Med. J. Cairo Univ., Vol. 90, No. 1, March: 367-376, 2022
www.medicaljournalofcairouniversity.net

Low Serum Spexin Levels may Underlie the Reciprocal Relation between Non-Alcoholic Fatty Liver and Polycystic Ovary Syndrome: An Observational Study

BASMA E. SAKR, M.D.*; MAHA M. RACHWAN, M.D.** and HATEM S. ALEGAILY, M.D.***

The Departments of Obstetrics & Gynecology, Clinical Pathology** and Hepatology, Gastroenterology & Infectious Diseases***, Faculty of Medicine, Benha University*

Abstract

Background: Polycystic ovary syndrome (PCOS) is a hormonal disorder common among women of reproductive age and affects over than 10% of these women. The hormonal derangement associated with PCOS is uniformly characterized by excess androgens and abnormal insulin activity.

Aim of Study: Estimation of serum levels of spexin (SPX) in women with polycystic ovary syndrome (PCOS) who had non-alcoholic fatty liver disease (NAFLD) and to evaluate its relation to the diagnostic markers of both diseases.

Patients and Methods: 102 PCOS women with NAFLD were evaluated clinically and by ultrasound for estimation of ovarian size and determination of liver steatosis grade. Blood samples were obtained for estimation of serum SPX, testosterone, dehydroepiandrosterone sulfate (DHEA-S), lipid profile, aspartate transaminase (AST) and alanine transaminase (ALT) and the AST/ALT ratio (AAR) was calculated.

Results: All women showed significantly high serum testosterone, DHEA-S, blood lipid profile with high homeostasis model assessment of IR (HOMA-IR) score, while showed significantly lower serum SPX levels than control women. Sixty-nine women had ovarian size of >10cm^3, and 36 and 5 patients had steatosis of grade 1 and 2, respectively. Serum SPX levels showed negative significant correlations with liver steatosis grade, HOMA-IR, ovarian size, BMI, serum testosterone, TG, TC and AAR; while showed positive significant correlation with serum HDL-c. Liver steatosis grade showed positive significant correlation with serum testosterone, ovarian size, BMI, AAR, HOMA-IR score, serum levels of DHEA-S, lipids. Regression analysis defined low SPX, high testosterone, LDL-c and DHEA-S levels are for high liver steatosis grade in PCOS women.

Conclusion: Serum SPX concentrations were significantly decreased in patients had PCOS and NAFLD and was correlated with their associated disturbances. Spexin could be considered as the cornerstone for regulation of the relation between PCOS and NAFLD.

Key Words: *Polycystic ovary syndrome – Non-alcoholic fatty liver disease – Spexin – Liver steatosis grade – Ovarian size.*

Correspondence to: Dr. Basma E. Sakr, The Department of Obstetrics & Gynecology, Faculty of Medicine, Benha University

Introduction

POLYCYSTIC ovary syndrome (PCOS) is a hormonal disorder common among women of reproductive age [1] and affects over than 10% of these women [2]. The hormonal derangement associated with PCOS is uniformly characterized by excess androgens and abnormal insulin activity [3]. PCOS presents with multiple clinical manifestations, which vary among patients, but is characterized by polycystic ovary morphology and rare or anovulation [4] and the altered hormonal milieu secondary to PCOS may extend during pregnancy and affect the normal fetal development [3].

Non-alcoholic fatty liver disease (NAFLD) is a highly prevalent major type of chronic liver disease [5] that may progress to end-stage liver diseases [6]. The pathophysiology underlying NAFLD involves a multitude of interlinked processes [7]. Inflammation plays key roles in NAFLD pathogeneses and continuous inflammation promotes the progression of nonalcoholic steatohepatitis [5]. Lipids are essential cellular components that participate in metabolic and endocrine regulation and reproductive functions [9], but altered oxidant/antioxidant balance causes chronic impairment of lipid metabolism [10].

Spexin (SPX) is a highly conservative amidated 14-amino acid peptide hormone that was discovered in 2007 by bioinformatics methods [11]. Animal studies detected expression of SPX gene and its protein product in a wide range of organs including, adrenal medulla [12], brain [13] and contribute to CNS-mediated control of arterial blood pressure and salt and water balance and modulate nociceptive responses [14]. Spexin mRNA and protein are associated with weight loss in rodents of diet-induced obesity [15] and are inversely related to blood glucose and lipids in T2DM, suggesting a

possible role for SPX in glucose and lipid metabolism [16].

Aim of the study:

Estimation of serum levels of spexin (SPX) in PCOS women with NAFLD and to evaluate its relation to the diagnostic markers of both diseases.

Patients and Methods

This Two-arm prospective observational study was conducted at Departments of Gynecology & Obstetrics, Hepatology and Clinical Pathology, Faculty of Medicine, Benha University, since Jan. 2019 till March 2020 after approval of the study protocol by the Local Ethical Committee. This study was designed as two-arm study; so, all women attending the Gynecology outpatient clinic complaining of infertility or amenorrhea were evaluated for the Rotterdam diagnostic criteria of PCOS and those fulfilling at least two of these three criteria were referred to Hepatology outpatient clinic for evaluation of liver for presence of NAFLD and were collected as group A. On the other arm, all women attending the Hepatology outpatient clinic with manifestation of NAFLD were asked about their fertility status and menstrual history and any woman with these problems were referred to Gynecology outpatient clinic for evaluation for Rotterdam diagnostic criteria of PCOS and were collected as group B. The study included 30 healthy fertile women of cross-matched age and BMI, and free of PCOS and NAFLD as control group for estimated serum spexin.

Evaluation parameters:

1- Obesity was evaluated depending on calculation of body mass index (BMI) according to the equation BMI (kg/m^2) = Weight (kg)/height (m^2) [17] and was graded according to WHO guidelines as average weight (BMI <24.9 kg/m^2), overweight (BMI=25-<30 kg/m^2) obese (BMI=30-<35 kg/m^2) and morbid obese (BMI >35kg/m^2) [18].

2- Diagnosis of PCOS: PCOS was diagnosed depending on the Rotterdam diagnostic criteria which included the following criteria: Amenorrhea of oligomenorrhea defined as less than 8 spontaneous menstrual cycles per year for at least 3 years before enrollment, serum total testosterone of >0.8ng/ml, and on ovarian transabdominal ultrasonography (TAU) or transvaginal ultrasonography (TVU) there were >12 follicles of 2–9mm range and/or an ovarian volume of > 10ml per ovary [19,20,21].

3- Diagnosis of NAFLD:

- Regarding the non-specific clinical presentation of patients with NAFLD [22], it can be diagnosed depending on the presence of abnormal liver enzyme serum levels especially aspartate transaminase (AST) and alanine transaminase (ALT). The AST/ALT ratio (AAR) was calculated to evaluate the presence of fibrosis, where AAR<1 indicated no or minimal fibrosis and AAR> 1 indicated presence of fibrosis-to-cirrhosis [23].

- TAU was performed to determine hepatic and renal parenchymal echogenic density on a grayscale and an average of three repeated measurements was used to calculate hepatorenal index (HRI) by the formula: HRI=Mean liver echogenicity divided by mean kidney echogenicity [24]. The optimal cutoff value for HRI to define significant steatosis was a value of > 1.27 that showed 100% sensitivity and negative predictive values with a specificity value of 54% [25] and a HRI in the range of 1-1.04 indicates normal liver, while hepatic steatosis was classified as mild if HRI was 1.05-1.24, moderate if HRI was 1.25-1.64 or severe if HRI was 1.65 [26].

4- Insulin resistance (IR) was evaluated using the homeostasis model assessment of IR (HOMA-IR) score that was calculated according to the formula: Fasting serum insulin (µU/ml) x [FBG (mg/ml)/18])/22.5; HOMA-IR score of >2 is considered abnormal [27].

Exclusion criteria:

Amenorrhea secondary to other pathologies, evident endocriopathy, diabetes mellitus, previous pelvic surgery involving the ovaries, hepatosplenomegaly, other causes of chronic liver diseases, hereditary lipoprotein disorders, PCOS without NAFLD, and NAFLD without PCOS.

Inclusion criteria:

All women with PCOS and NAFLD and accepted to sign the written fully informed consent were enrolled in the study. For comparative purposes, 30 fertile women with age- and BMI-matched and free of PCOS and NAFLD and of other exclusion criteria were collected from family planning clinic and enrolled as control group for US and laboratory data.

Blood sampling:

All enrolled women were asked to attend the hospital lab fasting for 12 hours and gave blood sample for estimation of blood lipid profile and to re-attend on the second day fasting 6 hours and

gave another blood sample for estimation of fasting blood glucose and hormonal profile. Blood samples were obtained under complete aseptic condition and divided into two parts:

1- The first part was put in a tube containing sodium fluoride (2mg sodium fluoride/ml blood) to prevent glycolysis for estimation of blood glucose levels.

2- The second part was collected in plain tube, allowed to clot, centrifuged at 1500xg for 15min and the serum samples were collected, divided into two parts; the first part was used for estimation of lipid profile and hormonal profiles and the 2nd part was collected in clean Eppindorff tube and stored at –20ºC for estimation of estimation of serum spexin levels.

Estimated parameters:

1- Blood glucose levels were estimated by glucose oxidase method by glucose oxidase method using BT 1500 Automatic biochemistry analyzer (SPAN Diagnostics, Gujarat India).

2- Serum levels of total cholesterol (TC), triglycerides (TG), high-density lipoprotein (HDL) and low-density lipoprotein (LDL), AST and ALT were estimated by photoluminescence methods using BT 1500 Automatic biochemistry analyzer (SPAN Diagnostics, Gujarat India).

3- Serum levels of insulin, testosterone (T), dehydroepiandrosterone sulfate (DHEA-S) and Prolactin using Automatic Immunoassay Analyzer (MAGLUMI 600, Snibe Diagnostic Co., Ltd., China).

4- Serum levels of human spexin was estimated using ELISA kit provided by Assay Genie Co (HUFI03307; Auckland, USA).

Study outcome:

Study outcome is the relation between serum levels of SPX and biochemical and ultrasonographic diagnostic markers for PCOS and NAFLD.

Statistical analysis:

Data are presented as mean, standard deviation (SD), numbers, percentages, median and interquartile range (IQR). Parametric results were analyzed one-way Anova test and non-parametric results were analyzed using Chi-square test and Mann-Whitney test. Correlation analysis was performed using Pearsons' correlation analysis and Regression analysis was performed using the Stepwise method. Statistical analysis was conducted using IBM® SPSS® Statistics (Version 22, 2015; Armonk, USA) for Windows statistical package. p-value <0.05 was considered statistically significant.

Results

During the study duration, 60 women of 325 women who had attended the Gynecology outpatient clinic, had at least two of the Rotterdam diagnostic criteria of PCOS and were found to have the diagnostic criteria for NAFLD for 18.5% incidence of NAFLD among PCOS women (Group A). On the other side, 42 women of 169 women who had attended the Hepatology outpatient clinic, had the diagnostic criteria of NAFLD and showed at least two of the Rotterdam diagnostic criteria of PCOS for 24.9% incidence of PCOS among women had NAFLD (Group B). These 102 women who had the diagnostic criteria of PCOS and NAFLD were enrolled in the study and underwent the study evaluation and investigations (Fig. 1). Demographic and clinical data evaluation showed non-significant (p>0.05) differences between patients of both groups (Table 1).

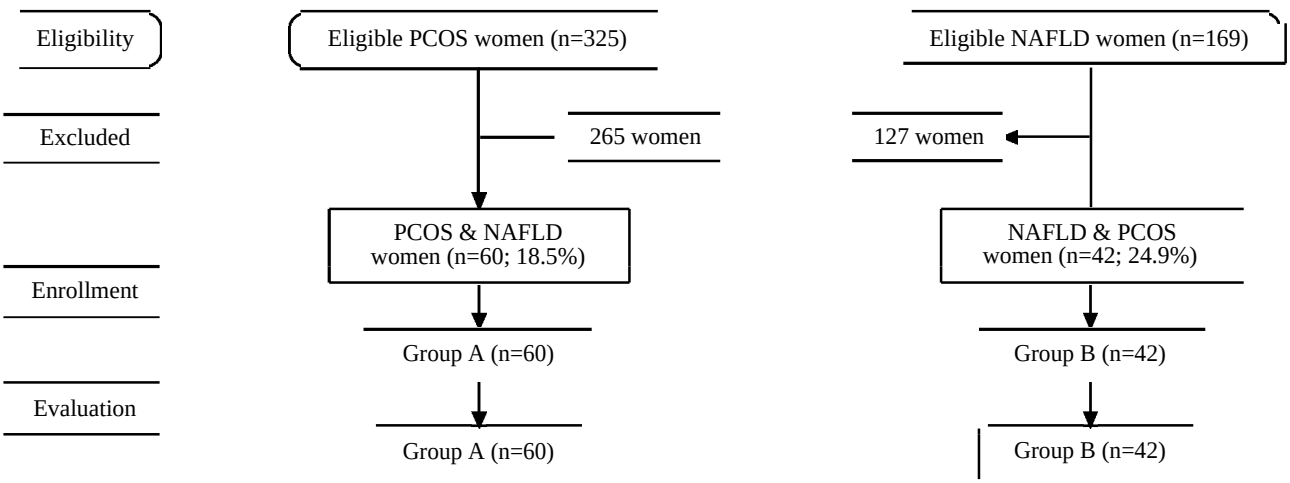

Fig. (1): Consort flow sheet.

Table (1): Demographic and clinical data of patients of both groups.

Data	Group A (n=60)	Group B (n=42)	*p*-value
Age (year):			
Categories:			
<20	3 (5%)	1 (2.4%)	0.526
20-25	26 (43.3%)	18 (42.9%)	
>25-30	24 (40%)	14 (33.3%)	
>30	7 (11.7%)	9 (21.4%)	
Mean (±SD)	26.2 (±3.9)	27 (±4.7)	0.354
Body mass index (kg/m^2):			
Categories:			
Overweight (24.9-29.9)	13 (21.7%)	8 (19%)	0.259
Obese (<30-34.9)	39 (65%)	23 (54.8%)	
Morbid obese (>30)	8 (13.3%)	11 (26.2%)	
Mean (±SD)	32.4 (±2.5)	33.4 (±2.7)	0.053
Marital history:			
Married	35 (58.3%)	30 (71.4%)	0.176
Single	25 (41.7%)	12 (28.6%)	
Fertility status of married:			
Fertile	14 (40%)	9 (30%)	0.321
Infertile			
• Primary	10 (28.6%)	14 (46.7%)	
• Secondary	11 (31.4%)	7 (23.3%)	

- Data are presented as mean, standard deviation (SD), range, numbers, percentages; *p*-value indicates the significance of difference between both groups; $p>0.05$ indicates non-significant difference.

The results of all laboratory investigations showed significant difference between patients and controls. Estimated FBG and serum insulin levels were non-significantly ($p=0.362$ & 0.288, respectively) higher in patients of group B in comparison to patients of group A. Moreover, 31 women of group A (51.7%) and 29 women of group B (69%) had HOMA-IR score >2, with non-significantly ($p=0.079$) higher frequency of IR among women of group B. Estimated levels of TC and LDL-c were significantly higher ($p=0.044$ & 0.026, respectively), while estimated levels of TG were non-significantly ($p=0.188$) higher, but estimated levels of HDL-c were non-significantly ($p=0.132$) lower in patients of group B in comparison to patients of group A. Moreover, estimated serum levels of testosterone and DHEA-S were significantly higher in patients in comparison to controls with non-significantly higher levels in women of group A than women of group B. On contrary, serum prolactin levels were non-significantly higher in patients of both groups in comparison to controls with non-significantly higher levels in patients of group B (Table 2).

Estimated serum AST and ALT levels were significantly higher in patients of both groups in comparison to control women with non–significantly higher levels in patients of group B in comparison to patients of group A. Considering AAR of <1 indicated no fibrosis; fortunately, all patients and controls had mean AAR of <1 despite of the significantly higher AAR in patients than controls and the non-significantly higher ration for patients of group B in comparison to patients of group A (Table 2).

All control women had unilateral ovarian size of <10cm^3, while only 33 of studied women had ovarian size of <10cm^3, while 69 women had ovarian size of more than 10cm^3. The frequency of women had ovarian size of <10cm^3 was significantly lower in patients of both groups in comparison to controls with non-significantly higher frequency among women of group A in comparison to women of group B. Ultrasonographic evaluation of liver steatosis grade defined 51 patients and 25 controls of grade 0, 36 patients and 5 controls of grade 1 steatosis, and 5 patients of grade 2 steatosis. The frequency of steatosis was significantly higher among patients of group A ($p=0.036$) and B (0.027) in comparison to control group with non-significantly higher frequency of steatosis among women of group B than women of group A (Table 3, Fig. 3).

Estimated serum spexin levels in patients of both groups were significantly ($p_1<0.0001$) lower in comparison to control levels. Interestingly, serum spexin levels in patients of group A were significantly ($p_2=0.005$) higher in comparison to its levels estimated in patients of group B (Table 4, Fig. 4).

Serum spexin levels showed negative significant correlations with liver steatosis grade, HOMA-IR, ovarian size, BMI, serum testosterone, TG, TC and AAR, in decreasing order of significance; while showed positive significant correlation with serum HDL-c. On contrary, liver steatosis grade showed positive significant correlation with serum testosterone, ovarian size, BMI, AAR, HOMA-IR score, serum levels of DHEA-S, TC, LDL-c and TG, in decreasing order of significance (Table 5). Regression analysis defined low serum spexin, high serum testosterone, LDL-c and DHEA-S as the predictors for high liver steatosis grade in PCOS women (Table 6).

Table (2): Laboratory data of patients of both groups.

Variables	Parameter	Control (n=30)	Group A (n=60)		Group B (n=42)
IR data	*FBG (mg/dl):*				
	Mean (±SD)	111.6 (8.1)	138.7 (15.8)		141.5 (14.2)
	p-value				
	p_1		<0.0001		<0.0001
	p_2			0.362	
	Serum insulin:				
	Mean (±SD)	1.735 (0.48)	5.545 (1.42)		5.83 (1.18)
	p-value				
	p_1		<0.0001		<0.0001
	p_2			0.288	
	HOMA-IR score:				
	<2	30 (100%)	31 (51.7%)		29 (69%)
	>2	0	29 (48.3%)		13 (31%)
	p-value				
	p_1		<0.0001		<0.0001
	p_2			0.079	
	Mean (±SD)	0.48 (0.14)	1.9 (0.56)		2.05 (0.5)
	p-value				
	p_1		<0.0001		<0.0001
	p_2				
Lipid profile	*TC (mg/dl):*				
	Mean (±SD)	159.5 (10.6)	183.6 (17.1)		190.2 (14.5)
	p-value				
	p_1		<0.0001		<0.0001
	p_2			0.044	
	TG (mg/dl):				
	Mean (±SD)	41 (11.8)	88.5 (15.5)		92.4 (13.2)
	p-value				
	p_1		<0.0001		<0.0001
	p_2			0.188	
	HDL-c (mg/dl):				
	Mean (±SD)	46.2 (4.6)	43.3 (6.1)		41.5 (5.5)
	p-value				
	p_1		0.03		0.0016
	p_2			0.132	
	LDL-c (mg/dl):				
	Mean (±SD)	72.4 (13.7)	122.6 (17.6)		130.2 (15.4)
	p-value				
	p_1		<0.0001		<0.0001
	p_2			0.026	
Hormonal profile	*Testosterone (ng/ml):*				
	Mean (±SD)	0.65 (0.16)	3.08 (0.7)		2.95 (0.46)
	p-value		<0.0001		
	p_1				<0.0001
	p_2			0.283	
	DHEA-S (µg/ml):		24.6 (3.77)		
	Mean (±SD)	15.1 (1.66)			23.1 (4.47)
	p-value		<0.0001		
	p_1				<0.0001
	p_2			0.068	
	Prolactin (ng/ml):		9.17 (1.6)		
	Mean (±SD)	9 (0.9)			9.35 (1.48)
	p-value		0.753		
	p_1				0.485
	p_2			0.565	
Liver enzymes	*AST (mg/ml):*				
	Mean (±SD)	19.4 (3.9)	25.3 (5.9)		25.8 (6.5)
	p-value				
	p_1		<0.0001		<0.0001
	p_2			0.658	
	ALT (mg/ml):				
	Mean (±SD)	24.6 (4.2)	30.6 (6.1)		31.1 (7.8)
	p-value				
	p_1		<0.0001		0.0001
	p_2			0.726	
	AAR:				
	Mean (±SD)	0.79 (0.058)	0.82 (0.076)		0.83 (0.06)
	p-value				
	p_1		0.027		0.0019
	p_2			0.493	

- Data are presented as mean, standard deviation (SD), range, median, interquartile range (IQR); FBG: Fasting blood glucose; HOMA-IR: Homeostasis model assessment of insulin resistance score; TC: Total cholesterol; TG: Triglycerides; HDL-c: High density lipoprotein cholesterol; LDL-c: low density lipoprotein cholesterol; DHEA-S: Dehydroepiandrosterone sulfate; AST: Aspartate transaminase; ALT: Alanine transaminase; AAR: AST/ALT ratio; $p1$ value indicates the significance of difference between both groups in comparison to control group; $p2$ value indicates the significance of difference between both groups; $p<0.05$ indicates significant difference; $p>0.05$ indicates non-significant difference.

Table (3): US data of enrolled patients.

Variable	Control (n=30)	Group A (n=60)		Group B (n=42)
Unilateral ovarian size:				
<10 cm3	30 (100%)	16 (26.7%)		17 (40.5%)
>10 cm3	0	44 (73.3%)		25 (59.5%)
p-value				
p_1		<0.0001		<0.0001
p_2			0.142	
Mean (±SD)	6.2 (0.6)	11.1 (1.7)		10.4 (1.7)
p-value				
p_1		<0.0001		<0.0001
p_2			0.060	
Liver steatosis grade:				
Grade 0	25 (83.3%)	35 (58.3%)		21 (50%)
Grade 1	5 (16.7%)	23 (38.4%)		18 (42.9%)
Grade 2		2 (3.3%)		3 (7.1%)
p-value				
p_1		0.046		0.027
p_2			0.557	

- Data are presented as mean, standard deviation (SD), range, numbers, percentages p 1 value indicates the significance of difference between both groups in comparison to control group; p2 value indicates the significance of difference between both groups; p<0.05 indicates significant difference; p>0.05 indicates non-significant difference.

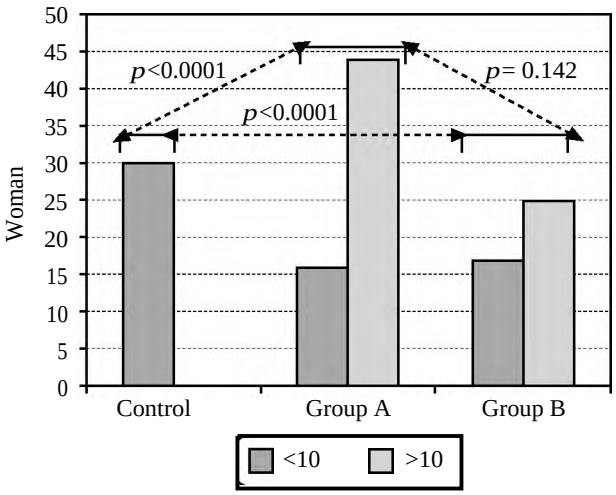

Fig. (2): Distribution of control and studied women according to US measurements of unilateral ovarian size.

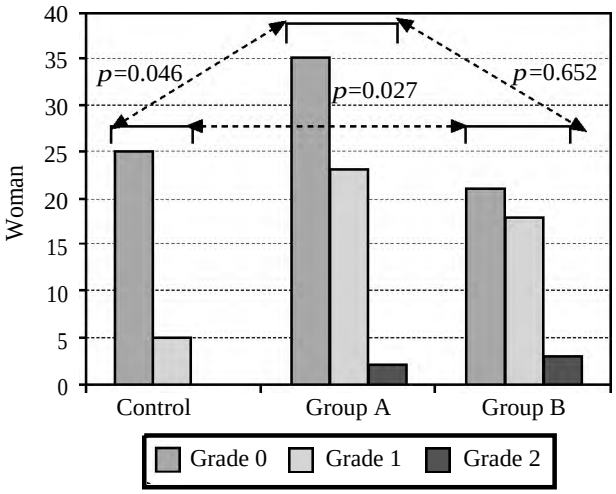

Fig. (3): Distribution of control and studied women among US grades of steatosis.

Table (4): Mean (±SD) of estimated serum spexin levels in patients of both groups in comparison to control levels.

	Control (n=30)	Group A (n=60)	Group B (n=42)
Mean (±SD)	3.76±0.66	3±0.62	2.66±0.58
p-value			
p_1		<0.0001	<0.0001
p_2		0.005	

- Data are presented as mean, standard deviation (SD); p 1 value indicates the significance of difference between both groups in comparison to control group; p2 value indicates the significance of difference between both groups; p<0.05 indicates significant difference; p>0.05 indicates non-significant difference.

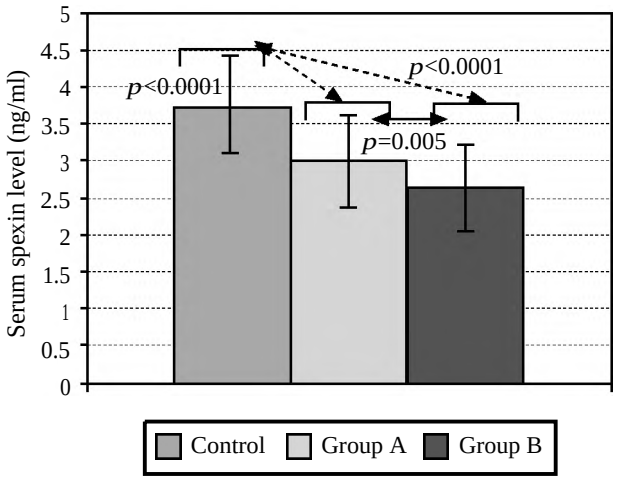

Fig. (4): Mean levels of serum spexin estimated in patients of both groups in comparison to control group.

Table (5): Correlation coefficient between serum spexin and liver steatosis grade and other studied parameters.

Variables / Parameters	Serum spexin		Liver steatosis grade	
	r	*p*	*r*	*p*
Body mass index	–0.352	<0.001	0.333	0.001
HOMA-IR	–0.407	<0.001	0.310	0.002
Ovarian size	–0.359	<0.001	0.368	<0.001
Total cholesterol	–0.248	0.012	0.240	0.015
High density lipoprotein	0.291	0.003	–0.141	0.156
Triglycerides	–0.329	0.001	0.227	0.022
Low density lipoprotein	–0.171	0.105	0.231	0.020
Testosterone	–0.335	0.001	0.413	<0.001
DHEA-S	–0.143	0.152	0.295	0.003
AST/ALT ratio	–0.226	0.022	0.313	0.001
Liver steatosis grade	–0.725	<0.001		

r=Pearson's correlation coefficient; HOMA-IR: Homeostasis model assessment of insulin resistance score; DHEA-S: Dehydroepiandrosterone sulfate; AST: Aspartate transaminase; ALT: Alanine transaminase; p-value indicates the significance of the coefficient; p<0.05 indicates significant difference; p>0.05 indicates non-significant difference.

Table (6): Regression analysis (Stepwise method) for studied parameters as predictors for liver steatosis in PCOS women.

Parameters	Standardized coefficient (β)	*t*-value	*p*-value
Serum spexin	–0.643	–9.561	<0.001
Serum testosterone	0.171	2.545	0.012
Serum Low density lipoprotein cholesterol	0.158	2419	0.017
Serum dehydroepiandrosterone sulfate	0.156	2.375	0.020

Discussion

All studied PCOS women and had NAFLD showed significant decrease of serum spexin (SPX). Moreover, Pearson's correlation analysis showed a negative significant correlation between serum SPX levels and BMI, HOMA-IR score and serum levels of TC and TG on one side and with serum testosterone and DHEA-S on the other side. These results point to a fact that serum SPX may be the coordinator of the relationship between PCOS and obesity. Similarly, Lin et al., **[28]** reported that in healthy adult females, serum SPX levels showed negative significant correlation with age, BMI, fasting glucose and TG levels and can independently predict the risk of high BMI and high fasting glucose. Thereafter, Guler & Demir **[29]** reported an inverse relation between serum SPX and androgens and the unfavorable metabolic profiles in PCOS women, and found the increased risk of having PCOS was in parallel with decreased SPX levels. In trial to explain these relations, Behrooz et al., **[30]** through a systemic review attributed the relation between SPX and BMI to its positive impact on overall metabolic status and appetite-regulating effect as it can act as an anorexigenic factor. Experimentally, Said et al., **[31]** found SPX attenuated the deleterious effects of metabolic syndrome induced in animal model by high-fructose diet and this effect was attributed to inhibition of inflammation and activation of peroxisome proliferator-activated receptors- γ and adenosine monophosphate-activated protein kinase. Also, El-Saka et al., **[32]** found SPX treatment of rats maintained on high fat/fructose diet improved insulin resistance (IR), dyslipidemia, oxidative stress and inflammation.

Moreover, the current study detected positive significant correlation between SPX and HDL-c levels; a finding that indicates a shift of cholesterol esterification from esterification with apolipoprotein B-100 towards with apo A-I and apo A-II with subsequent formation of HDL-c and decrease of LDL and VLDL. In line with these findings, Kumar et al., **[33]** detected increased serum SPX concentration 6-m after Roux-en-Y gastric bypass for girls with severe obesity with negative correlation with HOMA-IR and BMI. Thereafter, Atabey et al., **[34]** reported significant decrease of BMI, body fat, fasting glucose, total and LDL-c with increased HDL-c and plasma SPX levels at 3 months after laparoscopic sleeve gastrectomy for morbid obese patients.

Interestingly, the current study reported negative significant correlation between serum SPX and AAR and steatosis US grades. Moreover, steatosis US grade showed positive significant correlation with BMI, HOMA-IR and lipid profile. These findings illustrate the relation between SPX, obesity, IR and liver steatosis. In line with these data, Zhang et al., **[35]** detected a significantly lower SPX levels in patients with NAFLD in comparison to controls with significant correlation with HOMA-IR and this correlation remained significant after adjustment for gender and BMI.

The obtained results and these previous works point to a fact that serum SPX may be the modulator of the reciprocal relationship between PCOS and NAFLD. In line with this reciprocal relation between PCOS and NAFLD, Shengir et al., **[36]** reported that NAFLD is a frequent comorbidity in women with PCOS and is strongly associated with higher BMI and Taranto et al., **[37]** found women with PCOS had a high risk of NAFLD, and a combination of both was associated with central obesity, dyslipidemia, IR, and metabolic syndrome. Also, Asfari et al., **[38]** documented that in comparison to women free of PCOS, women with PCOS have four times higher risk of developing NAFLD. More recently, Won et al., **[39]** documented that metabolic syndrome diagnosis at PCOS diagnosis were risk factors associated with NAFLD.

Furthermore, there was positive significant correlation between serum levels of testosterone, DHEA-S and insulin with a significant correlation between levels of these hormone and presence of both PCOS and NAFLD. These results point to a possible role for the reported disturbed hormonal milieu and development of both pathologies and their possible upcoming complications.

In trial to explain this dilemma of disturbed hormonal milieu, experimentally, Cui et al., **[40]** using PCOS-like rat found long-term androgen excess affects mitochondrial function through induction of imbalance in apoptosis and autophagy and lead to hepatic lipid deposition and inflammation with subsequent IR and hepatic steatosis and

Siemienowicz et al., **[41]** detected decreased fibroblast growth factor 21 signaling in subcutaneous adipose tissue with increased hepatic triglyceride, reduced fatty acid oxidation capacity and increased hepatic expression of inflammatory markers inprenatally androgenized sheep.

Clinically, Qu & Donnelly **[42]** through a review of literature described a flow of events starting with obesity leading to development of IR and NAFLD with decreased circulating levels of sex hormone binding protein (SHBP] depending on the detected negative correlation between levels of SHBP and IR and markers of NAFLD and subsequently, the decreased levels of SHBP lead to increased bioavailability of androgens with subsequent progression of ovarian pathology, anovulation and the phenotypic characteristics of PCOS. As another explanation was provided by Bicer et al., **[43]** who detected increased levels of secreted frizzled-related protein 4, which is an adipokine involving in apoptotic process during ovulation and energy metabolism, and found these high levels were associated with higher possibility of having PCOS and were also related to IR, hyperandrogenism, ovarian follicular number and ovarian volume. On the other hand, Guler & Demir **[29]** found myonectin, a myokine involving in glucose and lipid metabolisms, is inversely associated with BMI, IR, TG and free androgen index, while it showed a positive association with HDL-C in women with PCOS.

Conclusion:

There is an evident reciprocal relationship between PCOS and NAFLD and both were correlated with disturbed hormonal milieu, lipid profile and insulin sensitivity. Serum spexin concentrations were significantly decreased in patients had both PCOS and NAFLD and was correlated with the associated disturbances.

Limitation:

Therelation between serum spexin levels and other adipocytokines was to be investigated. Also, the effect of dieting regimens, metformin, hormonal contraceptives and antilipotrophic factors need to be evaluated.

Recommendation:

Further wider scale studies are mandatory to establish the role of spexin in pathogenesis of both PCOS and NAFLD.

References

1- ÇELEBIER M., KAPLAN O., ÖZEL S¸., et al.: Polycystic ovary syndrome in adolescents: Q-TOF LC/MS analysis of human plasma metabolome. J. Pharm. Biomed Anal., Nov. 30 (191): 113543. Doi: 10.1016/j.jpba.2020.113543, 2020.

2- SIEMIENOWICZ K., FURMANSKA K., FILIS P., et al.: Pubertal FGF21 deficit is central in the metabolic pathophysiology of an ovine model of polycystic ovary syndrome. Mol. Cell Endocrinol., Apr. 5 (525): 111196. Doi: 10.1016/j.mce.2021.111196, 2021 .

3- LINDNER P.G.: Long-term Health of Offspring of Women with Polycystic Ovarian Syndrome. Clin. Obstet. Gynecol., Mar. 1; 64 (1):48-54. Doi: 10.1097/GRF.0000000000000598, 2021.

4- LIU M., ZHU H., HU X., et al.: Efficacy of coenzyme Q10 supplementation on glucose metabolism, lipid profiles, and biomarkers of inflammation in women with polycystic ovary syndrome: A protocol for a systematic review and meta-analysis. Medicine (Baltimore), Nov. 13; 99 (46): e23130. Doi: 10.1097/MD.0000000000023130, 2020.

5- WANG H., MEHAL W., NAGY L., et al.: Immunological mechanisms and therapeutic targets of fatty liver diseases. Cell Mol. Immunol., Jan. 18(1): 73-91. Doi: 10.1038/s41423-020-00579-3, 2021.

6- SRINIVAS A.N., SURESH D., SANTHEKADUR P., et al.: Extracellular Vesicles as Inflammatory Drivers in NAFLD. Front Immunol., Feb. 2; (11): 627424. Doi: 10.3389/fimmu.2020.627424, 2021.

7- KUCHAY M., CHOUDHARY N. and MISHRA S.: Pathophysiological mechanisms underlying MAFLD. Diabetes Metab. Syndr., Nov-Dec. 14 (6): 1875-1887. Doi: 10.1016/j.dsx.2020.09.026, 2020.

8- WANG C., WANG X., SONG G., et al.: A high-fructose diet in rats induces systemic iron deficiency and hepatic iron overload by an inflammation mechanism. J. Food Biochem., Jan. 45 (1): e13578. Doi: 10.1111/jfbc.13578, 2021.

9- FERRER M.J., SILVA A.F., ABRUZZESE G.A., et al.: Lipid Metabolism and Relevant Disorders to Female Reproductive Health. Curr. Med. Chem., Jan. 6. Doi: 10.2174/0929867328666210106142912, 2021.

10- ARROYAVE-OSPINA J., WU Z., GENG Y., et al.: Role of Oxidative Stress in the Pathogenesis of Non-Alcoholic Fatty Liver Disease: Implications for Prevention and Therapy. Antioxidants (Basel), Jan. 26; 10 (2): 174. Doi: 10.3390/antiox10020174, 2021.

11- MIRABEAU O., PERLAS E., SEVERINI C., et al.: Identification of novel peptide hormones in the human proteome by hidden Markov model screening. Genome Res., 17: 320-327, 2007.

12- RUCINSKI M., PORZIONATO A., ZIOLKOWSKA A., et al.: Expression of the spexin gene in the rat adrenal gland and evidences suggesting that spexin inhibits adrenocortical cell proliferation. Peptides, Apr. 31(4): 676-82. Doi: 10.1016/j.peptides.2009.12.025, 2010.

13- PORZIONATO A., RUCINSKI M., MACCHI V., et al.: Spexin expression in normal rat tissues. J. Histochem. Cytochem., 58: 825-837, 2010.

14- TOLL L, KHROYAN T, SONMEZ K, et al.: Peptides derived from the prohormone proNPQ/spexin are potent central modulators of cardiovascular and renal function

and nociception. FASEB J., Feb. 26 (2): 947-54. Doi: 10.1096/fj.11-192831, 2012.

15- GU L., MA Y., GU M., et al.: Spexin peptide is expressed in human endocrine and epithelial tissues and reduced after glucose load in type 2 diabetes. Peptides, Sep. 71: 232-9. Doi: 10.1016/j.peptides.2015.07.018, 2015.

16- MA A., BAI J., HE M., et al.: Spexin as a neuroendocrine signal with emerging functions. Gen. Comp. Endocrinol., Sep. 1; 265: 90-96. Doi: 10.1016/j.ygcen.2018.01.015, 2018.

17- BRAY G.A.: Pathophysiology of obesity. Am. J. Clin. Nutr., 55: 488S-94S, 1992.

18- WHO: Physical status: The use and interpretation of anthropometry. Report of a WHO Expert Committee. WHO Technical Report Series 854. Geneva: World Health Organization, 1995.

19- CHEN M.J., YANG W.S., YANG J.H., et al.: Low sex hormone-binding globulin is associated with low high-density lipoprotein cholesterol and metabolic syndrome in women with PCOS. Hum. Reprod, 21: 2266-71, 2006.

20- CHEN M.J., YANG W.S., YANG J.H., et al.: Relationship between androgen levels and blood pressure in young women with polycystic ovary syndrome. Hypertension, 49: 1442-7, 2007.

21- ANAFOROGLU I., ALGUN E., INCECAYIR O., et al.: Higher metabolic risk with National Institutes of Health versus Rotterdam diagnostic criteria for polycystic ovarian syndrome in Turkish women. Metab. Syndr. Relat. Disord., Oct. 9 (5): 375-80. Doi: 10.1089/met.2011.0019, 2011.

22- SMITH B.W. and ADAMS L.A.: Nonalcoholic fatty liver disease and diabetes mellitus: Pathogenesis and treatment. Hepatology, Dec. 30 (6): 1356-62, 1999.

23- ANGULO P., KEACH J.C., BATTS K.P., et al.: Independent predictors of liver fibrosis in patients with nonalcoholic steatohepatitis. Hepatology, Dec. 30 (6): 1356-62, 1999.

24- ISAKSEN V.T., LARSEN M.A., GOLL R., et al.: Hepatic steatosis, detected by hepatorenal index in ultrasonography, as a predictor of insulin resistance in obese subjects. BMC Obes., Sep. 20 (3): 39. doi: 10.1186/s40608-016-0118-0, 2016.

25- MARSHALL R.H., EISSA M., BLUTH E.I., et al.: Hepatorenal index as an accurate, simple, and effective tool in screening for steatosis. AJR Am. J. Roentgenol., Nov. 199 (5): 997-1002. doi: 10.2214/AJR.11.6677, 2012.

26- WEBB M., YESHUA H., ZELBER-SAGI S., et al.: Diagnostic value of a computerized hepatorenal index for sonographic quantification of liver steatosis. AJR Am. J. Roentgenol., Apr. 192 (4): 909-14, 2009.

27- MATTHEWS D.R., HOSKER J.P., RUDENSKI A.S., et al.: Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia., 28: 412-19, 1985.

28- LIN Y., LU H., CHEN J., et al.: Purple-leaf tea (Camellia sinensis L.) ameliorates high-fat diet induced obesity and metabolic disorder through the modulation of the gut microbiota in mice. BMC Complement Med. Ther., Dec. 10; 20 (1): 376. Doi: 10.1186/s12906-020-03171-4, 2020.

29- GULER A. and DEMIR I.: Decreased levels of spexin are associated with hormonal and metabolic disturbance in subjects with polycystic ovary syndrome J. Obstet. Gynaecol., Apr. 15; 1-6. Doi: 10.1080/01443615. 2020.1737660, 2020.

30- BEHROOZ M., VAGHEF-MEHRABANY E., MALEKI V., et al.: Spexin status in relation to obesity and its related comorbidities: A systematic review. J. Diabetes Metab. Disord., Nov. 7; 19 (2): 1943-1957. Doi: 10.1007/s40200-020-00636-8, 2020.

31- SAID M., NAFEH N. and ABDALLAH H.: Spexin alleviates hypertension, hyperuricaemia, dyslipidemia and insulin resistance in high fructose diet induced metabolic syndrome in rats via enhancing PPAR- y and AMPK and inhibiting IL-6 and TNF- a. Arch Physiol Biochem., Mar. 15: 1-6. Doi: 10.1080/13813455.2021.1899242, 2021.

32- EL-SAKA M., ABO EL GHEIT R., EL SAADANY A., et al.: Effect of spexin on renal dysfunction in experimentally obese rats: Potential mitigating mechanisms via galanin receptor-2. Arch. Physiol. Biochem., Feb. 25: 1-10. Doi: 10.1080/13813455.2021.1887265, 2021.

33- KUMAR S., HOSSAIN M., INGE T., et al.: Roux-en-Y gastric bypass surgery in youth with severe obesity: 1-year longitudinal changes in spexin. Surg Obes Relat Dis., Oct. 14 (10): 1537-1543. Doi: 10.1016/j.soard. 2018.07.007, 2018.

34- ATABEY M., AYKOTA M., ÖZEL M., et al.: Short-term changes and correlations of plasma spexin, kisspeptin, and galanin levels after laparoscopic sleeve gastrectomy. Surg. Today, Feb 8. Doi: 10.1007/s00595-021-02240-6, 2021.

35- ZHANG L., LI G., SHE Y., et al.: Low levels of spexin and adiponectin may predict insulin resistance in patients with non-alcoholic fatty liver. Pract Lab. Med., Feb. 7; 24: e00207. Doi: 10.1016/j.plabm. 2021.e00207, 2021.

36- SHENGIR M., KRISHNAMURTHY S., GHALI P., et al.: Prevalence and predictors of nonalcoholic fatty liver disease in South Asian women with polycystic ovary syndrome. World J. Gastroenterol., Nov. 28; 26 (44): 7046-7060. Doi: 10.3748/wjg. v26.i44.7046, 2020.

37- TARANTO D., GUIMARÃES T., COUTO C., et al.: Nonalcoholic fatty liver disease in women with polycystic ovary syndrome: associated factors and noninvasive fibrosis staging in a single Brazilian center. Arch. Endocrinol. Metab., May-Jun. 64 (3): 235-242. Doi: 10.20945/2359-3997000000242, 2020.

38- ASFARI M., SARMINI M., BAIDOUN F., et al.: Association of non-alcoholic fatty liver disease and polycystic ovarian syndrome. BMJ Open Gastroenterol., Aug. 7 (1): e000352. Doi: 10.1136/bmjgast-2019-000352, 2020.

39- WON Y., SEO S., YUN B., et al.: Non-alcoholic fatty liver disease in polycystic ovary syndrome women. Sci. Rep., Mar. 29; 11 (1): 7085. Doi: 10.1038/s41598-021-86697-y, 2021.

40- CUI P., HU W., MA T., et al.: Long-term androgen excess induces insulin resistance and non-alcoholic fatty liver disease in PCOS-like rats. J. Steroid Biochem. Mol. Biol., Apr. 208: 105829. Doi: 10.1016/j.jsbmb.2021.105829, 2021.

41- SIEMIENOWICZ K., FURMANSKA K., FILIS P., et al.: Pubertal FGF21 deficit is central in the metabolic pathophysiology of an ovine model of polycystic ovary syn-

drome. Mol. Cell. Endocrinol., Apr. 5 (525): 111196. Doi: 10.1016/j.mce.2021.111196, 2021.

42- QU X. and DONNELLY R.: Sex Hormone-Binding Globulin (SHBG) as an Early Biomarker and Therapeutic Target in Polycystic Ovary Syndrome. Int. J. Mol. Sci., Nov. 1; 21 (21): 8191. Doi: 10.3390/ijms21218191, 2020.

43- BICER M., ALARSLAN P., GULER A., et al.: Elevated circulating levels of secreted frizzled-related protein 4 in relation to insulin resistance and androgens in women with polycystic ovary syndrome. J. Endocrinol. Invest., Mar. 43 (3): 305-313. Doi: 10.1007/s40618-019-01108-4, 2020.

إنخفاض معدل السبيكسين قد يكون سبب العلاقة التبادلية بين الكبد الدهنى الغير كحولى ومتلازمة تكيس المبايض

\الأهداف: تقدير مستويات السبيكسين بمصل الدم فى النساء المصابة بمتلازمة تكيس المبايض الذين لديهم مرض الكبد الدهنى غير الكحولى، وتقييم علاقته بالمؤشرات التشخيصية لكلا منهما.

\المرضى والوسائل: تم تشخيص متلازمة تكيس المبايض عند ١٠٢ امرأة مصابة بمرض الكبد الدهنى غير الكحولى اعتماداً على معايير روتردام التشخيصية لمرض تكيس المبايض وعن طريق الفحص بالموجات فوق الصوتية من أجل تقدير حجم المبيض ودرجة التنكس الدهنى للكبد. بعد ذلك تم جمع عينات الدم من أجل تقدير مستويات السبيكسين، التستوستيرون، كبريتات الديهيدرو إيبى أندروستيرون، معدلات الدهون والانزيمات الناقلة لأمين الأسبارتات، أمين الألانين ثم حساب النسبة بين كلا الناقلين.

\النتائج: أظهر فحص مصل الدم إنخفاض ذو دلالة إحصائية لمستويات السبيكسين، وارتفا ع لمعدلات التستوستيرون، كبريتات أندروستيرون، الكوليسترول، الجلسريدات الثلاثية، البروتين الدهنى منخفض الكثافة ، مع ارتفا ع لمعدل مقياس مقاومة الأنسولين عند المقارنة بالمجموعة الضابطة. كان حجم المبيض عند ٦٩ امرأة أصغر من ١٠ سم٣، و كانت ٣٦، ٥ مريضات يعانين من الدرجة الأولى والثانية للتنكس الدهنى للكبد. وأرتبطت مستويات مصل السبيكسين سلبياً مع درجة التنكس الدهنى للكبد، معدل مقاومة الأنسولين، حجم المبيض، مؤشر كتلة الجسم، ومستوى التستوستيرون، الدهون، ونسبة ناقلات أمين الأسبارتات والآلانين، بينما أرتبطت إيجابياً مع البروتين الدهنى مرتفع الكثافة. وأرتبطت درجة التنكس الدهنى للكبد إيجابياً بمعدل التستوستيرون، حجم المبيض، مؤشر كتلة الجسم، نسبة ناقلات أمين الأسبارتات والألانين، معدل مقاومة الأنسولين، مستويات مصل كبريتات أندروستيرون، الكوليسترول، البروتين الدهنى منخفض الكثافة، والجلسريدات الثلاثية. حدد تحليل الانحدار معدل السبيكسين المنخفض، ومصل التستوستيرون المرتفع، والبروتين الدهنى منخفض الكثافة، ومستويات مصل كبريتات أندروستيرون على أنها مؤشرات لدرجة مرتفعة من التنكس الدهنى للكبد فى مرضى المتلازمة.

\الخلاصة: تركيزات السبيكسين بمصل الدم منخفضة إنخفاضاً ذو دلالة إحصائية عند النساء المصابة بمتلازمة تكيس المبايض والكبد الدهنى غير الكحولى، مع ارتباط بالاضطرابات الناتجة عنهما لذا يمكن اعتباره مسؤلا عن تنظيم العلاقة بين متلازمة تكيس المبايض ومرض الكبد الدهنى غير الكحولى والأعراض المصاحبة لهما.