Estimation the level of Gremlin in Polycystic ovarian syndrome patients

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Abstract. Polycystic ovary syndrome (PCOS) is a common endocrine disorder characterized by chronic ovulation dysfunction and overabundance of androgens; it affects women at reproductive age. The aim of the current study were to detect the analytic estimation of serum Gremlin as well as studying the relation between this biomarker and Body Mass Index (BMI), age, waist /hip ratio and type of infertility in Polycystic ovarian syndrome patients. Methods: The current study was conducted on 90 women (60 patients with polycystic ovary syndrome and 30 healthy control) ranging in their ages from 15-39 years in period from March to June, 2023. Blood samples were collected from women and serum samples prepare to estimate the serum levels of Gremlin. Results: The results indicated that there was a significant increase (P-Value< 0.05) in Gremlin levels in patients group when compare with control group. Also, after dividing the group of patients into three groups according to ages, we notice a significant increase (P-Value< 0.05) in Gremlin levels in age less than 20 years when compare with age 20-29 years and age 30-39 years, and a significant increase (P-Value< 0.05) in Gremlin levels in age 20-29 years when compare with age 30-39 years. On the other hand, when dividing the group of patients according to body mass index we notice a significant decrease (P-Value< 0.05) in Gremlin level in overweight group when compare with obese group and significant decrease (P-Value< 0.05) in Gremlin level in moderate W/H ratio group when compare with high W/H ratio group and no significant difference found between primary and secondary infertility. Conclusion: The current study concluded that a Gremlin is a prognostic marker in early detection of polycystic ovarian syndrome.

1 Introduction

Polycystic ovary syndrome (PCOS) is a common condition affecting up to 13% of reproductive-aged women [1]. It is diagnosed through the European Society for Human Reproduction and Embryology/American Society for Reproductive Medicine (ESRHE/ASRM) criteria, requiring two of the following features: polycystic ovaries on ultrasound, oligo ovulatory or an ovulatory cycles and biochemical or clinical hyperandrogenism [2]. Women with PCOS experience a combination of reproductive (infertility, pregnancy complications) [3, 4], metabolic (risk factors for and conditions of

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type 2 diabetes (T2DM) and cardiovascular disease (CVD)) [5, 6] and psychological (conditions including anxiety, depression, poor quality of life, disordered eating) comorbidities [7, 8]. Gremlin-1 is a secreted bone morphogenetic protein (BMP) antagonist playing a pivotal role in the regulation of tissue formation and embryonic development [9]. Since its first identification in 1997, gremlin-1 has been shown to be a multifunctional factor involved in wound healing, inflammation, cancer and tissue fibrosis [10]. Many growth factors and extracellular signaling molecules have a role in stimulating or preventing the transformation of primordial follicles essential for successful reproduction. One of the Differential screening-selected genes 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 [11]. 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 [12, 13].

2 Methods

2.1 Subject population

Ninety subject's women were concluded in this study, 60 as patient group with polycystic ovarian syndrome and 30 as control group which are apparently healthy women with ages range from [15 – 39] years. The Patient females were collected from private clinic for obstetrics and gynecology in AL-Hilla city were the patients are diagnosed by using of sonograph technique and clinical feature according to 2003 Rotterdam criteria [14] from March to June, 2023. Patients group were divided into subgroups according to age, body mass index, waist /hip ratio and type of infertility.

2.2 Exclusion criteria

Women with chronic diseases such as kidney or liver disease, hyperprolactinemia, diabetes, high blood pressure, or Cushing's syndrome, as well as women taking thyroid hormones or antithyroid medications, were excluded from the study. In addition, women who received hormonal therapy, including oral contraceptive pills, were also excluded.

2.3 Collection of blood samples

Blood samples were drawn from vein by using 5 ml disposable syringe. The samples were emptied in gel tube without of anticoagulant for preparing of serum. Blood was left at room temperature for 10 minutes for clotting then it will be Centrifuge (at 2000-3000 RPM) for 20 minutes, and then serum was separated and freezing at -80 °C until time for performed the laboratory analysis for study.

2.4 Body Mass Index (BMI)

The computation of BMI is performed by electronic balance and height device for calculating the weight and height. The most commonly used definitions, established by the World Health Organization [15] in 1995 and published in 2000, with some modification done in 2004, the equation is: BMI = weight (kg)/height (m2)

2.5 Waist /Hip ratio

The Waist Hip Ratio is calculated by dividing the waist measurement (just above the upper hip bone) by the hip measurement (widest part) by using measuring tape, The formula is: WHR= waist circumference / hip circumference. Ideally, women should have a waist-to-hip ratio of 0.8 or less, whereas men should have a waist-to-hip ratio of 0.95 or less [16].

2.6 Estimation of serum Gremlin concentration

The assessment of serum Gremlin concentration is performed by (Shanghai YL Biont/China) sandwich immunoassay technique (enzyme linked immunosorbent assay – automated microtiter plate), ELIZA reader (Bio kit YLA0195HU)

2.7 Statistical analysis

For statistical analysis, IBM-SPSS statistics 24 was used. T-test, one way ANOVA and Standard Error were applied to test the variability and the statistical significance of this experiment. In addition, effects are considered statistical significant at P value < 0.05 [17, 18].

3 Results

The result in figure (1) showed a significant increase (P value <0.05) in Gremlin concentration of PCOs group (6.07±0.22) ng/ml in comparison with that of control group (1.86±0.11) ng/ml.
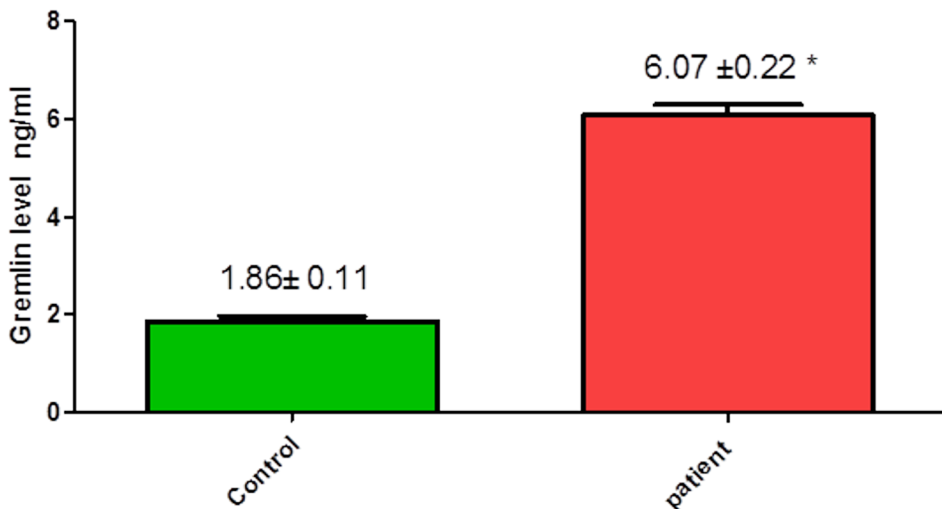
Fig. 1. Gremlin level (ng/ml) in patients group compare with control group. * refer to significant differences (P- Value <0.05) Patients n=60, Control n=30.

The level of Gremlin in three groups of patients as shown in figure (2). The results indicate the presence of a significant increase (P< 0.05) in serum Gremlin in age less than 20 (8.2 ±0.1) ng/ml in comparison with age 20-29 and age 30-39 groups (6.33±0.11) ng/ml

and (4.15±0.18) ng/ml respectively. Also there is a significant increase (P< 0.05) in age 20-29 (6.33±0.11) ng/ml when compare with 30-39 group (4.15±0.18) ng/ml.
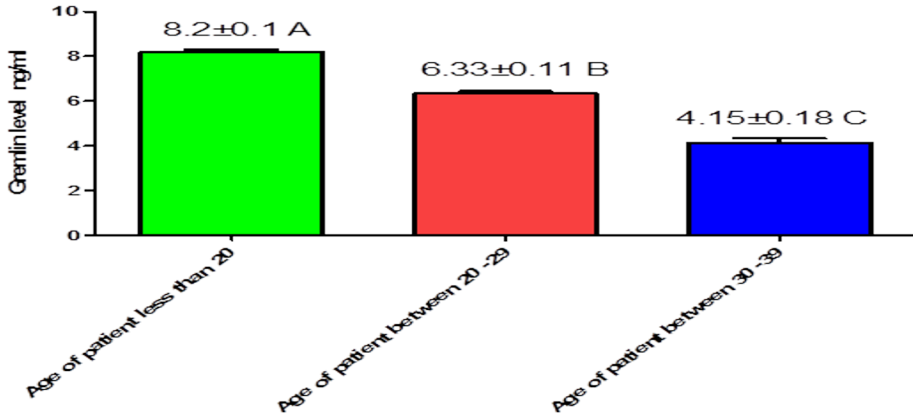

Fig. 2. Level of Gremlin (ng/ml) in age less than 20 , 20-29,30-39 group of patients with poly cystic ovary syndrome .Different letter refer to significant differences (P- Value <0.05). Age less than 20 n=15, 20-29 n=25, 30-39 n=20.

Figure (3) show to presence of significant increase (p<0.05) in Gremlin level in obese group (7.22±0.16) ng/ml as compared to overweight group (4.46±0.19) ng/ml of poly cystic ovary syndrome patients.
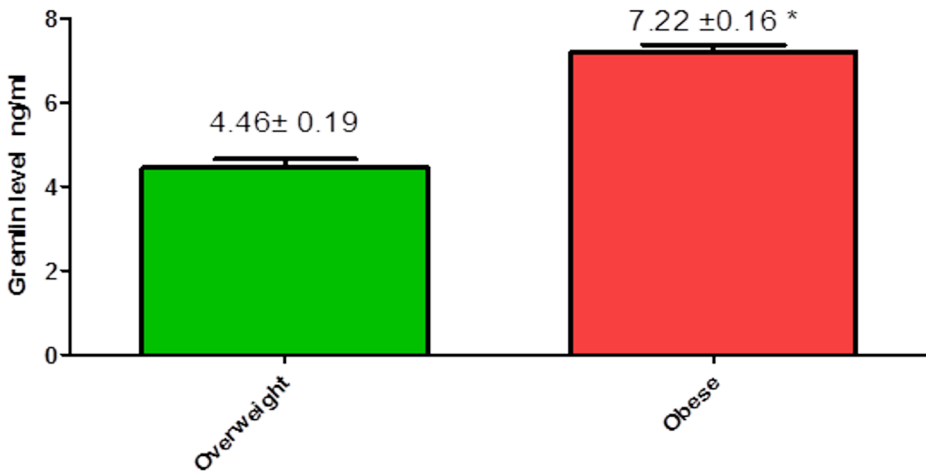
Fig. 3. Gremlin level (ng/ml) in obese group compare with overweight group of poly cystic ovary syndrome patients. * refer to significant differences (P- Value <0.05) obese n=35, overweight n=25.

Figure (4) show to presence of significant increase (p<0.05) in Gremlin level in high waist/hip ratio group (7.03±0.17) ng/ml as compared to moderate waist/hip ratio group (4.16±0.18) ng/ml of poly cystic ovary syndrome patients.
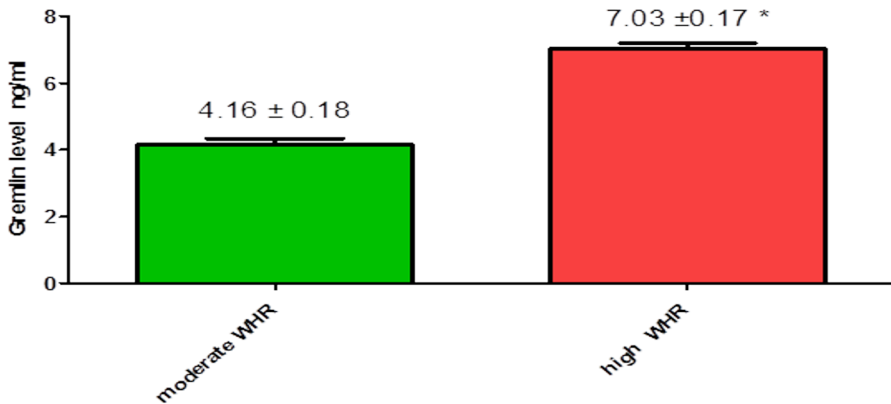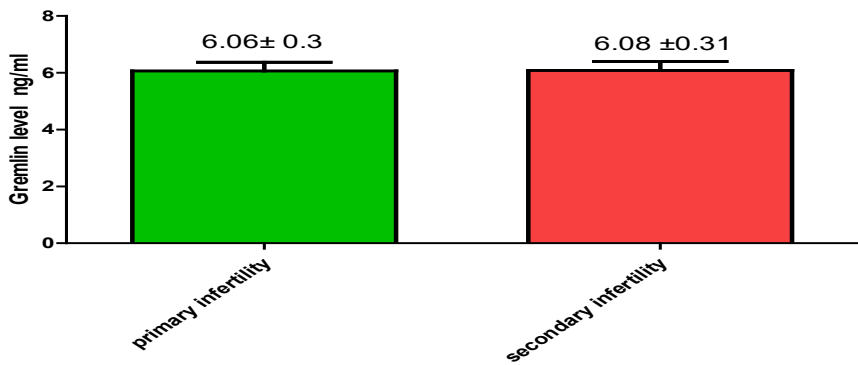
Fig. 4. Gremlin level (ng/ml) in high WHR compare with moderate WHR group of poly cystic ovary syndrome patients. * refer to significant differences (P- Value <0.05) moderate WHR n=20, high WHR n=40.

Figure (5) show no significant difference in Gremlin level in primary infertility group (6.06±0.3) ng/ml as compared to secondary infertility group (6.08±0.31) ng/ml of poly cystic ovary syndrome patients.

Fig. 5. Gremlin level (ng/ml) in primary infertility compare with secondary infertility group of poly cystic ovary syndrome patients. Primary infertility n=30, secondary infertility n=30.

4 Discussion

In figure (1) show a significant increase in gremlin level in polycystic ovarian syndrome and control group. Many studies has been postulated that gremlin is consider as Bone Morphogenic Protein (BMP) inhibitors play important roles in follicles development [19, 20]. Other recent studies has been indicate that many growth factors such as Growth Differentiation Factor (GDF) and Anti-Müllerian Hormone (AMH) initiate theca cell differentiation, follicles cells development and producing of androgen so that gremlin belonging to superfamily transforming growth factor may preventing by high level. A primordial follicles development by binding to BMP and blocking a receptors that responsible for growth factors and influencing on reproduction and fertility of PCOs patients [11,12].

Previous study has been proved strong association between gremlin in serum and follicular gremlin and concluded that a high level in both serum and follicular fluid in

gremlin may act as preventing of follicular development and can be useful for diagnostic markers [21].

Many previous studies has been shown that gremlin has an reversed effect on bone morphogenic protein -4(BMP4) to prevent a stimulation of follicles and both gremlin -1 and 2 were highly expressed in the ovary and play an important roles in transition of primordial to primary follicles [12,22].

Another former study has been shown that gremlin-1 nor gremlin-2 regulate the follicles development ,the other mechanism has been suggested is a role of gremlin-1 as inhibitory effect on AMH after binding [12].

Some immunohistochemical experiment studies has showed a high level of gremlin-1 and 2 in preantral follicles and developing follicles that have multiple layers of granulosa cells and less development, also gremlin -2 was detected with high expression that of grem-1 [12].

Recent studies has been suggested a mechanism of gremlin-1 effects on folliculogenesis by inhibiting of growth differentiation factor and by bone morphogenic protein 2 and showed a high expression of gremlin-1 and gremlin-2.

Many studies has been proved that growth differentiation factor 8 play a crucial role in up regulation of folliculogenesis in human by binding with type 1and 2 serine/ threonine kinase receptors which are more phosphorylate. Therefor gremlin-1 and 2 where known as BMP antagonist which contain cysteine knots compete with BMP2 ligand and inhibiting effect SMADs (SMAD6 and SMAD7) signaling inducing by BMP2 and BMP1 therefor gremlin-1 and 2 have an important roles as antagonist for members of BMP2 activity and GDF8 in human graulosa and leutin cells [23,24,25,26].

Some other studies disagree with present study and showed that global knockout mice after 48 hours of birth has abnormal ovary and delayed in folliculogenesis [27,28], also elevated of gremlin-1 and 2 associated with ovarian maturity [29,30]. Previous studies has been indicated a molecular mechanism of gremlin mediated a suppressive effect on GPF8 and suggested that a given of BMP2 up regulate a gremlin expression then antagonist to BMP2 receptors so that decrease in GPF8 that and SMAD signaling leading to decrease in folliculogenesis in ovarian human [31, 32, 33].

The gremlin-1 has crucial roles in PCOs women patients in present study by a signaling pathway mediate between BMP2 and BMP4 and suppressive effect on GDF8 then depletion in SMAD signaling and effect on follicles development lead to infertility in PCOs women.

The current results show a significant increase in gremlin-1 in ages less than 20 years than other (figure 2), no previous studies has been postulated the level of gremlin-1 in all ages of present study in PCOs patient's women. The explanation may be discussed depend on high up regulation gremlin in ages less than 20 also (20-29) years in compare with ages (30-39) years in which the binding of gremlin-1 on BMP receptors or affinity for gremlin-1 to these receptors are very high therefor antagonistic effect on a pathway in female ovary especially on granulosa or theca cell are highly inhibitor for SMAD pathway and GDF8 therefor a gremlin level is higher than significantly in ages 20 years than other.

The current results revealed that significant increase in gremlin-1 level in obese patient women than overweight in PCOs women, recent reviews has been showed that a central roles of gremlin-1 in adipose tissue homeostasis leading to dysfunction in obesity, diabetes and fatty liver disease than other [32].

Also another recent study has been indicated a positive correlation between gremlin-1 and obesity and with fat mass [35].

High gremlin level in ages less 20 years may be discuss as positive relation of gremlin-1 with cytokines secreted by adipose tissue such as adipokines and these adipokines associated with increase fat mass and obesity also with insulin resistance [36,37].

A significant increase in high W/H ratio in compare with moderate in PCOs women as in figure (4), no previous studies about gremlin-1 level with W/H ratio therefor the same explanation as in BMI (obesity) (figure 3) may be explained because a W/H ratio causes a central obesity may be related with a mechanism of gremlin binding antagonists for BMP and suppression of growth differentiation factor. The figure (5) showed a no significant difference between primary and secondary infertility in PCOs patients.

5 Conclusion

The current study concluded that Gremlin can be considering as a prognostic marker for detection of polycystic ovarian syndrome.

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