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Role of kisspeptin in polycystic ovary disease in patients of ALNajaf ALAshrif City, Iraq

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Abstract

The aim of the study was to arrive at some biochemical and hormonal evidence predicting ovarian polycystic ovaries in women with primary or secondary infertility.

Samples were collected at the fertility center laboratories in Sadr Medical City, Najaf, Iraq. The blood was taken for complete blood cell (CBC) testing on the samples . Samples were taken 88 samples (70 of which were samples of women with polycystic ovaries and 18 sample of control women) . A serum was then taken for biochemical tests, The hormones LH, FSH, prolactin, Estradiol, Kisspeptin1, Interleukin 37 were measured by ELISA method while lipid profile and total protein by spectrophotometer method.

The result showed a significant increase ($p<0.05$) of Kisspeptin-1 ,Prolactin, cholesterol ,triglyceride ,low density lipoprotein ,very low density lipoprotein, total protein and RBC in women with polycystic ovary disease compared with control group, While significant decrease ($p<0.05$) of high density lipoprotein and Interleukin-37 in women with polycystic disease compared with control group. And the results showed non-significant ($p > 0.05$) in both Luteinizing hormone, Follicle stimulation hormone, Estradiol hormone, platelet, WBC.

The significant increase in the level of Kisspeptin and some of the biochemical and physiological parameters measured in the present study may provide an indicator of increased infertility in women with ovarian disease.

Keywords: Infertility, kisspeptin, IL-37,cholestol, TG,HDL,LDL,VLDL, total protein,LH,FSH,E2, prolactin.

INTRODUCTION

Infertility is a typical disease in the reproductive system. This disease is known as the inability to deliver healthy one year after birth effectively in unprotected free endeavors [1].About 15% of couples on the planet have a problem and are facing failure Initial involvement in pregnancy. Therefore, these husbands can be interpreted as sinners [2]. The infertility in the men may be produced from the reduce the interleukin 17 level and reduced expression of CatSper1 protein in spermatozoa [3,4]. Polycystic ovary is a disorder in women, where the most prominent features of this disease is the increase of the hormone Androgen, problems in the process of ovulation, and the menstrual cycle irregular, and/or cysts in the ovaries or both, [5] and this disease prevents the development of follicle, a problem in ovulation, microcyte in the ovaries, and changes in the menstrual cycle, PCOS Affects more than 7% of adult women[6]. According to the Office of the National Institutes of Health and Disease Prevention, PCOS regularly affects 5 million women of reproductive age in the United States [7]. Research also suggests that women aged between 18-44 years old by 5% There will be among to 10% of infected with PCOS, making it the most common endocrine glands problems among Women in the period of fertility in the United States[8]. PCOS is often diagnosed in women who they have problems with obesity, acne, menopause or delayed menstruation, excessive hair growth, infertility, and women with polycystic ovaries have higher rates of endometrial cancer, cardiovascular disease, dyslipidemia, and type of diabetes than healthy women [9,10]. Kisspeptin (formerly known as metastatin) is a protein that is encoded by the KISS1 gene in humans. Kisspeptin is a protein that binds G-GPR54 protein receptors[11]. Kiss1 was originally identified to be of major importance in inhibiting the human genome causing malignant tumors, thus this property has the ability to suppress melanoma and malignant breast cancer, The gene kiss 1 is located on chromosome 1[12]. It is copied into the brain, adrenal gland, and pancreas, and is very expressive during gestation. In the early placenta, Kisspeptin and its receptor were also present at different sites in the kidneys, including in the blood vessels, as well as the smooth vascular muscles and renal tubular cells[13]. kisspeptin-GPR-54 signals play an important role in the initiation of GnRH in adulthood, puberty can also be affected by a range of environmental factors, and is known to be affected by the metabolic capacity of the person[14.] The secretion hormone is released from the hypothalamus to work on the anterior pituitary gland, stimulating the release of LH and follicle stimulating hormone (FSH)[15]. These hormones also lead to decreased sexual maturity and problems of the formation of gametes, and therefore we studied in this research the effect of infertility and infertility problems ovulation. [16] . IL-37 is a newly discovered protein belong to Interleukin-1 family member 7, This protein encoded by this gene is a member of the interleukin 1 cytokine family[17]. This cytokine can bind to and maybe a ligand for interleukin 18 receptor (IL18R1/IL-1Rrp), IL-1 cytokine interleukin 37 (IL-37) acts as a natural tonic for fungal infections and acquired immunity, Low IL-37 levels increase the production of cytokines induced by transient-like receptors (TLR) in human monocytes[18]. In addition, human IL-37 recombination into wild-type mice also suppresses inflammatory cytokines and inhibits excessive inflammation, for example in arthritis[19]. Recombinant IL-37 inhibits gene expression of NLRP3 and IL-1β after acute pneumonia, Recombinant IL-37 also increases insulin sensitivity in obesity induced by diet, [20] IL-37 also showed an important effect in curbing cancer cells in renal cell carcinoma and cervical cancer , Some studies have shown that IL-37 can inhibit cancer cell migration, proliferation, and cell proliferation on apoptosis perhaps by blocking the expression STAT3 and phosphorylation , For this reason, we used the IL-37 to find out the effect of the disease on the body's immunity and its relation to infertility and can be considered a diagnostic evidence of the disease [21,22].

MATERIALS AND METHODS

Blood and serum samples were collected from Infertility women with primary and secondary infertility disease in addition To control group (healthy women) who attended Fertility Center. The average age of infertility patients was 26.97 ± 0.69 years. All samples 215 were collected (Samples with interference to other diseases were excluded) and The samples that were tested 88. Control group (fertile and healthy women) were obtained from 18 samples and 70 samples were patients. After the hormones (LH ,FSH, Prolactin and Estradiol) and biomarker(Kisspeptin 1) were measured by using ELISA method (Huma Germany origin), while lipid profile and total protein were measured by using a spectrophotometer. Tests were conducted in laboratories of Department / Faculty of Science / University of Kufa. ELISA kits used in this study were LH (LH231F), FSH (FS232F) , Prolactin (PR234F) and Estradiol (ES180S) (CALBIOTECH company USA in Origin) and human Kisspeptin-1 (E-EL-H2129). While lipid profile and total protein kits was BIOLABO company French manufacturer of Reagents for Medical Biology.

Statistical analysis:

Statistical analyses of all result were carried out by the help of Graphpad prism version 5)software statistical package using t-test (with p value at level of significant less than 0.05) to compare values of result between groups . Result values were expressed as mean ± SE , number of patient , or percentage.

Table 1: levels of lipid and biochemical and hormonal markers in patient with PCOs compare with control.

Subject	Patient	Control	P –value (p< 0.05)
Cholesterol(mg/dl)	191.4 ± 3.970	140.6 ± 3.532	Significant
Triglyceride (mg/dl)	145.7 ± 3.985	98.50 ± 5.766	Significant
LDL (mg/dl)	123.2 ± 3.928	71.58 ± 3.735	Significant
VHDL (mg/dl)	29.09 ± 0.7955	19.70 ± 1.153	Significant
HDL (mg/dl)	40.90 ± 0.9307	49.33 ± 1.605	Significant
Hb (g/dl)	12.13 ± 0.1429	11.48 ± 0.1443	No significant
RBC (X 10^12/L)	4.336 ± 0.0510	3.987 ± 0.0762	Significant
LH (mlu/ml)	4.598 ± 0.5028	3.776 ± 0.3437	No significant
FSH(mlu/ml)	5.507 ± 0.7796	3.188 ± 0.2313	No significant
E2(pg/ml)	38.44 ± 3.360	34.14 ± 7.147	No significant
Prolactin (ng/ml)	25.41 ± 3.599	10.97 ± 0.7644	significant

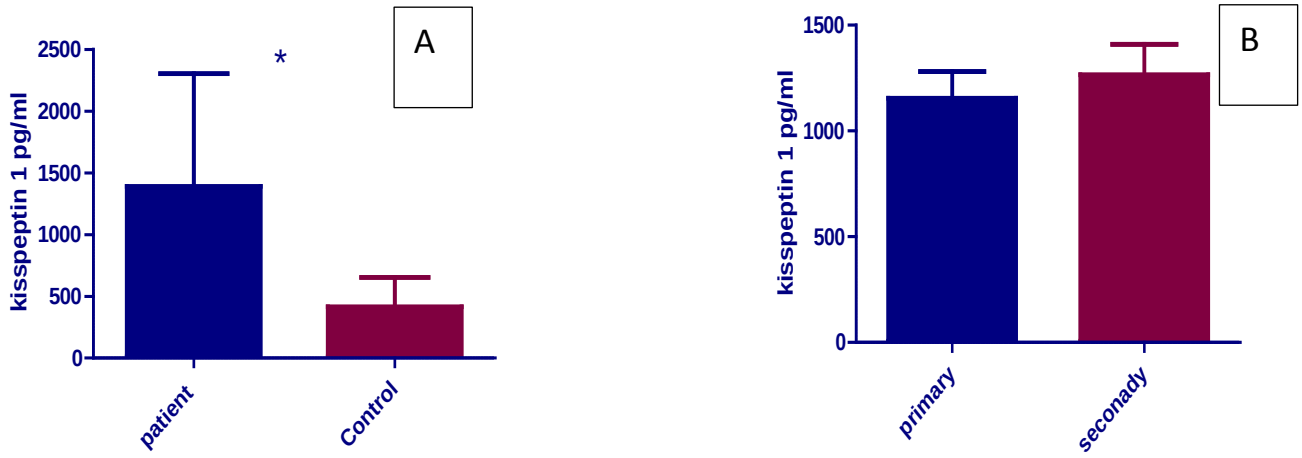

Figure 1: level of Kisspeptin in women with PCOs:

(A) Showed compare between patient and healthy : * significant difference ($p < 0.05$).

(B) Showed compare between type of PCOs disease (primary and secondary)

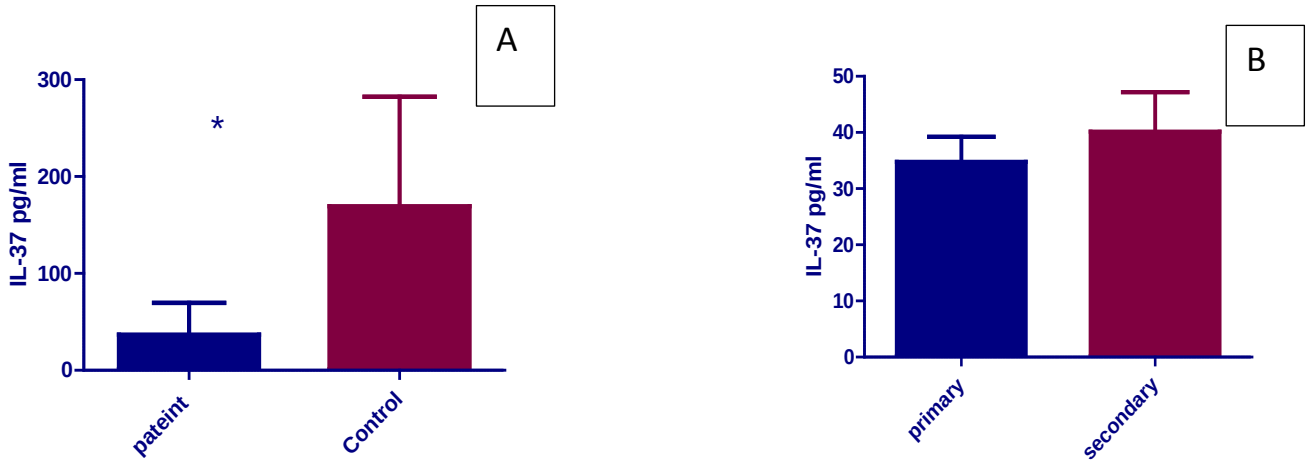

Figure 2: level of IL-37 in women with PCOs:

(A) Showed compare between patient and healthy : * significant difference ($p < 0.05$).

(B) Showed compare between type of PCOs disease (primary and secondary)

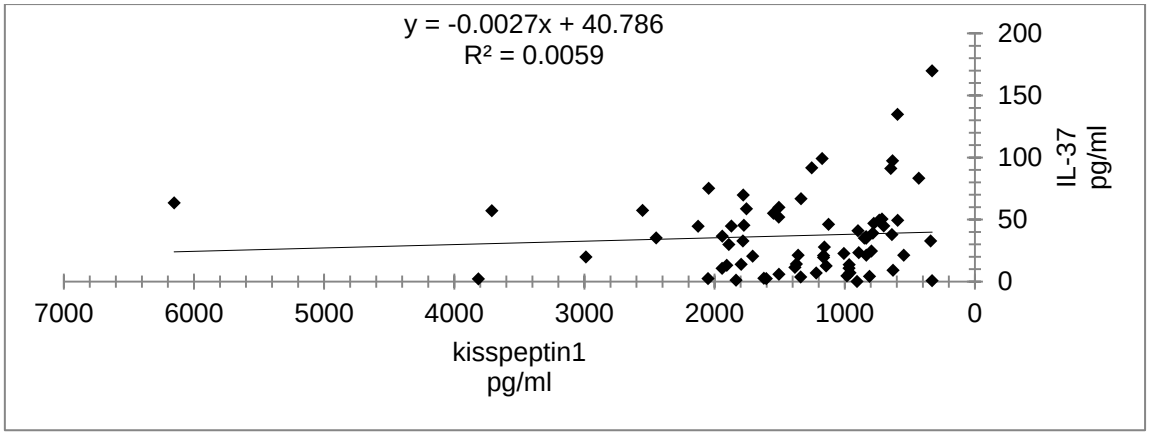

Figure 4: The correlation between kisspeptin-1 and IL-37 in women with polycystic ovary.

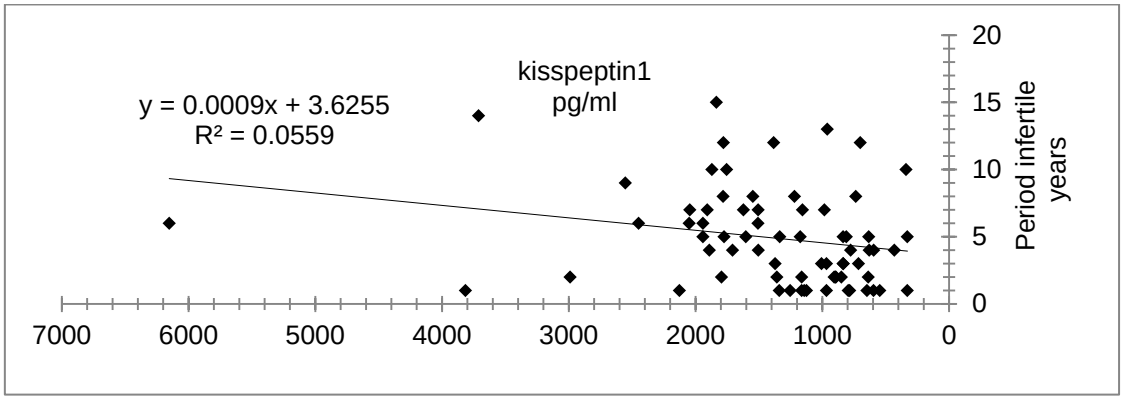

Figure 5: The correlation between kisspeptin-1 and fertility period in women with polycystic ovary.

RESULTS

The result showed a significant increase ($p<0.05$) of Kisspeptin in patients infertile women with polycystic ovary disease (1390 pg/ml ± 109.1) compared with control group (415.3pg/ml ± 56.06) showed in figure 1 A. The result showed a significant decrease ($p<0.05$) of IL-37 in patients infertile women with polycystic ovary disease (36.99 pg/ml ± 3.891) compared with control group (169.3 pg/ml ± 26.65) showed in figure 2A. The result in kisspeptin and IL-37 showed slightly increased in secondary infertility when compare with primary infertility but no significant ($p>0.05$) between them showed in figures (1B)(2B). The result showed a significant increase ($p<0.05$) of Prolactin hormone in patients infertile women with polycystic ovary disease (25.41 ng/ml ± 3.599) compared with control group (10.97 ng/ml ± 0.7644) ,but no significant ($p>0.05$) between primary and secondary infertility . While no significant in LH ,FSH, E2 between patient and control , the result showed a significant increase ($p<0.05$) of cholesterol, TG, LDL, VLDL and total protein in primary and secondary infertile women with polycystic ovary disease compared with control group. Also the result showed a significant decrease ($p<0.05$) of HDL in patients infertile women with polycystic ovary disease compared with control group, showed in table (1) .The result in lipid profile(TG ,cholesterol, LDL,VLDL) showed slightly increased in secondary infertility when compare with primary infertility while in HDL slightly decrease in secondary infertility when compare with primary infertility but no significant between them. Also the result showed significant increase ($p<0.05$) in Hb (12.13 ± 0.1429) and RBC (4.336 ± 0.0510) in women with polycystic ovary disease when compare with controls (11.48 ± 0.1443,3.987 ± 0.0762respectively), while no significant ($p> 0.05$) in WBC between patient and control. The result showed negative correlation between kisspeptin and IL-39 (figure 4) ,while positive correlation between kisspeptin and periods infertility (figure 5).

DISCUSSION

In the current study, the level of Kisspeptin-1 has a significant increase ($p<0.05$) in the serum of women with polycystic ovary disease compared with fertile women. The potential effect of kisspeptin in direct control of ovarian function may indicate that deregulation of the ovarian KISS1 system may participate in the ovarian phenotype, including ovulatory dysfunction and cystic ovaries (23). Gonadotropins control the ovarian expression of KISS1 as positively (24).On the other hand, local mediators also play important role in the control of ovarian KISS1 expression. All those observing suggest a possible role of locally produced kisspeptin on ovulation control. further, kisspeptin derived from ovaries have been newly suggested to play role in the adjustment of gonadotropin secretion (25,26).The study appeared that serum kisspeptin levels were significantly higher in women with PCO, the correlation between kisspeptin concentrations and serum FSH level were negatively correlated, this study agrees with Umit Gorkem (27). Other study showed The levels of kisspeptin were high in PCO disease and these levels had a positive relationship with LH levels. There are some data related to changes in kisspeptin levels and its relationship to metabolic and hormonal disorders in PCO disease (28). Some other studies confirmed higher kisspeptin levels in women with PCOS, in agreement with this study (29,30). Present study also understand that women with PCOS have higher levels of LH than ovulation without PCOS, this agree with Umit Gorkem (27).however, there is a need for other studies to obvious whether this phenomenon might play a role in the deregulation of gonadotropin secretion in PCOS. It appears possible that the human female kisspeptin system could

be a helpful therapeutic target in PCOS patients, Experimental studies have proposed that the expression hypothalamus KISS1 was sensitive to variation in the steroid environment and metabolism (23).On a functional level, sexual dimorphism is fully characterized by the menstrual cycle. LH surge is the more significant characteristic of this. Normally, the positive feedback of estrogen leading to create LH-peak. Kisspeptin relays this influence to the GnRH neurons, and thus to the LH cells (31), in our data showed higher kisspeptin1 in women with secondary polycystic ovary disease when compare with primary polycystic ovary disease. In the current study, the level of interleukin 37 has a significant increase ($p<0.05$) in the serum of women with polycystic ovary disease compared with fertile women. , IL-37 protein is present fundamentally in the cytoplasm of peripheral blood mononuclear cells (PBMC) and constitutively at low levels in normal people and can be upregulated by cytokines and inflammatory stimuli (32). The data in the present study confirm that PCOS is a proinflammatory state, IL-37 are recently discovered anti-inflammatory cytokines (33,34). Levels of cytokines (IL-37) were significantly decreased in obese and non-obese PCOS patients compared with obese and non-obese controls. These findings seem to be in line with the case that PCOS is a proinflammatory state because there shows to be an imbalance between pro and anti-inflammatory mediators in patients with PCOS. However, the increase in pro-inflammatory cytokines and referred to obesity, because there appears to be a strong association between inflammation and PCOS. Hence, inflammation seems to be affected both by obesity and PCOS. (35) , Although IL-37 is newly discovered from IL-1 family and according to results and studies it has been shown to decrease in women with PCOS,there is an inverse association with IL-1, according to studies, indicating that IL-1 is elevated in women with PCOS(36).Results of present study showed significant elevation in the serum level of LH ($p<0.0001$) and significant decline in the serum level of FSH ($p<0.05$) in infertile women with PCO in comparison with control group. Study the changes in the levels of LH (elevation) and FSH (decline) in PCO were recorded by many studies and considered as a characteristic feature of PCO (37,38).Low levels of gonadotropin can be refer to the increased production of androgen in PCO .Increasing the level of LH may directly promote the synthesis of androgen. However, it has been suggested that the height of LH levels result from weak negative reactions to LH secretion, due to excessive androgen action on the pituitary axis (39). Our results showed significant elevation in the serum level of LH ($p<0.0001$) and significant decline in the serum level of FSH ($p<0.05$) in infertile women with PCO in comparison with control group .In this study The frequency of hyperprolactinemia in PCO has increased in comparison with control. When a single blood sample was taken from the patient, 40% of PCO patients had hyperprolactinemia blood (40).Further, this study data indicate that hyperprolactinemia in PCO patients reflects just increased estrogen uptake since they have demonstrated a gradual increase in circulating estrogen levels. Prolactin Basically, this phenomenon is related to the increase in estrogen alone, since it was not possible to show statistically correct variations of estradiol. Taking into account that estrogen stimulates the Prolactin secretion (41), it can be assumed that hyperprolactinemia was found in patients who have greater secretion of estrogens, especially esters. Further , our data don't allow us to determine the development of estrogen in such patients because estrogen is largely the product of peripheral androstenone conversion. Which can be produced from the ovary and adrenal glands (42). In our data no highly significant between primary and secondary polycystic disease .In many study to note the effects of polycystic ovaries on the lipid profile, total cholesterol, TG, and LDL levels were statistically higher, in our data higher in secondary polycystic disease compare with primary polycystic disease , and HDL was lower in PCOS patients than healthy females with close proximity to age groups, in our data lower in secondary polycystic disease compare with primary polycystic disease. In another study similar to those observed in PCOS patients. Furthermore, similar results were found in some studies that PCOS patients were had high level in lipid profile such as high total cholesterol, LDL, TGs concentrations and low levels of HDL control (43,44,45) These studies are consistent with our results. Our results showed a significant elevation in the serum level of viscosity ($p<0.05$) in infertile women with PCO in comparison with the control group, Increased viscosity of blood observed in young women with PCOS is clearly an early detection marker that contributes to cardiovascular risk (46). The study concluded that the length of the period of infertility, the significant increase in the level of kisspeptin and some of the biochemical and physiological parameters measured in this study may provide an indication of increased infertility in women with ovarian disease.

REFERENCES

1. World Health Organization WHO. Towards more objectivity in diagnosis and management of male infertility, Int. J. Androl. 2012.
2. Haider, A.; Fauzdar, A. and Kumar, A. Serum inhibin B and follicle-stimulating hormone levels as markers in the evaluation of azoospermic men: a comparison. 2010.
3. AL-Msaid, H. and AL-Sallami ,A. Study the Level of Cytokine in Unexplained and Idiopathic Infertile Men. J. Pharm. Sci. & Res.; 2018, Vol. 10(4): 808-811.
4. AL-Msaid, H. and AL-Sallami ,A. Study of Catsper1 Protein Levels in Unexplained and Idiopathic Infertile Men. International Journal of Pharmaceutical Quality Assurance ; 2018, 9(2) : 195-198.
5. Christopher R. McCartney, M.D., and John C. Marshall, M.B., Ch.B., M.D. Polycystic Ovary Syndrome, the New England Journal of Medicine. 2016.
6. Umland EM, Weinstein LC, Buchanan EM.,Menstruation-related disorders. In: DiPiro JT, Talbert RL, Yee GC, et al., editors. Pharmacotherapy:A Pathophysiologic Approach. 8th ed. New York: McGraw-Hill. 2011.
7. Lin LH, Baracat MC, Gustavo AR, et al. Androgen receptor gene polymorphism and polycystic ovary syndrome. Int J Gynaecol Obstet. 2013.
8. American Congress of Obstetricians and Gynecologists., Polycystic Ovary Syndrome. ACOG Practice Bulletin No. 108: Obstet Gynecol. 2009.
9. National Institutes of Health Department of Health and Human Services, Polycystic Ovary Syndrome (PCOS) NIH ,Beyond Infertility:Pub. 2013.
10. McFarland C. Treating polycystic ovary syndrome and infertility. MCN Am J Matern Child Nurs. 2012,
11. Messager S, Chatzidaki EE, Ma D, Hendrick AG, Zahn D, Dixon J, Thresher RR, Malinge I, Lomet D, Carlton MB, Colledge WH, Caraty A, Aparicio SA Kisspeptin directly stimulates gonadotropin-releasing hormone release via G protein-coupled receptor 54. Proceedings of the National Academy of Sciences of the United States of America. 2005.
12. Lee JH, Miele ME, Hicks DJ, Phillips KK, Trent JM, Weissman BE, Welch DR KiSS-1, a novel human malignant melanoma metastasis-suppressor gene. Journal of the National Cancer Institute. 1996.
13. Skorupskaite K, George JT, Anderson RA The kisspeptin-GnRH pathway in human reproductive health and disease. Human Reproduction Update. 2014.
14. Mead EJ, Maguire JJ, Kuc RE, Davenport AP Kisspeptins: a multifunctional peptide system with a role in reproduction, cancer and the cardiovascular system. British Journal of Pharmacology. 2007.
15. Rhie YJ Kisspeptin/G protein-coupled receptor-54 system as an essential gatekeeper of pubertal development. Annals of Pediatric Endocrinology & Metabolism. 2013.
16. Bhattacharya M, Babwah AV Kisspeptin: beyond the brain. Endocrinology. 2015.

17. Charles A. Dinarello ,Claudia Nold-Petry ,Marcel Nold ,Mayumi Fujita ,Suzhao Li ,Soohyun Kim ,Philip Bufler. Suppression of innate inflammation and immunity by interleukin-37. Eur J Immunol 2016, 46(5):1067–1081
18. Giulio Cavalli Marije Koenders Vassili Kalabokis Jihye Kim Aik Choon Tan Cecilia Garlanda Alberto Mantovani Lorenzo Dagna Leo A. B. Joosten Charles A. Dinarello. Treating experimental arthritis with the innate immune inhibitor interleukin-37 reduces joint and systemic inflammation. Rheumatology (Oxford) 2016, 55(12):2220–2229.
19. Moretti S, Bozza S, Oikonomou V, Renga G, Casagrande A, Iannitti RG, Puccetti M, Garlanda C, Kim S, Li S, van de Veerdonk FL, Dinarello CA, Romani L . IL-37 inhibits inflammasome activation and disease severity in murine aspergillosis. PLoS Pathog 2014, 10(11):e1004462.
20. Nold-Petry CA, Lo CY, Rudloff I, Elgass KD, Li S, Gantier MP, Lotz-Havla AS, Gersting SW, Cho SX, Lao JC, Ellisdon AM, Rotter B, Azam T, Mangan NE, Rossello FJ, Whisstock JC, Bufler P, Garlanda C, Mantovani A, Dinarello CA, Nold MF IL-37 requires the receptors IL-18Rα and IL-1R8 (SIGIRR) to carry out its multifaceted anti-inflammatory program upon innate signal transduction. Nat Immunol 2015, 16(4):354–365.
21. Yazhuo Jiang ,Yili Wang, Liang Liang ,Yang Gao, Juan Chen ,Yi Sun ,Yongyi Cheng, Yonggang Xu. “IL-37 mediates the antitumor activity in renal cell carcinoma,” Medical Oncology, 2015, vol. 32, no. 11.
22. Sen Wang, Weifang An, Yunhong Yao, Renhuai Chen, Xiaoxuan Zheng, Wanyong Yang, Yi Zhao, Xinrong Hu, Enping Jiang, Yanhong Bie, Zhangquan Chen, Ping Ouyang,He Zhang,and Hui Xiong . “Interleukin 37 expression inhibits STAT3 to suppress the proliferation and invasion of human cervical cancer cells,” Journal of Cancer, 2015, vol. 6, no. 10, pp. 962–969.
23. Witchel SF, Tena-Sempere M. The Kiss1 system and polycystic ovary syndrome: lessons from physiology and putative pathophysiologic implications. Fertil Steril. 2013.
24. Castellano JM, Gaytan M, Roa J, et al. Expression of KiSS-1 in rat ovary: putative local regulator of ovulation? Endocrinology. 2006.
25. Balasch J, Fabregues F, Carmona F, et al. Ovarian luteinizing hormone priming preceding follicle-stimulating hormone stimulation: clinical and endocrine effects in women with long-term hypogonadotropic hypogonadism. J Clin Endocrinol Metab. 2009.
26. Gaytan F, Gaytan M, Castellano JM, et al. KiSS-1 in the mammalian ovary: distribution of kisspeptin in human and marmoset and alterations in KiSS-1 mRNA levels in a rat model of ovulatory dysfunction. Am J Physiol Endocrinol Metab. 2009.
27. Umit Gorkem, Cihan Togrul, Emine Arslan, Ayla Sargin Oruc & Nuriye Buyukkayaci Duman Is there a role for kisspeptin in pathogenesis of polycystic ovary syndrome?, Gynecological Endocrinology. 2018.
28. Ozlen Emekci Ozay, Ali Cenk Ozay, Berrin Acar, Erkan Cagliyan, Mustafa Seçil & Tuncay Küme: Role of kisspeptin in polycystic ovary syndrome. 2016.
29. Chen X, Mo Y, Li Y, Yang D. Increased plasma metastin levels in adolescent women with polycystic ovary syndrome. Eur J Obstet Gynecol Reprod Biol. 2010.
30. Jeon YE, Lee KE, Jung JA, et al. Kisspeptin, leptin, and retinol-binding protein 4 in women with polycystic ovary syndrome. Gynecol Obstet. 2013.
31. kauffman, a. S. Coming of age in the kisspeptin era: sex differences, development, and puberty. Molecular and cellular endocrinology. 2010.
32. Kumar S., Hanning C. R., Brigham-Burke M. R. et al., Interleukin-1F7b (IL-1H4/IL-1F7) is processed by caspase-1 and mature IL-1F7b binds to the IL-18 receptor but does not induce IFN-γ production,” Cytokine, 2002, .vol. 18, no. 2, pp. 61–71.
33. Collison LW, Workman CJ, Kuo TT, et al. The inhibitory cytokine IL-35 contributes to regulatory T-cell function. Nature; 2007, .450: 566–9.
34. Nold, M.F., Nold-Petry, C.A., Zepp, J.A., Palmer, B.E., Bufler, P., Dinarello, C.A., IL37 is a fundamental inhibitor of innate immunity. Nat. Immunol. 2010, 11, 1014–1022.
35. Asli N., Ercan B., Irem D., Huri B., Murat D., and Faruk B., Relationship between hyperandrogenism, obesity, inflammation and polycystic ovary syndrome. Gynecol Endocrinol,: 2016, 1,5
36. Farideh Zafari Zangeneh1 Ph.D., Mohammad Mehdi Naghizadeh2 M.Sc., Masoumeh Masoumi1 M.Sc. Polycystic ovary syndrome and circulating inflammatory markers .International Journal of Reproductive BioMedicine, 2017, Vol. 15. No. 6. pp: 375-382
37. Diamanti, K.E.; Baillargeon, J.P.; Iuorno, M.; Jakubowicz, D. and Nestler, J. A modern medical quandary: polycystic ovary syndrome, insulin resistance and oral contraceptive pills. J Clin Endoc Metab . 2003.
38. Farideh, Z. ; Bagher, M. ; Ashraf, A. ; Akram ,A., and Kazem, M. Effects of chamomile extract on biochemical and clinical parameters in a rat model of polycystic ovary syndrome. J Reprod Infertil. 2010.
39. Jonard, S. and Dewailly, D. The follicular excess in polycystic ovaries, due to intraovarian hyperandrogenism, may be the main culprit for the follicular arrest. Hum Reprod Update. 2004..
40. Goldzieher JW, Dozier TS, Smith KE, Steinberger E., Improving the diagnostic reliability of rapidly fluctuating plasma hormone levels by optimized multiple-sampling techniques. J Clin Endocrinol Metab. 1976.
41. Yen S S C, Ehara Y & Siler J M Augmentation of prolactin secretion by estrogen in hypogonadal women.J Clin. 1974.
42. Hatch R, Rosenfield R L, Kim M H & Tredway D Hirsutismimplications, etiology and management. Am J Obstet Gynecol. 1981.
43. Orio F Jr, Palomba S, Spineli L, Cascella T, Tauchmanova L, Zullo F, et al. , The cardiovascular risk of young women with polycystic ovary syndrome: an observational, analytical prospective case-control study. J Clin Endocrinol Metab. 2004.
44. Valkenburg O, Steegers-Theunissen RP, Smedts HP, Dallinga- Thie GM, Fauser BC, Westerveld EH, et al. A more atherogenic serum lipoprotein profile is present in women with polycystic ovary syndrome: a case-control study. J Clin Endocrinol. 2008.
45. Manjunatha S, Amruta S Bennal, Shaktiprasad Hiremath, Veena H C. Effect of PCOS on Lipid Profile. Scholars Journal of Applied Medical Sciences (SJAMS).Sch. J. 2014.
46. Sloop G, Holsworth RE Jr., Weidman JJ, St Cyr JA. The role of chronic hyperviscosity in vascular disease. Therapeutic advances in cardiovascular disease.; 2015, 9(1):19–25.