significant BMI: Body mass index; FBG: Fasting blood glucose; HOMA-IR: Homeostasis model assessment of insulin resistance; HDL: High density lipoprotein cholesterol; LDL: Low density lipoprotein cholesterol; FSH: Follicle stimulating Hormone; LH: Luteinizing Hormone; PCOS: Polycystic ovary syndrome

Table 3. Correlation analysis of spexin with metabolic and hormonal parameters among cases

VariableSerum	Spexin (ng/ml)	
	r-value	p-value
BMI (Kg/m²)	-0.297	<0.05*
FBG (mg/dl)	-0.495	<0.001*
Serum Insulin (µIU/ml)	-0.408	<0.001*
HOMA-IR	-0.601	<0.001*
Cholesterol (mg/dl)	-0.527	<0.001*
Triglycerides (mg/dl)	-0.535	<0.001*
HDL (mg/dl)	0.310	<0.05*
LDL (mg/dl)	0.154	0.171
FSH (mIU/ml)	0.038	0.739
LH (mIU/ml)	-0.346	<0.05*
Testosterone(ng/ml)	-0.079	0.483

Pearson's correlation coefficient (r) used.

Discussion

In the present study, we have evaluated circulating levels of Spexin among women with PCOS and healthy controls. We found circulating Spexin levels to be significantly decreased in women with PCOS as compared to controls. The results of our study is similar to another study, which also documents decrease serum levels of Spexin among PCOS women[11].

However, no difference was found between serum Spexin levels in PCOS group and control group in another study[12]. We have found statistically significant difference in metabolic parameters between both groups. The difference in glycemic parameters among controls such as fasting blood glucose, Serum Insulin and HOMA-IR was found to be statistically significant ($p<0.001$) when compared with PCOS group. We also found inverse correlation of Spexin with fasting blood glucose, Serum Insulin and HOMA-IR suggesting the link of Spexin in glucose metabolism. Women with PCOS frequently exhibit increased insulin secretion, which occurs as a consequence of insulin resistance. Spexin is involved in regulation of insulin secretion[11]. We discovered in the current study that women with PCOS had higher levels of insulin in their blood and a greater degree of insulin resistance than the controls. The production of hormones by the endocrine portion of the pancreas plays an essential role in maintaining metabolic homeostasis. Spexin and insulin co-localization in beta cells of pancreas has been demonstrated in humans[2]. Several human studies have also reported the role of Spexin in lipid metabolism. Some stu-dies showed an inverse correlation of Spexin with lipid profile[13,14]. In our study, parameters of lipid metabolism have also found to be altered in PCOS women as compared to the controls. Serum Cholesterol and Triglycerides levels were found to be increased while HDL levels have been found to be decreased in PCOS women ($p<0.001$) when compared with healthy controls. We also found negative association of Spexin with unfavorable lipid profile and positive association with HDL. In agreement with our study, similar results have been reported in a recent study in Egypt[13]. Accumulative data also have reported raised serum levels of Insulin and degree of Insulin resistance in PCOS women[11,15]. In some studies significant association has been reported between Spexin and glycemic parameters[13,16]. These findings are in favor of Spexin having role in metabolic health.These results imply that decreased Spexin levels may have a role in development of hyperinsulinemia and insulin resistance thus serving as a valuable biomarker of an individual's metabolic health status. Spexin may act as a key biomarker in the early alteration in the cardiometabolic health and the development of related comorbidities such as cardiovascular diseases and diabetes. However some studies found no association between Spexin with metabolic parameters[17,18].

There is imbalance in Hypothalamic-pituitary-ovarian axis which is an important pathophysiology leading to PCOS. A mounting evidence suggests that in women with PCOS higher GnRH pulse frequency and strength can favour LH generation over FSH production leading to a high LH to FSH ratio[19]. LH production promotes the androgen synthesis in ovarian theca cells leading to hyperandrogenemia and perturbed follicular development along with inhibition of ovulation due to increase secretion of LH, thus promoting the formation of polycystic ovarian morphology among PCOS females[20.]. In our study, significantly increased levels of LH among PCOS females as compared to controls ($p<0.001$) along with inverse association of Spexin with LH have been found. Similar, negative associ-ation of Spexin with LH has also been docume-nted in various studies[11,21]. These findings imply the role of Spexin in regulating the hypothalamic-pituitary-ovarian axis through its association with LH.

The focus of treatment in PCOS has mostly been on menstrual irregularities, fertility problems, acne and hirsutism with less focus on long term cardiometabolic risks. The possibility of Spexin as a therapeutic target will be helpful in the development of innovative novel therapies in the future for the treatment of metabolic disorders and obesity.

Conclusion

In conclusion, the diminished presence of Spexin in females with PCOS indicates that Spexin could potentially play a role in connecting the different pathophysiological mechanisms of PCOS. It is possible that Spexin serves as a significant mediator in individuals with metabolic obesity, potentially enhancing their metabolic well-being. However, additional research is necessary to establish the exact nature of the association between Spexin and PC OS.

Reference

1. Assefa F, Kim JA, Lim J, Nam SH, Shin HI, Park EK. The neuropeptide spexin promotes the osteoblast differentiation of MC3T3-E1 cells via the MEK/ERK pathway and bone regeneration in a mouse calvarial defect model. Tissue Engineering and Regenerative Medicine 2022;1:1-4. [DOI: 10.1007/s13770-021-00408-2]. Available from: https://europepmc.org/article/med/34951679. Accessed on 28th November 2023.

2. Sassek M, Kolodziejski PA, Szczepankiewicz D, Pruszynska-Oszmalek E. Spexin in the physiology of pancreatic islets-mutual interactions with insulin. Endocrine 2019;63:513-9. [DOI: 10.1007/s12020-018-1766-2]. Available from: https://link. springer.com/article/10.1007/s12020-018-1766-2. Accessed on 28th November 2023.

3. Jeong B, Kim KK, Lee TH, Kim HR, Park BS, Park JW,et al. Spexin Regulates hypothalamic leptin action on feeding behavior. Biomolecules 2022 ;12(2):236. [DOI: 10.3390/biom12020236]. Available from: https://www.mdpi.com/2218-273X/12/2/236. Accessed on 28th November 2023.

4. Walewski JL, Ge F, Lobdell IV H, Levin N, Schwartz GJ, Vasselli JR, et al. Spexin is a novel human peptide that reduces adipocyte uptake of long chain fatty acids and causes weight loss in rodents with diet induced obesity. Obesity. 2o14;22(7):1643-52. [DOI: 10.1002/oby.20725]. Available from: https://onlinelibrary.wiley.com/doi/10.1002/oby.20725. Accessed on 28th November 2023.

5. Respekta N, Maœlanka A, Mlyczyñska E, Billert M, Szlaga A, Sambak P, et al. Levels of spexin and its receptors GALR2 and GALR3 in the hypothalamus and ovary of letrozole-induced polycystic ovary syndrome in rats. Biochem Biophys Res Commun 2022;627:207-13. [DOI: 10.1016/j.bbrc.2022.08.059]. Avaialbe from: https://www. sciencedirect.com/science/article/abs/pii/S0006291X22011895?via%3Dihub. Accessed on 28th November 2023.

6. Ortiz-Flores AE, Luque-Ramírez M, Escobar-Morreale HF. Polycystic ovary syndrome in adult women. Med Clín (English Edition). 2019; 152(11):450-7. [DOI:10.1016/j.medcli.2018.11.019]. Available from: https://www.elsevier.es/es-revista-medicina-clinica-2-linkresolver-sindrome-ovario-poliquistico-mujer-adulta-S0025775318307474. Accessed on 28th November 2023.

7. Kostopoulou E, Anagnostis P, Bosdou JK, Spiliotis BE, Goulis DG. Polycystic ovary syndrome in adolescents: pitfalls in diagnosis and management. Current Obesity Reports. 2020;9:193-203. [DOI: 10.1007/s13679-020-00388-9]. Avaialbe from: https://link.springer.com/article/10.1007/s13679-020-00388-9. Accessed on 28th November 2023.

8. Unluhizarci K, Karaca Z, Kelestimur F. Role of insulin and insulin resistance in androgen excess disorders. World J Diabetes. 2021;12(5):616-29. [DOI: 10.4239/wjd.v12.i5.616]. Avaialble form: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8107978/. Accessed on 28th Novemver 2023.

9. Vatier C, Christin-Maitre S, Vigouroux C. Role of insulin resistance on fertility–Focus on polycystic ovary syndrome. InAnnales d'Endocrinologie. 2022;28(3):199-202. Elsevier Masson. [DOI: 10.1016/j.ando.2022.04.004]. Available from: https://www.sciencedirect.com/science/article/abs/pii/S0003426622000518?via%3Dihub. Accessed on 28th November 2023.

10. Rotterdam ESHRE/ASRM Sponsored PCOS Consensus Workshop Group. Revised 2003 consensus on diagnostic criteria and long term health risks related to polycystic ovary syndrome (PCOS). Hum Reprod 2004;19(1):41-7. [DOI: 10.1093/humrep/deh098]. Available from: https://academic.oup.com/humrep/article/19/1/41/690226?login=false. Accessed on 28th November 2023.

11. Guler A, Demir Ý. Decreased levels of spexin are associated with hormonal and metabolic disturbance in subjects with polycystic ovary syndrome. Journal of Obstetrics and Gynaecology. 2021;41 (3):408-13. [DOI: 10.1080/01443615.2020.1737660]. Available from: https://www.tandfonline.com/doi/abs/10.1080/01443615.2020.1737660. Accessed on 28th Novemer 2023.

12. Beyazit F, Hiz MM, Turkon H, Unsal MA. Serum spexin, adiponectin and leptin levels in polycystic ovarian syndrome in association with FTO gene polymorphism. Ginekologia Polska. 2021;92 (10) :682-8. [DOI: 10.5603/GP.a2020.0176]. Available from: https://journals.viamedica.pl/ginekologia_polska/article/view/69496. Accessed on 28th November 2023.

13. Albeltagy ES, Abd Elbaky NM. Association of lower circulating Spexin levels with higher body mass indices and glucose metabolic profiles in adult subjects in Egypt. Human Nutrition & Metabolism. 2022;27:200137. [DOI: /10.1016/j.hnm.2021.200137]. Available from: https://www.sciencedirect.com/science/article/pii/S2666149721000190. Accessed on 28th November 2023.

14. Arshad FA, Mehmood R, Kausar N, Bibi A, Khan MA, Hussain S, Perveen S. Assessment and association between lipid and hormonal profile in nonpregnant females having polycystic ovarian syndrome. Endocrinol Metab Syndr 2019;8(1):1-5. Available from: https://www.longdom.org/open-access/assessment-and-association-between-lipid-and-hormonal-profile-in-nonpregnant-females-having-polycystic-ovarian-syndrome-25541.html. Accessed on 28th November 2023.

15. Dahan MH, Reaven G. Relationship among obesity, insulin resistance, and hyperinsulinemia in the polycystic ovary syndrome. Endocrine. 2019 Jun 15;64:685-9. [DOI: 10.1007/s12020-019-01899-9]. Available from: https://link.springer.com/article/10.1007/s12020-019-01899-9. Accessed on 28th November 2023.

16. Al-Daghri NM, Sabico S, Al-Hazmi H, Alenad AM, Al-Amro A, Al-Ghamdi A, Hussain SD, Chrousos G, Alokail MS. Circulating spexin levels are influenced by the presence or absence of gestational diabetes. Cytokine 2019;113:291-5. [DOI; 10.1016/j.cyto.2018.07.023]. Available from: https://www.sciencedirect.com/science/article/abs/pii/S1043466618303223?via%3Dihub. Accessed on 28th November 2023.

17. Hodges SK, Teague AM, Dasari PS, Short KR. Effect of obesity and type 2 diabetes, and glucose ingestion on circulating spexin concentration in adolescents. Pediatr Diabetes 2018;19(2):212-6. [DOI: 10.1111/pedi.12549.]. Available from: https://onlinelibrary.wiley.com/doi/10.1111/pedi.12549. Accessed on 28th November 2023.

18. Karaca A, Bakar-Ates F, Ersoz-Gulcelik N. Decreased spexin levels in patients with type 1 and type 2 diabetes. Medical Principles and Practice. 2019;27(6):549-54. [DOI: 10.1159/000493482]. Available from: https://karger.com/mpp/article/27/6/549/204421/Decreased-Spexin-Levels-in-Patients-with-Type-1. Accessed on 28th November 2023.

19. Okigbo CC, Gill S, Hall JE. The Hypothalamic-Pituitary Axis in PCOS. InPolycystic Ovary Syndrome: Current and Emerging Concepts. Springer 2022;73-9.

20. Kumariya S, Ubba V, Jha RK, Gayen JR. Autophagy in ovary and polycystic ovary syndrome: role, dispute and future perspective. Autophagy. 2021 Oct 3;17(10):2706-33. [DOI: 10.1080/15548627.2021.1938914]. Available from: https://www.tandfonline.com/doi/full/10.1080/15548627.2021.1938914. Accessed on 28th November 2023.

21. Sherman SB. The Role of Neuropeptide Spexin in the Modulation of Metabolism and Behaviors. The University of Toledo. OhioLINK Electronic Theses and Dissertations Center 2020; [DOI: 000-0002-7248-8565]. Available from: https://etd.ohiolink.edu/acprod/odb_etd/etd/r/1501/10?clear=10&p10_accession_num=mco1596840064046446. Accessed on 28th November 2023.