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The impact of long non-coding RNA H19 on metabolic features and reproductive phenotypes of Egyptian women with polycystic ovary syndrome

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Abstract

Background Polycystic ovary syndrome (PCOS) is known as the most common endocrine/metabolic disorder in women of reproductive age. Long non-coding RNAs (lncRNAs) regulate a wide range of physiological and pathological processes. We designed this study to evaluate lncRNA H19 relative expression in patients with PCOS and to evaluate its impact on metabolic features and reproductive phenotypes of Egyptian women with polycystic ovaries.

Material and methods The case–control study enrolled 50 control groups and 50 patients, with PCOS. The selection of patients with PCOS depended on the diagnosis according to the Rotterdam Consensus (2004). The lncRNA H19 were measured by real-time quantitative polymerase chain reaction (RT-qPCR).

Results The lncRNA H19 level was significantly higher in the PCOS group (1.71 ± 0.48) compared to controls (0.924 ± 0.081). Furthermore, lncRNA H19 levels were significantly positively correlated with anthropometric and metabolic parameters including BMI, waist/hip ratio, TC, TG, LDL, FPG, FSI, HbA1c, and HOMA-IR. Regarding reproductive phenotypes features, hirsutism score, and AFC levels were significantly positively correlated with lncRNA H19 levels. The linear regression test revealed that BMI and AFC were the only parameters independently associated with lncRNA H19 among other studied parameters. Interestingly, receiver operating characteristic curve (ROC) analysis detected that the area under the curve (AUC) for the lncRNA H19 was 0.925 (95% CI = 0.856–0.955) with sensitivity = 96.4%, specificity = 96%, and the cutoff values (1.08). Thus, the predictive power of lncRNA H19 of PCOS was highly sensitive and specific.

Conclusion PCOS patients had significantly higher lncRNA H19 levels than controls. lncRNA H19 levels were significantly positively correlated with metabolic risk factors as well as clinical and laboratory features of PCOS.

Keywords lncRNA, PCOS, Metabolic features, Reproductive phenotypes

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Introduction

Polycystic ovary syndrome (PCOS) is a common reproductive endocrine disease; it is associated with a range of reproductive, obstetric, metabolic [1], and psychological features. Reproductive and obstetric manifestations include hyperandrogenism, menstrual dysfunction, infertility, and pregnancy complications [2].

Approximately, 50% of PCOS women are either overweight or obese [3]. Obesity is associated with multiple factors that may influence hypothalamic-pituitary function, particularly insulin resistance, its cardinal features are oligo- or anovulation, clinical and/or biochemical signs of hyperandrogenism, and polycystic ovaries [4].

PCOS may present with other disorders, including insulin resistance (IR), obesity, type 2 diabetes, cardiovascular disease, and anxiety. Given the diversities and complications of this condition, a broader picture of its etiology needs to be elucidated [5].

An emerging body of evidence suggests that the understanding of PCOS has been enhanced by genome and transcriptomic analyses in PCOS patients. However, it is still a challenge to characterize the full processes of multiple molecular interactions on gene regulatory networks in PCOS, specifically in understanding the spectrum of DNA, RNA, and protein interactions [6].

There are interesting reports suggesting that long non-coding RNAs (lncRNAs) have an important role in the pathogenesis of PCOS [7]. lncRNA BANCR has been exposed to be involved in the progression of PCOS by promoting cell apoptosis [8]. Additionally, important research detected that the upregulation of lncRNA H19 is associated with a decrease the insulin resistance levels in women with PCOS [9].

Considerable research showed that lncRNAs have emerged as critical regulators of adipose tissue subsequently they can modulate gene expression at the epigenetic, transcriptional, and post-transcriptional levels [10]. Additionally, they interact with DNA, RNA, protein complexes, other non-coding RNAs, and microRNAs to regulate a wide range of physiological and pathological processes. To test this hypothesis, in the present study, we investigated lncRNA H19 in patients with PCOS and to evaluate its impact on metabolic features and reproductive phenotypes of Egyptian women with polycystic ovaries.

Subject and methods

A case–control study was conducted on 50 obese patients with PCO, BMI ˃ 30 kg/m^2, and 50 healthy patients

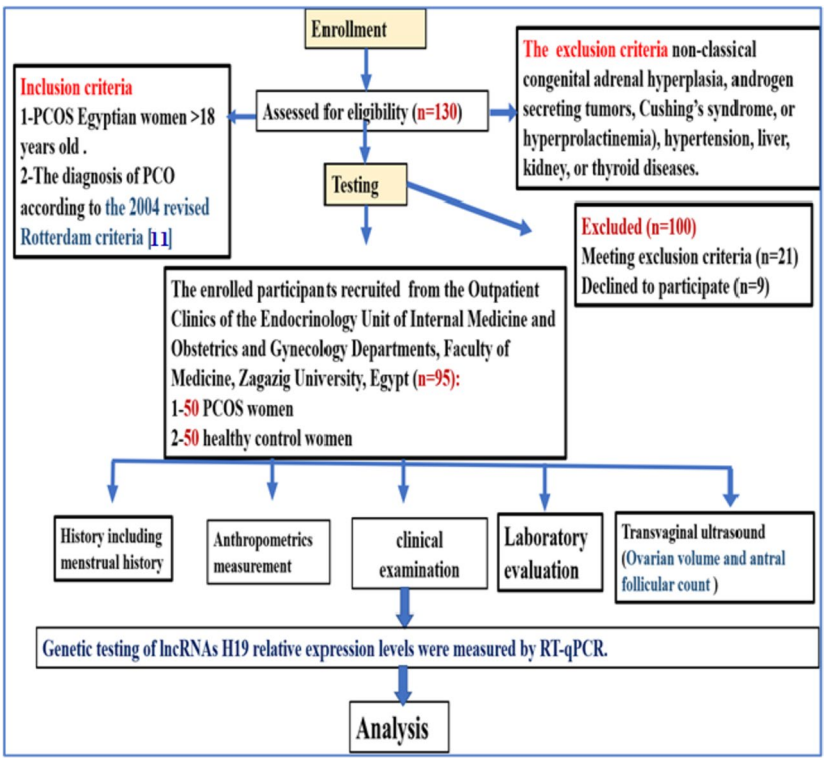

Fig. 1 The flowchart of the study [11]

matched for age. The design and the methodology of the study are shown in the flowchart (Fig. 1). After being informed of the purpose and procedures of the study, all subjects signed an informed consent form.

The ethical committee of Faculties of Medicine, Zagazig University, approved the study.

Laboratory evaluation was done for the studied participants enrolled from the Departments of Internal Medicine and Obstetrics and Gynaecology. Testing was done according to operating techniques in the Medical Biochemistry Department and Zagazig University Hospital and medical microbiology and immunology laboratories.

Ethics approval and consent to participate

The study protocol was approved by the Ethical Committee of the Faculty of Medicine, Zagazig University, the reference number was IRB (Ethics number. 10628), and each participant signed a written informed consent document.

Quantitative real-time RT-PCR for assessment

The RNA was obtained from EDTA peripheral blood samples according to the company's directions. The relative expression of H19 was calculated using $2^{-\triangle\triangle Ct}$ (Ct, cycle threshold) with GAPDH used as an internal control. Upstream of H19: 5′-GCCTTGACGTGCTGGATC T-3′, downstream of H19: 5′-TCCGATGCTTTACTC AAGAAGTT-3′, Upstream of GAPDH: 5′-GGGAAA CTGTGGCGTGAT-3′, and downstream of GAPDH: 5'-GAGTGGGTGTCGCTGTTGA-3′.

Statistical analysis

Statistical analyses were performed using the Statistical Package for the Social Sciences for Windows (version 26.0; SPSS Inc., Chicago, IL, USA). Data were expressed using descriptive statistics (mean ± standard deviation) and were analyzed using the *t* test. The Pearson correlation coefficient was used to assess the association between. Receiver operating characteristic (ROC) analysis was performed to assess the predective power of lncRNA H19 for the prediction of PCOS, the area under the curve (AUC), and the cutoff values were tested. We considered *P* to be significant at < 0.05 with a 95% confidence interval (CI).

Results

Clinical and anthropometric characteristics of the studied groups

In the obese group, as expected we found significantly higher levels of metabolic risk factors for example, BMI, waist/hip ratio, systolic blood, and diastolic blood pressure, compared to control group ($P < 0.001^*$, Table 1).

Laboratory characteristics of the studied groups

In the obese group, TC, TG, LDL, FPG, FSI, HbA1c, HOMA β, and HOMA-IR compared to control group. PCOS phenotype including total testosterone, androstenedione, hirsutism score, ovarian volume FSH, LH, LH/FSH, AMH,DHEA-S, total testosterone, and AFC values were significantly high in PCOS women compared to controls. On the contrary, we detected significantly lower HDL in PCOS patients than in those healthy women ($< 0.001^*$, Table 2).

Comparison of lncRNA H19 relative expression in studied groups

In the PCO group, we found significantly higher values of lncRNA H19 (1.71 ± 0.48) compared to controls (0.924 ± 0.081) ($P < 0.001$*, Fig. 2).

Correlation between lncRNA H19 and clinical and laboratory parameters among PCOS women

Among the PCOS group ($n = 50$), lncRNA H19 levels were significantly positively correlated with anthropometric and metabolic parameters including BMI, waist/hip ratio, TC, TG, LDL, FPG, FSI, HbA1c,

Table 1 Clinical, anthropometric characteristics of studied groups

	Control group (mean ± SD) ($n = 50$)	PCOS group (mean ± SD) ($n = 50$)	*P*
Age (years)	29.07 ± 6.4	30.05 ± 6.473	0.265
Body mass index (kg/m^2)	24.55 ± 2.51	37.32 ± 2.16	$< 0.001^*$
Waist/hip ratio	0.93 ± 0.22	1.44 ± 0.358	< 0.001*
Systolic blood pressure (mm Hg)	127.68 ± 6.54	130.95 ± 6.8	< 0.05*
Diastolic blood pressure (mm Hg)	75.18 ± 5.88	85.04 ± 3.320	< 0.05*
Hirsutism score	4.42 ± 0.981	20.84 ± 4.12	< 0.001*
Ovarian volume	5.15 ± 0.89	8.783 ± 3.96	< 0.001*
AFC	11.1 ± 3.42	24.6 ± 4.52	< 0.001*

AFC Antral follicle cells

Table 2 Laboratory characteristics of the studied patients

	Control group (mean ± SD) (*n* = 50)	PCOS group (mean ± SD) (*n* = 50)	*P*
Total cholesterol (mg/dL)	126.7 ± 20.65	199.9 ± 12.17	< 0.001*
Triglycerides (mg/dL)	139.6 ± 11.19	240.5 ± 91.46	< 0.001*
LDL cholesterol (mg/dL)	105.8 ± 4.32	152.7 ± 13.77	< 0.001*
HDL cholesterol (mg/dL)	52.26 ± 4.44	34.76 ± 4.1	< 0.001*
Fasting plasma glucose (mg/dL)	75.12 ± 8.95	94.11 ± 6.81	< 0.001*
Fasting serum insulin (IU/mL)	6.85 ± 1.45	16.1 ± 7.41	< 0.001*
HbA1c (%)	4.77 ± 0.147	5.9 ± 0.12	< 0.001*
HOMA-IR	1.4 ± 0.85	4.63 ± 2.12	< 0.001*
HOMA β	164.5 ± 22.1	196.8 ± 31.11	< 0.001*
FSH (mIU/mL)	5.89 ± 1.01	5.06 ± 1.49	< 0.05*
LH (mIU/mL)	3.54 ± 1.22	5.439 ± 1.5	< 0.001*
LH/FSH	0.53 ± 0.030	1.15 ± 0.18	< 0.001*
AMH (ng/ml)	2.54 ± 0.4	5.339 ± 0.61	< 0.001*
DHEA-S (mg/mL)	0.92 ± 0.48	1.45 ± 0.84	< 0.001*
Total testosterone (ng/mL)	0.51 ± 0.15	1.53 ± 0.13	< 0.001*

HOMA-IR Homeostasis model assessments of insulin resistance, *DHEA* Dehydroepiandrosterone

* Statistically significant ($P < 0.05$)

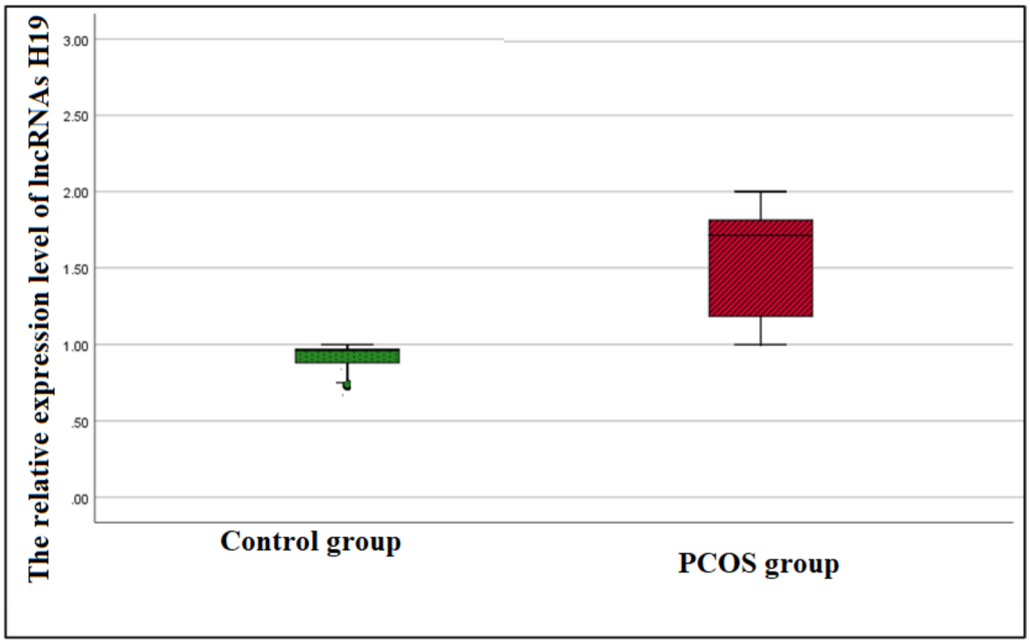

Fig. 2 Comparison of lncRNA H19 relative expression levels in studied groups

and HOMA-IR (Table 2, $P < 0.001$*). Concerning PCOS phenotyping features, hirsutism score, and AFC levels were significantly positively correlated with lncRNA H19 (Table 2, $P < 0.001$*). Although other studied parameters were non-significantly correlated with lncRNA H19 level, $P > 0.05$.

Linear regression analysis

For further assessment of the current findings of the present study, we applied linear regression, and we detected that BMI and AFC were independently associated with lncRNA H19 (Table 3 and 4, $P < 0.05$).

Table 3 Pearson correlation coefficient between lncRNA H19 relative expression with clinical, anthropometric, laboratory, and phenotype characteristics of the PCOS group

	r	*P*
Body mass index (kg/m^2)	0.642	< 0.001*
Waist/hip ratio	0.351	< 0.001*
Systolic blood pressure (mm Hg)	0.108	0.261
Diastolic blood pressure (mm Hg)	0.238	0.017
Hirsutism score	0.424	< 0.001*
Ovarian volume	0.127	0.208
AFC	0.449	< 0.001*
Total cholesterol (mg/dL)	0.345	< 0.001*
Triglycerides (mg/dL)	0.777	< 0.001*
LDL cholesterol (mg/dL)	0.570	< 0.001*
HDL cholesterol (mg/dL)	− 0.041	0.667
Fasting plasma glucose (mg/dL)	0.351	< 0.001*
Fasting serum insulin (IU/mL)	0.238	< 0.001*
HbA1c (%)	0.365	< 0.001*
HOMA-IR	0.345	< 0.001*
HOMA B	0.111	0.248
FSH (mIU/mL)	0.187	0.051
LH (mIU/mL)	0.119	0.214
LH/FSH	0.027	0.787
DHEA-S (mg/mL)	0.218	0.209
Total testosterone (ng/mL)	0.1237	0.173

* Statistically significant ($P < 0.05$)

The accuracy of lncRNA H19 for prediction of PCOS by ROC analysis

The power of lncRNA H19 to the prediction of PCOS among the studied group was evaluated using ROC analysis. The AUC was 0.925 (95% CI = 0.856–0.955) with sensitivity = 96.4%, specificity = 96%, and the cutoff values (1.08) (Fig. 3).

Discussion

Polycystic ovary syndrome (PCOS) has been realized as an endocrinopathy and metabolic disturbance with an incidence of 5–10% in reproductive-age women [12, 13]. Despite the controversial opinions of current diagnostic criteria of PCOS, blood tests for PCOS are mainly based on hormone levels and endocrine function, and there is a growing clinical need for biomarkers that are both sensitive and specific enough for PCOS diagnosis [14].

LncRNAs have been characterized as functional RNA, involved in biological, developmental, and pathological processes, acting through mechanisms such as epigenetics, cis regulation at enhancers, and post-transcriptional regulation of mRNA processing. More recently, studies concerned the functions of lncRNAs in PCOS [6].

Even though the pathogenesis of PCOS is yet to be fully understood, it leads to treatment difficulties [15]. It is well-known that alterations in apoptosis are a pathology in the progress of PCOS [16]. Compelling evidence indicates that the development of PCOS is accompanied not only by changes in protein expression but also by changes in non-coding RNA expression, such as lncRNA [17]. Recently, there has been a growing realization that H19 regulates androgen synthesis, granulosa cell proliferation, follicular development, and ovarian function [18].

The current research enrolled 50 patients with PCOS and 50 controls As expected the metabolic and phenotypic features were significantly higher in the PCOS group compared to the healthy control group. Intriguingly, the main findings of the current research were that we observed significantly higher values of lncRNA H19 in the PCOS group compared to controls. Moreover, lncRNA H19 levels were significantly positively correlated with anthropometric and metabolic parameters including BMI, waist/hip ratio, TC, TG, LDL, FPG, FSI, HbA1c, and HOMA-IR. Regarding PCOS

Table 4 Linear regression analyses in PCOS women to test the influence of the main independent variables against lncRNA H19 levels (dependent variable)

Model		Unstandardized coefficients		Standardized coefficients	*t*	*P* value	95% C.I	
		B	SE	Beta			Lower bound	Upper bound
1	Constant	− 2.217	0.939		− 2.362	0.020	− 4.081	− 0.353
	HbA1c	0.001	0.003	0.044	0.397	0.693	− 0.005	0.007
	HOMA-IR	0.011	0.007	0.131	1.523	0.131	− 0.003	0.025
	Waist/hip ratio	0.002	0.002	0.128	0.987	0.326	− 0.002	0.007
	BMI	0.583	0.147	0.318	3.963	< 0.001*	0.291	0.875
	TG	− 0.030	0.038	− 0.107	− 0.803	0.424	− 0.105	0.045
	AFC	1.065	0.250	0.439	4.256	< 0.001*	0.568	1.562
	Hirsutism score	0.059	0.129	0.047	0.460	0.646	− 0.196	0.314

* Statistically significant ($P < 0.05$)

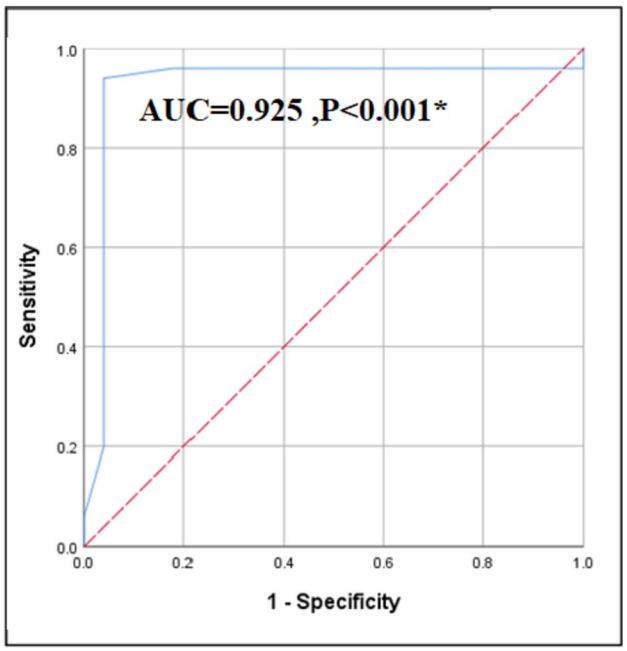

Fig. 3 The accuracy of lncRNA H19 relative expression for prediction of PCOS by ROC analysis

phenotyping features, hirsutism score, and AFC levels were significantly positively correlated with lncRNA H19 levels. Furthermore, we used a linear regression test to further analyze our results and detected that BMI and AFC were independently associated with lncRNA H19.

Similarly, Qin et al. have reported that H19 expression is highly upregulated in the circulation of patients with PCOS compared with those of controls [19]. Consistent with this finding, Chen et al. confirmed that H19 is a key target of metformin in PCOS treatment [20].

In line with this idea, Li et al. detected increased expression of H19 in ovarian tissues and granulosa cells of patients with PCOS [21]. Similar results were observed by Wang et al. they found overexpression of lncRNA H19 in PCOS follicular fluid exosome samples than in non-PCOS follicular fluid exosome samples [22].

Many studies studied the role of lncRNA H19 dysregulation in infertility [23]. H19 expression level was found to be associated with low ovarian reserve [24] and uterine fibroids [25]. A noteworthy result of Korucuoglu et al. research detected lower IGF1 and H19 expression in the endometrium of women with unexplained infertility which leads to proliferation as well as apoptosis of endometrial stromal cells [26].

Our study is unique in that a sensitive, real-time PCR assay was used to assess the role of lncRNA H19 in PCOS prediction and its association with metabolic and reproductive phenotype features of PCOS. The data presented here reinforces the argument that lncRNA H19 expression profiling may generate a unique molecular signature for PCOS. The AUC of lncRNA H19 to predict PCOS among the studied group was 0.925 with a sensitivity of 96.4% and specificity of 96% at the cutoff values (1.08).

Limitations of the study

The findings of this study have to be seen in light of some limitations, firstly, a small sample size. Secondly, this study was conducted on Egyptian women only. Lastly, this study did not compare tissue and circulatory lncRNA H19 expression levels. In the future, we will conduct a large sample study and include other ethnicities than Egyptian.

Conclusion

In conclusion, the current study provided unequivocal evidence that PCOS patients had significantly higher lncRNA H19 level than controls. lncRNA H19 levels were significantly positively correlated with positively correlated with metabolic risk factors as well as clinical and laboratory features of PCOS. Thus, we suggested that lncRNA H19 could be non-invasive biomarker of PCOS and further study indeed to support the current finding.

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Authors' contributions
Nearmeen M. Rashad, Walid Mohamed Elnagar, Dina Ahmed Seleem, and Hoda Afifi collected patients' samples and clinical data. Marwa H.S. Hussien and Rehab M. Atef prepared samples for laboratory investigations. Nearmeen M. Rashad wrote the paper. Statistical analysis, interpretation of data, and preparation of the paper for submitting international was done by Nearmeen M. Rashad. Critical revision of the manuscript was performed by all of the authors.

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Availability of data and materials
All data supporting the findings of this study are available within the paper.

Declarations

Consent for publication
Not applicable.

Competing interests
None.

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