RESEARCH ARTICLE

# Gremlin-1 level in polycystic ovary syndrome and its clinical correlations; A case control study

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## Abstract

**Objective:** To evaluate the involvement of the level of Gremlin-1 in serum and follicular fluid in the diagnosis of polycystic ovary syndrome.

**Methods:** The case-control study was conducted at the Kafrelsheikh University Hospital, Egypt, from September 2021 to February 2022, and comprised women with polycystic ovary syndrome and healthy controls. All participants were subjected to a detailed clinical assessment, complete clinical examination and hormonal profile assessment. Gremlin-1 concentrations in plasma and follicular fluid samples were assessed by a double-antibody sandwich enzyme-linked immunosorbent assay kit. Data was analysed using SPSS 20.

**Results:** Of the 90 subjects, 45(50%) were patients with a mean age of 29.53±4.82 years, and 45(50%) were controls with a mean age of 30.89±6.08 ($p>0.05$). Mean weight, body mass index, waist circumference and waist-hip ratio were significantly higher in patients compared to controls ($p<0.05$). Serum and follicular fluid Gremlin-1 levels were significantly higher in the patient group ($p<0.05$). The best cutoff of serum Gremlin-1 in the diagnosis of polycystic ovary syndrome was ≥1.325ng/ml with area under curve 0.857, sensitivity 93.3%, specificity 68.9%, positive predictive value 75%, negative predictive value 91.2% and overall accuracy 81.1%. The best cutoff of follicular fluid Gremlin-1 in the diagnosis of polycystic ovary syndrome was ≥1.725ng/ml with area under curve 0.789, sensitivity 73.3%, specificity 68.9%, positive predictive value 70.2%, negative predictive value 72.1% and overall accuracy 71.1%.

**Conclusion:** There was a strong correlation between serum and follicular Gremlin-1 levels in polycystic ovary syndrome patients.

**Keywords:** Polycystic ovary syndrome, Hyperandrogenism, Follicular fluid, Anovulation, Predictive value, Infertility.

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## Introduction

Approximately 6-10% of females of reproductive age present with polycystic ovarian syndrome (PCOS).[1] It is common for cardiometabolic dysfunction to be a substantial comorbidity, which has long-term repercussions on cardiovascular health.[1]

In order to understand the pathophysiology of PCOS, it is necessary to look at a wide range of cases. Predicted ovarian-related and non-ovarian perturbations have been linked to PCOS, but it is not clear which of these are the primary causes and which are indirect repercussions.[2]

First activation or recruitment of primordial cells into growing primary follicles in women of reproductive age does not need serum gonadotropins. Cell connections between mesenchymal and epithelial cells, paracrine signals from the ovary, and oocyte-secreted substances keep primordial cells dormant. Anti-Müllerian Hormone (AMH), a stimulant of granulosa cell differentiation, and Growth Differentiation Factor (GDF), an initiator of theca cell differentiation, are important elements in follicular development and control. In addition to producing androgen, primordial theca cells possess Luteinizing Hormone (LH) receptors. As a result, an increase in the number of tiny follicles is thought to be the cause of PCOS's enhanced follicular recruitment.[3]

Many growth factors and extracellular signalling molecules have a role in stimulating or preventing the transformation of primordial follicles essential for successful reproduction. One of the Differential screening-selected gene Aberrant in Neuroblastoma (DAN) family of bone morphogenetic protein (BMP) inhibitors, Gremlin-1 has a similar bioactivity and is an antagonist of BMPs belonging to the Transforming Growth Factor Beta (TGF-β) superfamily of BMPs[4], As a result of their binding to BMPs, they are thought to have a significant influence on reproduction by blocking growth factors from activating their receptors.[5]

Human transition of primordial cells to primary follicles is not yet known to be regulated by these inhibitors[5], which

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have been shown to be generated in the ovary of animals, and to control later stages of their follicle development.[6, 7]

Gender-specific cancers, infertility and embryological ovarian growth have all been linked to Gremlin-1 protein expressions.[4, 8, 9]

PCOS is characterised by anomalies in the development of the ovarian follicles. The current study was planned to evaluate the involvement of Gremlin-1 level in serum and follicular fluid in PCOS diagnosis.

## Patients and Methods

The case-control study was conducted at the Kafrelsheikh University Hospital, Egypt, from September 2021 to February 2022. After approval from the institutional ethics review committee, the sample was raised by approaching all patients who met the inclusion criteria during the study timeframe as a comprehensive sample. Those included were women aged 17-40 years with normal thyroid and prolactin levels, and having at least two elements of the Rotterdam criteria,[10] which comprises oligo/anovulation, hyperandrogenism, acne, androgenic alopecia and/or biochemical markers of hyperandrogenism and morphology of PCOS on ultrasound.[10] Women having history of ovarian surgery, endometrioma, congenital adrenal hyperplasia, and diabetes mellitus, as well as smokers and current contraceptive users were excluded.

Women of the same age group, with regular menstrual cycles, normal ovarian shape and no signs of hyperandrogenism were enrolled as the healthy controls. Written informed consent was obtained from all the participants.

In order to classify hirsutism, Ferriman Gallwey scores (FGS)[11] was used. The participants' body mass index (BMI), weight, height, waist circumference (WC), and hip circumference (HC) were measured.

Total testosterone, Follicle Stimulating Hormone (FSH), LH, estradiol[2], high-density lipoprotein (HDL), low-density lipoprotein (LDL), total cholesterol (TC) and triglyceride (TG) levels were measured using venous blood.

A Voluson E6 Expert ultrasound (U/S) equipment (General Electric®, Fairfield, CT, USA) was used for transvaginal ultrasonography on day 2 of the menstrual cycle to check for PCOS U/S criteria and to rule out any uterine or pelvic diseases. Ultrasonographic assessment was done using 5.0-7.5MHz endovaginal probe. Mean ovarian volume, calculated as the average of the two ovaries, was determined by measuring each ovary in three planes and calculating the volume using the prolate ellipsoid formula V = D1 x D2 x D3 x 0.523 (12); antral follicular count was determined using the method developed by Broekmans et al.[13] for counting follicular structures with a diameter of 2-10mm using the internal diameter of the sonolucent area.

From day 2 of menses or withdrawal bleeding after 5-day treatment with oral norethisterone acetate 5mg (Steronate®, Hi-Pharm, Egypt) twice per day, all patients received antagonist protocol of induction with 300 units gonadotropins, Gonadotropin Hormone-Releasing Hormone (GnRh) antagonist by flexible method when follicle reached 14mm, and both GnRh antagonist and gonadotropins continued till the day of triggering by beta human chorionic gonadotropin (HCG). All participants were advised about the time of egg retrieval and then serum and follicular fluid samples were taken.

Ethylenediaminetetraacetic acid (EDTA) tubes were used to collect peripheral venous blood and follicular fluid samples which were then centrifuged at room temperature for 20 minutes before being stored at -30°C as EDTA plasma to assess Gremlin-1 levels.

Automated enzyme-linked immunosorbent assay (ELISA) (Best 2000 Biokit) was used to quantify Gremlin-1 levels in plasma and follicular fluid with a double-antibody sandwich (Human GREM1 ELISA kit, Catalog No. E2735Hu).

Data was analysed using SPSS 20. Quantitative data was expressed as mean and standard deviation, while qualitative data was expressed as frequencies and percentages. Student's t-test was used to compare the mean of two sets of quantitative data. Chi-square test and Fisher's exact test were used to compare categorical data, as appropriate. Connections between different variables were explored using the correlation coefficient $P<0.05$ was considered statistically significant and $p<0.01$ was deemed highly significant.

## Results

Of the 90 subjects, 45(50%) were patients with a mean age of 29.53±4.82 years, and 45(50%) were controls with a mean age of 30.89±6.08 ($p>0.05$). Serum and follicular Gremlin- levels, FGS, LH, FSH, TG, TC, LDL, weight, BMI, WC and waist-hip ratio (WHR) varied significantly between the groups ($p<0.05$) (Table 1).

The best cut-off value of follicular fluid Gremlin-1 in the diagnosis of PCO was ≥1.725ng/ml with area under curve (AUC) 0.789, sensitivity 73.3%, specificity 68.9%, positive predictive value (PPV) 70.2%, negative predictive value (NPV) 72.1% and overall accuracy 71.1% (Figure). There was a highly significant positive correlation between serum and follicular fluid Gremlin-1 (r 0.359**,** $p<0.001$).

Moreover, a significant positive correlation between serum

**Table-1:** Comparison between patients and controls regarding clinical data.

| Parameter | Group<br>Patient group (n=45)<br>Mean ± SD | Control group(n=45)<br>Mean ± SD | *t-value* | *p-value* |
|---|---|---|---|---|
| Mean Age (year) | 29.53 ± 4.82 | 30.89 ± 6.08 | -1.172 | 0.244 |
| Mean Weight (kg) | 78.6 ± 11.5 | 65.2 ± 7.71 | 6.493 | <0.001** |
| Mean Height (cm) | 163.31 ± 5.13 | 165.2 ± 5.62 | -1.665 | 0.099 |
| BMI (kg/m2) | 29.65 ± 4.48 | 23.86 ± 4.48 | 7.484 | <0.001** |
| Waist circumference (cm) | 92.22 ± 14.93 | 83.04 ± 7.85 | 3.365 | <0.001** |
| Hip circumference (cm) | 109.31 ± 18.51 | 103.69 ± 11.77 | 1.719 | 0.09 |
| Waist/hip ratio | 0.85 ± 0.06 | 0.81 ± 0.07 | 3.109 | 0.003* |
| FGS | 13.27 ± 3.35 | 10.56 ± 2.68 | 4.239 | <0.001** |
| FBG (mg/dl) | 88.44 ± 13.33 | 83.4 ± 10.08 | 1.932 | 0.0537 |
| Triglycerides (mg/dl) | 118.09 ± 21.69 | 103.87 ± 25.23 | 2.868 | 0.003* |
| Total cholesterol (mg/dl) | 159.53 ± 17.86 | 146.84 ± 21.69 | 3.053 | 0.003* |
| HDL (mg/dl) | 69.89 ± 12.09 | 67.78 ± 14.49 | 0.751 | 0.455 |
| LDL (mg/dl) | 78.73 ± 17.74 | 68.51 ± 14.38 | 3.002 | 0.003* |

**t Independent sample t test *p<0.05 is statistically significant**

**SD:** Standard deviation, BMI: Body mass index, FGS: Ferriman Galway Score, FBG: Fasting blood glucose, HDL: High-density lipoprotein, LDL: Low-density lipoprotein.

**Table-2:** Correlation of serum and follicular Germlin-1 with patients' clinical data.

| Parameter | Serum Gremlin-1 | | | |
|---|---|---|---|---|
| | *r-value* | *p-value* | *r-value* | *p-value* |
| Age (year) | -0.006 | 0.959 | -0.198 | 0.062 |
| Weight (kg) | 0.202 | 0.056 | 0.384 | <0.001** |
| Height (cm) | -0.068 | 0.523 | 0.061 | 0.566 |
| BMI (kg/m2) | 0.296 | 0.005* | 0.385 | <0.001** |
| Waist circumference (cm) | 0.157 | 0.139 | 0.288 | 0.006* |
| Hip circumference (cm) | 0.005 | 0.965 | 0.141 | 0.184 |
| Waist/hip ratio | 0.299 | 0.004* | 0.273 | <0.001** |
| Fasting blood glucose(mg/dl) | -0.086 | 0.421 | 0.041 | 0.704 |
| FSH (mIU/mL) | 0.366 | <0.001** | 0.297 | 0.004* |
| LH (mIU/mL) | 0.454 | <0.001** | 0.437 | <0.001** |
| E2 (pg/mL) | 0.362 | <0.001** | 0.101 | 0.344 |
| Total testosterone (ng/dl) | 0.208 | 0.049* | -0.093 | 0.383 |
| Triglycerides (mg/dl) | 0.114 | 0.285 | 0.114 | 0.285 |
| Total cholesterol (mg/dl) | 0.068 | 0.523 | 0.071 | 0.504 |
| HDL (mg/dl) | -0.216 | 0.041* | 0.108 | 0.309 |
| LDL (mg/dl) | 0.374 | <0.001** | 0.01 | 0.924 |
| FGS | 0.191 | 0.071 | 0.271 | 0.01* |

**r Spearman rank correlation coefficient*p<0.05 is statistically significant**

**BMI:** Body mass index, FSH: Follicle stimulating hormone, LH: Luteinizing hormone, E2: Estradiol, HDL: High-density lipoprotein, LDL: Low-density lipoprotein; FGS: Ferriman Galway Score.

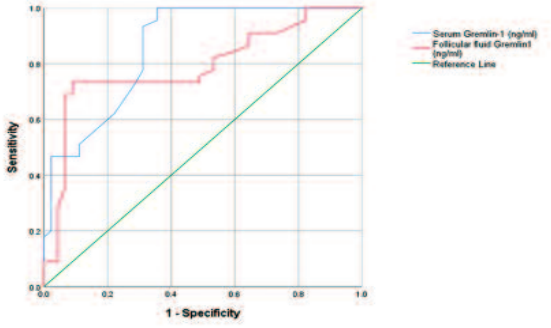


**Figure:** Receiver operating characteristic (ROC) curve showing performance of serum and follicular fluid Gremlin-1 in the diagnosis of polycystic ovary syndrome (PCOS).

Gremlin-1 levels and BMI, WHR, FSH, LH, E2, total testosterone and LDL was found, while HDL, age, weight, height, HC and FGS had significant negative correlations with serum Gremlin-1 ($p<0.05$).

Also, there was a significant positive correlation between follicular Gremlin-1 level and BMI, weight, WC, WHR, FSH, LH and FGS, and a negative correlation between follicular Gremlin-1 and age, height, HC, TC, TG, LDL, HDL, E2 and total testosterone (Table 2).

## Discussion

The production of too much LH by the anterior pituitary gland or hyperinsulinaemia that cause PCOS, stimulates the ovaries to create excessive amounts of male hormones(androgens), mainly testosterone.[6] It is also possible that PCOS runs in the family. There has been a higher incidence in women whose mothers (24-52%) and sisters (32-66%) had PCOS.[7]

Gremlin-1, a BMP extracellular antagonist, blocks BMP signalling by neutralising its ligands.[14,15] Early embryonic development is controlled by a variety of biological processes that are regulated by BMPs and their antagonists.[16-19]

As a consequence, addressing the underlying characteristics in PCOS patients might aid in the prevention of illness or improving therapeutic results. In order to achieve effective reproduction, the primordial follicle transition must be stimulated or inhibited by a variety of growth factors and extracellular signalling molecules. One of the DAN family of BMP inhibitors, Gremlin-1, is a TGF-β superfamily antagonist and has similar bioactivities.[4]

Serum and follicular fluid Gremlin-1 levels varied significantly between the two study groups, and there was a significant association between serum Gremlin-1 and follicular fluid. No prior data on this link could be identified in literature, making the present study unique in this regard.

The current findings are in agreement with Nadiye Koroglu et al.[20] related to the inter-group difference in the levels of Gremlin-1, FGS, LH and FSH/LH.

In the light of the findings, Gremlin-1 seems to be a critical regulator in the aetiology of PCOS. Future research on Gremlin-1 may provide useful information for improving PCOS therapy.

The current study has some limitations as the sample size was too small to allow generalisation of the findings. Also, the sample size was not calculated. Besides, the presence of confounding variables in the study may have led to bias.

Longitudinal, multicentre studies with larger samples are needed to elucidate the clinical implications of the current findings.

## Conclusion

There was a strong correlation between serum and follicular Gremlin-1 levels in PCOS patients, and can be as a useful PCOS diagnostic tool.

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**Conflict of Interest:** None.

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