CEOG Clinical and Experimental Obstetrics & Gynecology

Original Research

Long non-coding RNA steroid receptor activator in polycystic ovary syndrome: possible association with metabolic syndrome

H.M.G. Youssef[1], E.S. Marei[1], L.A. Rashed[2]

[1]Health Radiation Research Department, National Center for Radiation Research and Technology, Egyptian Atomic Energy Authority, Cairo
[2]Faculty of Medicine, Cairo University, Cairo (Egypt)

Summary

Introduction: Polycystic ovary syndrome (PCOS) is a metabolic and endocrine disorder which affects women of reproductive age with a prevalence of 8–18% and implies various severe consequences to female health, including alarming rates of infertility, which accounts for approximately 75% of anovulatory infertility. Long non-coding RNA steroid receptor activator (lncRNA-SRA) affects multiple biological processes. However, it is not known whether lncRNA-SRA is associated with PCOS and the possible accompanied metabolic syndrome (MetS). *Material and Methods:* The expression levels of lncRNA-SRA were measured by real-time quantitative-PCR in PCOS patients (n=30 women), and in control group (n=20 women), patients with metabolic syndrome were diagnosed, and the association between lncRNA-SRA and metabolic syndrome parameters in PCOS group and control were analyzed. *Results:* LncRNA-SRA expression mean level was significantly higher in the women with PCOS than that in the control group. There was a high significant positive correlation between lncRNA-SRA expression and hirsutism, BMI, waist circumference (WC), and insulin levels, also with significant positive correlation between lncRNA-SRA with HOMA/IR and testosterone in PCOS group. *Conclusion:* The authors found that the lncRNA-SRA expression is potentially increased with PCOS and it has positive correlation with hirsutism, obesity, testosterone, and insulin resistance in PCOS. LncRNA-SRA is significantly increased in MetS subgroup of PCOS, thereby suggesting that elevated lncRNA-SRA might be an important mediator not only associated to hormonal and clinical parameters but also associates metabolic syndrome in PCOS and may be a mediator in the pathogenesis of both syndromes.

Key words: LncRNA-SRA; Polycystic ovary syndrome; Metabolic syndrome; Insulin resistance; Hirsutism.

Introduction

Polycystic ovary syndrome (PCOS) is the most common endocrine disorder affecting women of reproductive age [1]. Patients with PCOS are characterized by obesity, hirsutism, insulin resistance, and increased risks of metabolic syndrome [2]. Metabolic syndrome (MetS) is a cluster of endocrinopathic and metabolic disturbances including hyperglycemia/insulin resistance, central obesity, dyslipidemia, and hypertension [3]. It is associated with a two-fold increased risk of cardiovascular disease and a five-fold increased risk of type 2 diabetes [4]. Several features of metabolic disturbances, insulin resistance, and hyperinsulinemia particularly have been observed also in most women with PCOS [5]. Obesity and visceral obesity are common among PCOS women associated with metabolic syndrome (MetS) [6]. Some studies report that MetS is more common among women with PCOS due to the high prevalence of insulin resistance, obesity, and visceral obesity in these patients [7, 8].

There are number of the genes associated with PCOS and also involved in the insulin receptor signaling pathway, steroid biosynthesis, and in the regulation of gonadotropin secretion [9]. lncRNAs have vital role in reproduction, such as germ cell specification, sex determination and gonadogenesis, sex hormone responses, meiosis, gametogenesis, placentation, non-genetic inheritance, and pathologies affecting reproductive tissues [10]. The lncRNA-steroid receptor activator (SRA) is a unique coregulator that functions as a non-coding RNA [11]. LncRNA-SRA has since been shown to modulate the activity of androgen, estrogen ESR1, and progesterone receptors through direct association with hormone receptors [11, 12]. Steroid receptor RNA activator (SRA) expressed by the SRA1 gene (5q31.3), 0.87 kB in size, is initially characterized as long non-coding RNA by Lanz *et al.* in 1999 [11]. LncRNA SRA is able to coordinate the functions of various transcription factors and coregulators, and enhance the transcriptional activity of steroid receptors on reporter genes, serving as a distinct scaffold [13, 14]. The aim of this study was to compare the expression level of lncRNA-SRA in PCOS patients and healthy control women, then to elucidate the association between lncRNA-SRA and MetS in PCOS.

Revised manuscript accepted for publication August 29, 2018

Clin. Exp. Obstet. Gynecol. - ISSN: 0390-6663
XLVI, n. 5, 2019
doi: 10.12891/ceog4774.2019

7847050 Canada Inc.
www.irog.net

Published: 10 October 2019

Table 1. — *Clinical data, laboratory investigations, hormonal investigations, and concentration of lnc RNA (steroid receptor activator) in PCOS and controls.*

Parameters	PCOS (n=30) Mean ± SD	Controls(n=20) Mean ± SD	*p*-values
Age (years)	28.20 ± 3.10	27.90 ± 3.90	NS
Hirsutism	26.76 ± 3.58	7.60 ± 1.95	0.0001
BMI (kg/m²)	35.57 ± 3.53	27.44 ±1.85	0.001
WC (cm)	85.66 ± 6.13	76.20 ± 5.51	0.001
FBG (mmol/l)	5.98 ±1.16	5.09 ± 0.99	0.05
Insulin(mIu/l)	23.21 ± 5.91	13.02 ± 2.44	0.001
HOMA/IR	6.21 ± 2.49	2.82 ± 0.48	0.001
Cholesterol (mg/dl)	220.41 ± 19.80	172.10 ± 17.79	0.001
TG (mg/dl)	115.53 ±23.99	95.10 ± 28.52	0.01
HDL (mg/dl)	40.70 ± 7.41	55.60 ± 4.9	0.001
FSH (Iu/ml)	6.84 ±1.54	3.61 ± 0.55	0.001
LH (Iu/ml)	8.40 ± 4.32	6.08 ± 1.04	0.01
Prolactin (ng/ml)	9.13± 5.04	8.49 ± 2.16	NS
Testosterone (ng/ml)	0.79 ± 0.32	0.36 ± 0.12	0.001
lnc RNA steroid receptor activator (relative expression)	9.89 ± 3.80	1.04 ± 0.06	0.0001

Materials and Methods

This case-control study was carried out on 30 PCOS patients and 20 healthy women in the middle age group. The study was conducted from June to December 2016 in Ain Shams Maternity Hospital. All participants gave informed consent. The procedures followed were in accordance with the ethical standards of the hospital and approved by ethical committee of National Center for Radiation Research and Technology (NCRRT). The studied subjects were classified into two groups; PCOS group: 30 women with PCOS were characterized by (OA + HA + PCOM). Oligoanovulation (OA) was defined as self-reported menstrual cycle length of ≥ 35 days or < 10 menstrual periods per year. Diagnosis of PCOS was based on Rotterdam criteria with two of (1) irregular menstrual cycles (oligoanovulation) OA (≥ 35 days), (2) clinical (hirsutism, acne) or biochemical hyperandrogenism (HA) (increase in at least one circulating ovarian androgen), and (3) polycystic ovarian morphology (PCOM) by ultrasound and with exclusion of related disorders [15]. PCOM is indicated by the presence of ≥ 12 follicles measuring 2 to 9 mm in the entire ovary or by the finding of increased ovarian size (≥ 10 mL) [16]. Twenty healthy women were selected as control group that had regular menstrual cycles and normal ovaries, and showed no clinical signs of hyperandrogenism. Subjects with history of ovarian surgery or previously treated with cytotoxic drugs, pelvic radiation, receiving hormonal therapy within three months before the beginning of the study, or had another endocrinological disorder were excluded. Patients and controls were subjected to complete history taking to clarify irregularity of menstrual cycles. Hormonal assays for all the subjects were performed in the early follicular phase of the menstrual cycle (days 3–5) or randomly in amenorrhea patients. PCOM were diagnosed by ultrasound which was performed with Logiq Book XP with a vaginal probe of 7.5 MHz.

Follicle stimulating hormone and luteinizing hormone, fasting plasma glucose, and fasting insulin were determined by chemiluminescent-immunometric assays. Testosterone was measured with an analyzer using an automated competitive binding immunoenzymatic assay. Cholesterol, high-density lipoprotein cholesterol (HDL-C), and triglycerides (TG) were measured using an enzymatic colorimetric method. HOMA-IR was calculated as fasting serum insulin (mIU/l) × fasting blood glucose (FBG) (mmol/l)/22.5]. The authors measured height and weight to calculate BMI, which was calculated as weight (kg)/ height in meters squared (kg/m²).

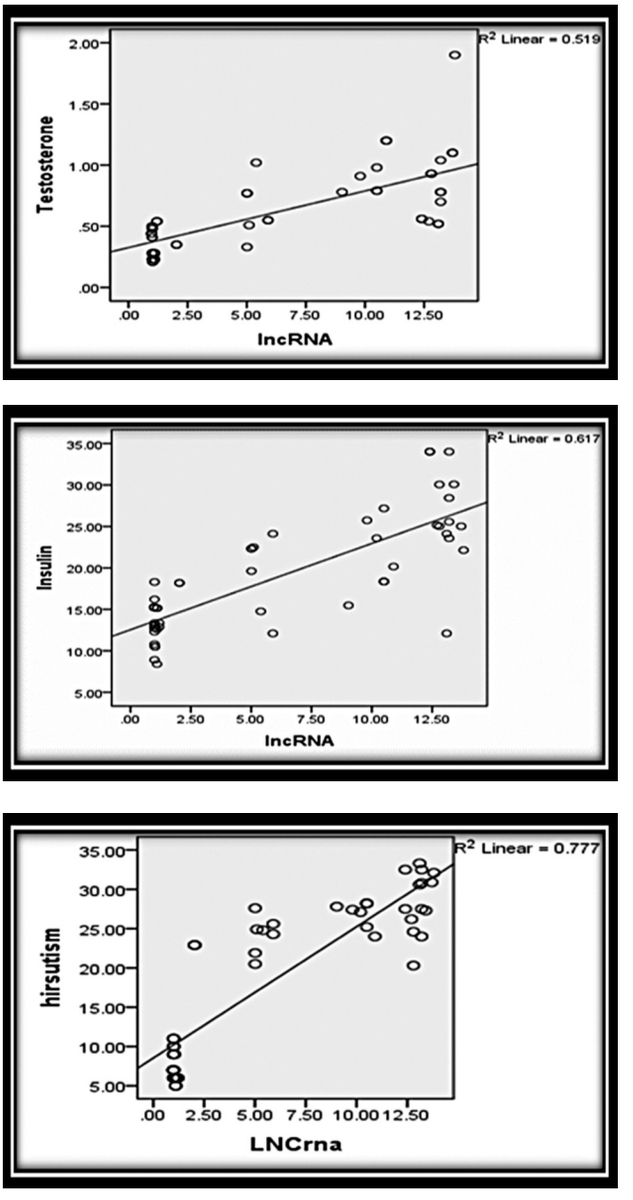

Figure 1. — The figure shows correlation between lncRNA-SRA and testosterone, insulin, and hirsutism, respectively.

Metabolic syndrome was defined according to the modified American Heart Association/National Heart Lung Blood Institute AHA/NHLBI (ATP III 2005) definition. It was diagnosed if at least three of the following five features were present (1) waist circumference of ≥80 cm or more, (2) blood pressure of ≥ 130/85 mmHg, (3) fasting blood sugar of ≥ 100 mg/dL, (4) triglycerides of ≥150 mg/dL, and (5) HDL of ≤ 50 mg/dl.

Quantitative analysis of lncRNA by real-time quantitative RT-PCR: total RNA was extracted using an SV total RNA isolation system according to manufacturer's instruction. The total RNA (2 μg) was used for cDNA conversion using high capacity cDNA re-

Table 2. — *Correlation between lnc RNA steroid receptor activator with different clinical and laboratory investigated parameters in PCOS and in control groups.*

Pearson correlation	Hirsutism	BMI	WC	FBS	Insulin	HOMA-IR	Cholesterol	TG	FSH	Testosterone
lncRNA (PCOS) r	0.583	0.489	0.570	0.153	0.505	0.390	-0.282	0.118	0.063	0.457
p-values	0.001*	0.006*	0.001*	0.650	0.001*	0.033*	0.131	0.305	0.739	0.011*
Significance (2-tailed)	HS	HS	HS	NS	HS	Sig.	NS	NS	NS	Sig.
lncRNA (control) r	-0.548	-0.050	-0.457	-0.255	-0.318	-0.400	0.141	-0.169	0.339	0.129
p-values	0.012*	0.830	0.04*	0.278	0.171	0.080	0.552	0.476	0.144	0.587
Significance (2-tailed)	Sig.	NS	Sig.	NS	NS	NS	NS	NS	NS	NS

Table 3. — *Number, percentage of women and the mean value of LncRNA steroid receptor activator within MetS and without MetS subgroups in PCOS and control groups.*

PCOS group	n	Percentage	lncRNA mean	Std. Deviation	Std. Error	95% Confidence interval for mean		Minimum	Maximum
						Lower bound	Upper bound		
With MetS	19	63.3%	11.0337	3.58933	.82345	9.3037	12.7637	2.03	13.80
Without MetS	11	36.7%	7.8345	3.37181	1.0166	5.5693	10.0998	2.03	13.10
Total	30	100%	9.8607	3.79151	0.69223	8.4449	11.2764	2.03	13.80
Control group						Lower bound	Upper bound		
With MetS	4	20%	1.0250	0.05000	0.02500	0.9454	1.1046	1.00	1.10
Without MetS	16	80%	1.0438	0.07347	0.01837	1.0046	1.0829	0.98	1.20
Total	20	100%	1.0400	0.06867	0.01536	1.0079	1.0721	0.98	1.20

Table 4. — *Comparison of clinical, laboratory findings and lncRNA between polycystic ovary syndrome patients with MetS and without metabolic syndrome*

Parameters	PCOS with MetS (n=19) Mean ± SD	PCOS without MetS (n=11) Mean ± SD	*p*-value	Sig.
Age (years)	28.52 ± 3.5	27.63 ± 2.1	0.392	NS
Hirsutism	26.90 ± 4.8	26.56 ± 2.53	0.779	NS
BMI (kg/m²)	36.83 ± 2.86	33.40 ± 3.64	0.01	Sig.
WC (cm)	89.00 ± 4.77	80.60 ± 3.38	0.001	HS
FBG (mmol/l)	6.53 ± 1.05	5.04 ± 0.60	0.001	HS
Insulin (mIu/l)	25.54 ± 5.38	19.18 ± 4.57	0.002	HS
HOMA/IR	7.29 ± 2.33	4.36 ± 1.47	0.001	HS
Cholesterol (mg/dl)	216.95 ± 17.44	226.38 ± 22.97	0.255	NS
TG (mg/dl)	122.84 ± 26.16	106.36 ± 17.04	0.05	Sig.
HDL (mg/dl)	37.87 ± 5.84	45.59 ± 7.52	0.009	HS
LH (Iu/ml)	8.36 ± 4.95	8. 45 ± 3.18	0.954	NS
FSH (Iu/ml)	6.43 ± 1.35	7.55 ± 1.66	0.074	NS
Prolactin (ng/ml)	8.20 ± 4.90	10.73 ± 5.08	0.197	NS
Testosterone (ng/ml)	0.84 ± 0.38	0.69 ± 0.16	0.148	NS
LncRNA (relative expression)	11.03 ± 3.58	7.83 ± 3.37	0.02	Sig.

verse transcription kit. Real-time qPCR amplification and analysis were performed using an applied biosystem with software version 3.1. The reaction contained SYBR Green Master Mix, gene-specific primer pairs for long non-coding RNA and GAPDH with following sequence forward 5- ACAACCGAAGGTAACCAGGC-3, reverse 5- TGGGCACGTTTGGGATCTTG-3 and forward 5-TGTTGCCATCAATGACCCCTT-3, and reverse 5-CTCCACGACGTACTCAGCG-3, respectively. cDNA and nuclease-free water. With cycling conditions (10 minutes at 95°C followed by 40 cycles of 15 seconds at 95°C and 60 seconds at 60°C). Data were analysed with a sequence detection system software and quantified using the v1.7 sequence detection software. Relative expression of studied genes was calculated using the comparative threshold cycle method. All values were normalized to the GAPDH which was used as the control housekeeping gene. After the concentration and purity of RNA were evaluated using a spectrophotometer and denaturing gel electrophoresis, the RNA was used to reverse transcribed into cDNA using a reagent kit. Real-time PCR system with the following parameters: 15-minute RT reaction at 37°C, 5 s reverse transcriptase inactivation reaction at 85°C and refrigeration at 4°C. Quantitative real time-polymerase chain reaction (qRT-PCR) was conducted to measure the levels of lncRNA-SRA by using the 10.0ml SYBR Premix Ex Taq II, 2 mL 5100 ng total cDNA, each forward and reverse primers 0.8 and 20.0 mL total volume following the manufacturer's protocol. Reactions were performed using a RT-PCR system with the following reaction profile: pre-denaturation for 30 seconds at 95°C, and PCR amplification for 45 cycles with 5 seconds at 95°C, 60 seconds at 60°C. PCR was followed by a melt curve analysis to determine the reaction specificity and agarose gel electrophoresis

to confirm the size of PCR product.

The study followed the principles of the Declaration of Helsinki II for Medical Research. Written informed consent was obtained from all participants. Data were anonymized and sensitive data were kept in a separate document. The Scientific Ethics Committee of National Center for Radiation Research and Technology (REC-NCRRT) approved the study (13H/17).

Differences between the two groups PCOS and control were determined using Independent test. Relationships between variables were assessed using two-tailed Pearson's correlation coefficient (r). All statistical analysis were performed using SPSS 20.0 software with statistical significance accepted when $p < 0.05$.

Results

Table 1 shows the significant increase in FBS, TG, and LH in PCOS group. There was also a high significant increase in PCOS group with regards to hirsutism, BMI, waist circumference (WC), insulin, HOMA/IR, cholesterol, HDL, FSH, and testosterone. There was also a high significant increase in lncRNA-SRA relative expression level in PCOS group which was 9.89 ± 3.80, compared to the control group which was 1.04 ± 0.06 relative expression. Table 2 shows that in the PCOS group there was a direct high significant correlation among lncRNA-SRA and hirsutism, BMI, WC, insulin, HOMA/IR, and testosterone. There was a non-significant correlation with cholesterol, HDL, TG, and FSH. In control group there was an inverse significant correlation with hirsutism and WC.

Table 3 shows that the number of PCOS patients with MetS was 19 women with a percentage of 63.3%, and the patients without MetS were 11 with a percentage of 36.7%. LncRNA-SRA was correlated directly with metabolic syndrome; Pearson correlation between lncRNA-SRA and MetS was 0.414 ($p = 0.023$).

Metabolic syndrome was represented by only four (20%) women of the control group and 16 (80%) women were free of MetS. There was non-significant correlation between LncRNA-SRA and MetS in control group. There was also a non-significant difference between lncRNA-SRA in both subgroups.

Table 4 shows that in PCOS group, there were significant increases in BMI, TG, and lncRNA-SRA in MetS subgroup compared to non-MetS subgroup. There were also high significant increases in WC, FBS, insulin, HOMA/IR, and HDL in MetS subgroup than in non-MetS. There were also non-significant differences in both subgroups with regards to age, hirsutism, cholesterol, LH, FSH, prolactin, and testosterone. In PCOS, lncRNA-SRA relative expression in MetS was 11.03 ± 3.58 and in non-MetS it was 7.83 ± 3.37. Figure 1 shows the direct positive significant correlation between lncRNA-SRA with testosterone, insulin, and hirsutism, respectively.

Discussion

PCOS is one of the most common endocrine disorders in women characterized by chronic anovulation, oligomenorrhea or amenorrhea, hyperandrogenism, and polycystic ovary morphology with strong association of metabolic syndrome [17]. MetS is one type of endocrinological disturbance that consists of insulin resistance, dyslipidemia, obesity, central adiposity, and hypertension that has been shown to be associated with a two-fold increased risk of cardiovascular disease and a five-fold increased risk of type 2 diabetes [18].

In this study, there is non-significant difference with regards to age between both studied groups. PCOS patients had menstrual irregularity at 5.53 ± 2.03 years, lasting for a minimum of two years and a maximum of ten years of menstrual irregularities. A family history of diabetes mellitus presented in 33.3% of the cases, hypertensive disorders in 53%, family members with obesity in 20%, and a history of ischemic heart disease within the family in 16.6%.

In the PCOS group, there were abnormalities in WC in 20 (66.6%) patients, high-density lipoprotein cholesterol (HDL-C) in 28 (93.3%), high blood pressure in 15 (50%), higher triglycerides in 7 (23.3%), diabetes in 6 (20%), and impaired glucose in 8 (36.6%) patients. There was significant increase in FBS, TG, and LH in PCOS than control group; there was also a high significant increase in PCOS with regards to hirsutism, BMI, WC, insulin, HOMA/IR, cholesterol, HDL, FSH, and testosterone compared to the control group. BMI in PCOS group was 35.57 ± 3.53 kg/m^2 which put them in the obese category. Barber *et al.* reported that adiposity plays crucial role in the development and maintenance of PCOS and strongly influences the severity of its clinical and endocrine features [19]. An elevated expression of lncRNA-SRA was observed in PCOS patients (9.89 ± 3.80) and in control group (1.04 ± 0.06) relative expressions. In PCOS, there was a positively high significant correlation between lncRNA-SRA and hirsutism, BMI, WC, insulin, HOMA/IR, and testosterone and there was insignificant correlation with FBG, cholesterol, HDL, TG, and FSH. Liu *et al.* [20] reported an increase lncRNA-SRA in PCOS but no association between lncRNA-SRA and HOMA-IR, fasting glucose, and fasting insulin was observed in both PCOS and the control. This is inconsistent with the present study, where there was a significant association between lncRNA-SRA with HOMA/IR and insulin in PCOS, but in the control group the results were similar to others.

In PCOS patients, lncRNA-SRA was positively correlated with MetS ($r = 0.414$ and $p = 0.02$). This positive correlation with MetS indicated that lncRNA-SRA might regulate several receptors. Coley *et al.* implicate steroid receptor RNA activator (SRA) as a nuclear receptor coregulator with multiple partners, and it regulates the activities of multiple nuclear receptors mainly in its RNA forms [13]. SRA was described for the first time as a molecule regulating adipogenesis. It was shown that lncRNA-SRA, initially identified as a coactivator of steroid receptors, also pro-

motes adipocyte differentiation [21].

The prevalence of metabolic syndrome in PCOS was studied by Jamil *et al.* [22] who reported (58.3%). In the present study the prevalence of MetS in PCOS reached 63.3% which is higher than other studies and this attracts attention that PCOS patients in Egypt have higher incidence of metabolic syndrome which requires screening of in all PCOS patients to assure prophylaxis from diabetes and cardiovascular consequences. Incidence of MetS in PCOS was 46% in the study conducted by Gluek *et al.* [23], who recommended diet and metformin for those patients. According to Mandrelle *et al.* [24] the overall prevalence of metabolic syndrome according to the modified AHA/NHLBI ATP III (2005) criteria was 37.5%; 5.8% cases were detected to have diabetes mellitus, 11.7% had impaired fasting glucose, dyslipidemia was present in 93.3%, but in the present study, 22% of PCOS patients were diabetics, 36.6% had impaired FBG, and 93.3% dyslipidemia.

In PCOS with MetS, WC was 89 ± 4.77 cm, BMI was 36.83 ± 2.86 kg/m², which is considered obese, triglycerides was 122.84 ± 26.16 mg/dL, HDL-C was 37.87 ± 5.84 mg/dL which represented high risk, systolic blood pressure was 130 ± 15 mmHg, diastolic blood pressure was 85 ± 7 mmHg, and FBS was 6.53 ± 1.05 mmol/L, which was considered within the impaired fasting glucose levels. Serum insulin was 25.54 ± 5.38 mIu/L and HOMA/IR was 7.29 ± 2.33, which was considered insulin resistant. Alebić *et al.* [25] investigated whether insulin resistance in PCOS is exclusively associated with BMI and suggested specific HOMA-IR cutoff value ≥ 3.15 for PCOS. HOMA/IR in all PCOS in this study was 6.21 ± 2.49 which was approximately 2.2-fold than that in control group (2.82 ± 0.48). Impaired fasting glucose (IFG) was diagnosed when FBG was between 5.6–6.9 mmol/L, diabetes mellitus was confirmed by FBG ≥ 7.0 mmol/L [26], and IR was diagnosed by the presence of one or more of the following: fasting plasma insulin ≥ 20 μU/ml and a cut-off value of HOMA-IR ≥ 3.8 [27]. Therefore, according to recorded cut-off level of HOMA/IR in PCOS by Alebić *et al.,* 84.7% had a higher HOMA/IR in their PCOS group. With PCOS patients are considered obese as their mean BMI is 35.57 ± 3.53 kg/m². This is consistent with Ehrmann who reported that in PCOS patients, the syndrome can be regarded as an obesity-related disorders more than 50% are obese or overweight that predisposed them to metabolic disorders [28].

With PCOS there are significant increases in BMI, TG, and lncRNA-SRA, high significant increases in WC, FBS, insulin, HOMA/IR, and HDL in MetS subgroup than in non-MetS subgroup. There was no significant differences in both subgroups with regards to age, hirsutism, cholesterol, LH, FSH, prolactin, and testosterone.

In PCOS subgroups, lncRNA-SRA in MetS represents significant higher expression level (11.03 ± 3.58) than in non-MetS (7.83 ± 3.37), with a relative expression significance of $p = 0.02$. In control group, there was no significant correlation between lncRNA-SRA and MetS, and there was no significant difference in lncRNA-SRA in both subgroups.

Multiple linear regression analysis revealed that lncRNA-SRA relative expressions contributed positively and significantly to BMI, testosterone, and hirsutism in the PCOS group. For BMI, $r^2 = 0.239$, $f = 8.801$, $p = 0.006$ and for testosterone $r^2 = 0.358$, $f = 7.515$, and $p = 0.003$, and for hirsutism $r^2 = 0.340$, $f = 14.409$, and $p = 0.001$. The present data demonstrated a high significant association of obesity and androgenicity in PCOS with high expression of lncRNA-SRA. The high expression of lncRNA-SRA explains the high androgenicity due to activation of steroid receptors including androgen receptors. This finding is consistent with that reported that lncRNA-SRA is an important regulator of adipose tissue mass and function in diet-induced obesity [29].

Research showed that lncRNA-SRA affects multiple biological processes such as steroid receptors signaling, steroidogenesis, adipogenesis, and insulin sensitivity whose functions might play an important role in the pathophysiology of PCOS [14, 21]. Lanz *et al.* characterized lncRNA- SRA as a RNA molecule which enhances the activity of steroid receptors through a ribonucleoprotein complex [11]; recently, lncRNA-SRA has been seen to participate extensively in nuclear receptors' coactivation in several hormone-related systems, including non-steroid nuclear receptors and other transcription factors. Elevated lncRNA-SRA expression contributes to steroid hormone receptors (AR, ER, and PR) hyperactivity and also contributes to steroidogenesis abnormality, affecting endocrinological and reproductive-related genes' expression and worsened the abnormal metabolic type of PCOS [30]. This explains why in MetS PCOS, lncRNA-SRA level is higher than in non-MetS. Research suggests a bivalent state for lncRNA-SRA at certain sites, which explains why in non-MetS PCOS, lncRNA-SRA activates steroid receptors while it and at represses other receptors.

Conclusion

The present study includes evidence that lncRNA-SRA is upregulated in PCOS and is higher in PCOS with metabolic syndrome.

References

[1] Franks S.: "Polycystic Ovary Syndrome". *Med. Prog.*, 1995, *333*, 853.

[2] Kandaraki E., Christakou C., Diamanti-Kandarakis E.: "Metabolic syndrome and polycystic ovary syndrome… vice versa". *Arq. Bras. Endocrinol. Metabol.*, 2009, *53*, 227.

[3] Huang P.: "A comprehensive definition for metabolic syndrome". *Dis. Model. Mech.*, 2009, *2*, 231.

[4] Grundy S., Cleeman J., Daniels S., Donato K., Eckel R., Franklin B., *et al.*: "Diagnosis and management of the metabolic syndrome: An American Heart Association / National Heart, Lung, and Blood Institute Scientific Statement". *Circulation*, 2005, *112*, 2735.

[5] Behboudi-Gandevani S., Ramezani Tehrani F., Rostami Dovom M., Farahmand M., Bahri Khomami M., Noroozzadeh M., *et al.*: "Insulin resistance in obesity and polycystic ovary syndrome: systematic review and meta-analysis of observational studies". *Gynecol. Endocrinol.*, 2016, *32*, 343.

[6] Lim S., Davies M., Norman R., Moran L.: "Overweight, obesity and central obesity in women with polycystic ovary syndrome: a systematic review and meta-analysis". *Hum. Reprod. Update*, 2012, *18*, 618.

[7] Moran L., Misso M., Wild R., Norman R.: "Impaired glucose tolerance, type 2 diabetes and metabolic syndrome in polycystic ovary syndrome: a systematic review and meta-analysis". *Hum. Reprod. Update*, 2010, *16*, 347.

[8] Behboudi-Gandevani S., Amiri M., Yarand R., Noroozzadeh M., Farahmand M., Dovom M., *et al.*: "The risk of metabolic syndrome in polycystic ovary syndrome: A systematic review and meta-analysis". *Clin. Endocrinol. (Oxf.)*, 2018, *88*, 169.

[9] Mohamed-Hussein Z., Harun S.: "Construction of a polycystic ovarian syndrome (PCOS) pathway based on the interactions of PCOS-related proteins retrieved from bibliomic data". *Theor. Biol. Med. Model*, 2009, *6*, 18.

[10] Taylor D., Chu E., Spektor R., Soloway P.: "Long Non-Coding RNA Regulation of Reproduction and Development". *Mol. Reprod. Dev.*, 2015, *82*, 932.

[11] Lanz R., McKenna N., Onate S., Albrecht U., Wong J., Tsai S., *et al.*: "A steroid receptor coactivator SRA, functions as an RNA and is present in an SRC-1 complex". *Cell*, 1999, *97*, 17.

[12] Lanz R., Razani B., Goldberg A., O'Malley B.: "Distinct RNA motifs are important for coactivation of steroid hormone receptors by steroid receptor RNA activator (SRA)". *Proc. Natl. Acad. Sci. USA*, 2002, *99*, 16081.

[13] Colley S., Leedman P.: "Steroid receptor RNA activator – a nuclear receptor coregulator with multiple partners: insights and challenges". *Biochimie*, 2011, *93*, 1966.

[14] Xu B., Yang W.H., Gerin I., Hu C.D., Hammer G.D., Koenig R.J.: "Dax-1 and steroid receptor RNA activator (SRA) function as transcriptional coactivators for steroidogenic factor 1 in steroidogenesis". *Mol. Cell. Biol.*, 2009, *29*, 1719.

[15] ESHRE/ASRM: "Revised Rotterdam 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome (PCOS)". *Hum. Reprod.*, 2004, *19*, 41.

[16] Balen A., Laven J., Tan S., Dewailly D.: "Ultrasound assessment of the polycystic ovary: international consensus definitions". *Hum. Reprod. Update*, 2003, *9*, 505.

[17] Korhonen S., Hippelainen M., Niskanen L., Vanhala M., Saarikoski S.: "Relationship of the metabolic syndrome and obesity to polycystic ovary syndrome; a controlled, population-based study". *Am. J. Obstet. Gynecol.*, 2001, *184*, 289.

[18] Soares E., Azevedo G., Gadelha R., Lemos T., Maranhao T.: "Prevalence of the metabolic syndrome and its components in Brazilian women with polycystic ovary syndrome". *Fertil. Steril.*, 2008, *89*, 649.

[19] Barber T., McCarthy M., Wass J., Franks S.: "Obesity and polycystic ovary syndrome". *Clin. Endocrinol. (Oxf.)*, 2006, *65*, 137.

[20] Liu Z., Hao C., Huang X., Zhang N., Bao H., Qu Q.: "Peripheral blood leukocyte expression level of lncRNA steroid receptor RNA activator (SRA) and its association with polycystic ovary syndrome: a case control study". *Gynecol. Endocrinol.*, 2015, *31*, 363.

[21] Xu B., Gerin I., Miao H., Vu-Phan D., Johnson C.N., Xu R., *et al.*: "Multiple roles for the non-coding RNA SRA in regulation of adipogenesis and insulin sensitivity". *PLoS One*, 2010, *5*, e14199.

[22] Jamil A., Alalaf S., Al-Tawil N., Al-Shawaf T.: "A case-control observational study of insulin resistance and metabolic syndrome among the four phenotypes of polycystic ovary syndrome based on Rotterdam criteria". *Reprod. Health*, 2015, *12*, 7.

[23] Glueck C., Papanna R., Wang P., Goldenberg N., Sieve-Smith L.: "Incidence and treatment of metabolic syndrome in newly referred women with confirmed polycystic ovarian syndrome". *Metabolism Clin. Exp.*, 2003, *52*, 908.

[24] Mandrelle K., Kamath M., Bondu D., Chandy A., Aleyamma T., George K.: "Prevalence of metabolic syndrome in women with polycystic ovary syndrome attending an infertility clinic in a tertiary care hospital in south India". J. Hum. Reprod. Sci., 2012, *5*, 26.

[25] Alebić M., Bulum T., Stojanović N., Duvnjak L.: "Definition of insulin resistance using the homeostasis model assessment (HOMA-IR) in IVF patients diagnosed with polycystic ovary syndrome (PCOS) according to the Rotterdam criteria". *Endocrine*, 2014, *47*, 625.

[26] American Diabetes Association: "Standards of medical care in diabetes". *Diabetes Care*, 2013, *1*, S11.

[27] Legro R., Castracane V., Kauffman R.: "Detecting insulin resistance in polycystic ovary syndrome: purposes and pitfalls". *Obstet. Gynecol. Survey*, 2004, *59*, 141.

[28] Ehrmann D.: "Polycystic ovary syndrome". *N. Engl. J. Med.*, 2005, *352*, 1223.

[29] Liu S., Sheng L., Miao H., Saunders T.L., MacDougald O.A., Koenig R.J., Xu B.: "SRA gene knockout protects against diet-induced obesity and improves glucose tolerance". *J. Biol. Chem.*, 2014, *289*, 13000.

[30] Fatica A., Bozzoni I.: "Long non-coding RNAs: new players in cell differentiation and development". *Nat. Rev. Genet.*, 2014, *15*, 7.

Corresponding Author:
HANAN MOHAMED GABR YOUSSEF, M.D.
Egyptian Atomic Energy Authority
Health Radiation Research Department
National Center for Radiation Research and Technology
Ahmad El-Zomor Street
Nasr city, Cairo (Egypt)
e-mail: hanangabr62@gmail.com