** زانستی**

**Ministry of Higher Education &**

**وه‌زاره‌تی خوێندنی باڵا و تۆێژینه‌وه‌ی Scientific Research**

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| **پرۆپۆزەلى توێژینه‌وه‌ بۆ به‌ده‌ستهێنانی بروانامه‌ی دکتۆرا PhD Research Proposal** | | |
| **ناونيشانی پرۆپۆزه‌لی تۆێژینه‌وه‌ی پێشنیازکراو 1. Title of PhD research proposal**  **Association between Insulin Resistance, Serum Interleukins, gene polymorphism and obesity in Polycystic Ovary Syndrome Women Kurdish Patients** | | |
| **زانیاری گشتی 2. General information** | | |
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| **3. Summary (Abstract) of PhD research proposal**    Polycystic ovarian syndrome (PCOS) is the most prevalent endocrinopathy of reproductive years. Salient features in presentation of patients PCOS include menstrual dysfunction, hyperandrogenism and/or polycystic appearance of ovaries on ultrasound. While the diagnosis of PCOS depends on presence of specified criteria, misdiagnoses are common. Despite years of extensive research, the exact etiology of PCOS remains largely unknown. In the past decade, apart from insulin resistance and hyperandrogenemia, anti-mullerian hormone (AMH), an important marker of ovarian reserve, and vascular endothelial growth factor (VEGF), a crucial factor in angiogenesis, have been examined as plausible players of causative relevance for PCOS. Vitamin D, a sex-steroid hormone that is universally known for its relevance for skeletal health, has received increasing attention due to growing evidence supporting its pivotal in reproductive physiology and in PCOS.  There is evidence that PCOS is also a proinflammatory disorder, characterized by the presence of chronic low-grade inflammation and there is increased level of several inflammatory cytokines that associated with IR and Obesity such as IL-18, IL-17, TNF-a which stimulate the synthesis of IL-6 and IL-6 adjust the synthesis of C-reactive protein (CRP) within the liver | | |
| **4.Introduction**  Polycystic ovarian syndrome (PCOS) is a complex, enigmatic, and common disease. It is the most common endocrinopathy faced by reproductive-aged women, and affects up to 1 in 5 women. Women frequently have anovulatory menstrual cycles, hirsutism, obesity, and are at risk for diabetes mellitus, hypertension, lipid abnormalities, sleep disorders, depression, and metabolic syndrome. Persistent anovulation also increases risk of endometrial cancer.  The exact mechanism of PCOS development is not yet completely elucidated,  however, there are several hallmarks of abnormal function in women with PCOS.  Pathophysiological abnormalities in gonadotropin secretion, and ovarian folliculo-genesis are well-known, however, steroidogenesis, abnormal or impaired insulin secretion or action, and abnormal adipose tissue function, have also been described in PCOS. In the hypothalamus and pituitary, women with PCOS have increased gonadotrophin secretion of luteinizing hormone (LH), as well as increased LH pulse amplitude and frequency, increased LH pulses and overall increased daytime secretion of LH is observed early during puberty in girls with hyperandrogenism, which may indicate that abnormalities of LH may be a primary defect in PCOS. This increased LH secretion leads to stimulate increased androgen production in the ovarian theca cells, which leads to hyperandrogenism in these females. Follicles within the ovary have also been noted to have increased resistance to follicle stimulating hormone (FSH), which may contribute to the pathophysiology of PCOS. Ultimately in most women with PCOS, LH to FSH ratios is inverted from normal, with LH increasing, usually 3 times that of FSH. In addition, the ovaries excrete high levels of anti-Mullerian hormone, a glycoprotein made in granulosa cells by preantral follicles, which may contribute to the disorder.  PCOS presents in women in a myriad of ways, as this condition is a spectrum of clinical signs and symptoms. Clinical or biochemical hyperandrogenism, oligo anovulation and polycystic morphology are the generally accepted diagnostic criteria. In general, women with PCOS have 2 main phenotypes: lean and obese. A small portion of patients with PCOS present with a normal body mass index (BMI; ≤25 kg/m2), and are classified as “lean PCOS.” Recent research suggests that metabolic, hormonal, and hematological abnormalities are similar to women with “obese PCOS,” however they are usually more subtle and less-severe.  Obesity is commonly seen in women with PCOS, although not all women with PCOS are obese. Obesity has shown to contribute to PCOS symptoms, and the amount of visceral fat, in particular, has been shown to play a key role. Visceral adipose tissue releases several adipokines, including adiponectin. Adiponectin has decreased expression in obesity, and has been linked to insulin resistance. Adiponectin is an insulin-sensitizing, anti-inflammatory molecule. Dyslipidemia is common in women with PCOS compared with weight-matched controls. Typically, women have higher levels of triglycerides and lower high density lipoprotein levels. This association is independent of BMI; however, obesity has been shown to worsen lipid profiles. Insulin resistance appears to also play a role in hyperlipidemia, by stimulation of lipolysis and altered expression of lipoprotein lipase and hepatic lipase.  Insulin resistance plays a large role in the pathophysiology of PCOS and associated conditions, such as metabolic syndrome and cardiovascular disease. The pathogenesis of insulin resistance in PCOS is incompletely understood,  Metabolic syndrome comprises of multiple metabolic disorders that directly increase the risk of diabetes mellitus type 2, cardiovascular disease and coronary artery disease. Pharmacologic treatment for hypertension, abnormal glucose, or dyslipidemia counts for one of the diagnostic criteria. There is an increased risk of metabolic syndrome in women with PCOS. A recent study noted the risk of metabolic syndrome is 11-fold higher in women with PCOS compared with age matched controls.  Inflammatory cytokines may be important factors in the pathogenesis of polycystic ovary syndrome. There is convincing evidence describing the influence of low-grade inflammation and cytokines in polycystic ovary syndrome. Ample evidence considered that PCOS is a proinflammatory disorder associated with chronic low grade inflammation persistence, with the presence of elevated levels of several inflammatory cytokines correlated with insulin resistance  The current study has the aim of evaluation of some hormonal, physiological, haematological, determination of enzymatic antioxidant such as catalase, glutathione peroxidase, superoxide dismutase, vitamin C and E, and biochemical parameters in PCOS, study mutations at the level of nucleotide sequences, determine the alteration in the expression of the[CYP11, CYP17, CYP19, CYP20 and CYP21 families](https://www.guidetopharmacology.org/GRAC/FamilyDisplayForward?familyId=266) examined genes, also investigate the serum level of some cytokines under study such as TNF-α, IL-17, C3 and CRP,with investigate the association of gene polymorphisms of these cytokines in the obese Kurdish women with PCOS in Erbil city. | | |
| **5. Research objectives**  1. Determination of some hematological parameters and relation between some factors which alter in these patients with hormonal test.  2.Assesment of antioxidant aspect and some biochemical test in PCOS.  3.Genotyping of selected genes polymorphism will perform and nucleotide sequencing of the PCR products for the  [CYP11, CYP17, CYP19, CYP20 and CYP21 families](https://www.guidetopharmacology.org/GRAC/FamilyDisplayForward?familyId=266) selected genes are detecting using Sanger sequencing analyzer.  4.Blood samples were collected from obese women with POS, and transferred into ten milliliters (10 mls) sterile tubes let to be a clot in order to separate a serum and centrifuged at 3000 rpm for three minutes and serum was separate from whole blood and stored in deep freeze (- 70°C) in aliquot into several Eppendorf tubes until assayed. Kits were used in the study, such as IL-17, Il-18 and we used ELISA to investigate the levels of these cytokines.  5. The polymorphisms of these cytokines will be analyze by PCR technique. DNA was extracted from 2 ml of 2 ml of blood samples with EDTA using Genomic DNA Extraction Kit. | | |
| **6. Methodology and data collection**  This project has been designed to analyze two important hormones in patients and comparing them with the healthy individuals, one of them is adiponectin, and the second one is anti-Mullerian hormone (AMH) from the serum of PCOS and control group in Erbil province hospitals.  The first task is to take a blood from PCOS patients at hospitals in Erbil, Duhok, Sulaimani and Halabja cities. Venous blood will aspirate into a 5 ml syringe, then place in plain tubes as well as anticoagulant tubes, and maintain at room temperature. The serums of plain tubes preserved in Eppendorf tubes and store at -60oC in the deep freezer until assay. The anticoagulant tubes will further analyze by molecular methods using PCR.  Complete blood count used to determine the relationship between some parameters to able for distinguish the primary reason of PCOS.  Serum used for evaluation some anti-oxidant test, with insulin resistance marker, dyslipidemia or hyperlipidemia, obesity marker, metabolic syndrome, liver and renal function test in PCOS patients with different types of this disorder.  **Mutation and polymorphism analysis using Sanger sequencing technique**  Genotyping of selected genes polymorphism will perform by using tetra-primer amplification refractory mutation system (T-ARMS), which is a rapid and simple technique for recognition of SNP. Polymerase chain reaction (PCR) will perform using commercially available according to the manufacturer procedure. The PCR products are analyzing by DNA electrophoresis gels stained with SYBR Safe DNA Gel Stain and the imaging, and digital documentation will perform  The nucleotide sequencing of the PCR products for the selected genes is detecting using DNA fragments sequencing primers used for amplification. The obtained will analyze. Then, the DNA will get from peripheral blood samples, and they are examining by next-generation sequencing | | |
| **7. Scope and limit to the research**  Little attention has been paid to the role of gene polymorphisms and receptor gene mutations in PCOS; In this study we will cover these parts. | | |
| **8. Duration and timeline**  **Phase1**: 12 months for sample collection  **Phase2**: 12 months for tests and experiment  **Phase3**: 12 months for data analysis, writing up, and publications | | |
| **9. Conclusions**  PCOS is a frequently-encountered condition by the Obstetrician and Gynecologist. Women may present with a spectrum of symptoms, linked to ovulatory dysfunction, insulin resistance and need complete evaluation. A thorough evaluation should exclude secondary causes for oligo-ovulation and hyperandrogenism. Women may present in adolescence, and this provides a unique opportunity to inform the patient of PCOS and the associated risks. Patients need counseling regarding risk of obesity, metabolic syndrome, and life-long risk of development of cardiovascular disease, coronary artery disease, diabetes mellitus type 2, obesity, infertility, depression, anxiety, sleep disorders, and endometrial cancer. Early education and counseling allow for women to make health lifestyle interventions and modifications. | | |
| **10. References**  Kalyanaraman, R and Lubna Pal (2021) A Narrative Review of Current Understanding of the Pathophysiology of Polycystic Ovary Syndrome: Focus on Plausible Relevance of Vitamin D. Int. J. Mol. Sci., 22, 4905.  Shahana Sh., Aysha Noor, Jahan, S. (2019). Polycystic Ovary Syndrome: A Brief Review with Recent Updates. Delta Med Col J. Jul;7(2):84-99  Calcaterra, V.; Verduci, E. Cena, H.; Magenes, V.; Todisco, C.; Tenuta, E.; Gregorio, C.; Giuseppe, R.; Bosetti, A.; Profio, E. and Zuccotti, G.(2021) Polycystic Ovary Syndrome in Insulin-Resistant Adolescents with Obesity: The Role of Nutrition Therapy and Food Supplements as a Strategy to Protect Fertility. Nutrients, 13, 1848.  Teede H, Deeks A, Moran L. (2010) Polycystic ovary syndrome: a complex condition with psychological, reproductive and metabolic manifestations  that impacts on health across the lifespan. BMC Med.; 8:41.  Azziz R.(2018) Polycystic ovary syndrome. Obstet Gynecol.; 132:321–336.  Azziz R, Carmina E, Chen Z, et al. (2016) Polycystic ovary syndrome. Nat Rev Dis Primers. 2:16057.  Toosy S, Sodi R, Pappachan JM. (2018) Lean polycystic ovary syndrome (PCOS): an evidence-based practical approach. J Diabetes Metab Disord.  17:277–285.  Jeanes YM, Reeves S.(2017) Metabolic consequences of obesity and insulin resistance in polycystic ovary syndrome: diagnostic and methodological challenges. Nutr Res Rev. 30:97–105.  Wild RA, Painter PC, Coulson PB, et al.(1985) Lipoprotein lipid concentrations and cardiovascular risk in women with polycystic ovary syndrome.  J Clin Endocrinol Metab. 61:946–951.  Dokras A, BochnerM, Hollinrake E, et al.(2005) Screening women with polycystic ovary syndrome for metabolic syndrome. Obstet Gynecol.106:131–137.  Blankenberg S, Tiret L, Bickel C, Peetz D, Cambien F, Meyer J and Rupprecht H J (2002) Interleukin-18 is a strong predictor of cardiovascular death in stable and unstable angina. *Circulation* 106(1), 24-30.  Palomba S., Falbo A., Chiossi G., Orio F., Tolino A., Colao A., La Sala G. and Zullo F.(2014) Low-grade chronic inflammation in pregnant women with polycystic ovary syndrome: a prospective controlled clinical study. J. Clin. Endocrinol Metab 2 ; 99: 2942-2951.  Akbarzadeh, S., Ghasemi, S. and Kalantarhormozi, M. **2012**. Relationship among plasma adipokines,insulin and androgens level as well as biochemical glycemic and lipidemic markers with incidence of PCOS in women with normal BMI. *Gynecology Endocrinol*, **28**:521–524. | | |
| **11. General notes:** هەر زانیارییەکی گشتی دیکە کە سەرپەرشتیار بە گرنگی بزانێت | | |
| **12.**  **په‌سه‌ندكردنی پرۆپۆزەل له‌ لایه‌ن لیژنه‌ی زانستی به‌ش**  ژماره‌ی كۆنووسی كۆبوونه‌وه‌:  رێكه‌وتی كۆبوونه‌وه‌:  بریار: په‌سه‌ند كرا په‌سه‌ند نه‌كرا    ناوی سیانی و واژووی لیژنه‌ی زانستی به‌ش  واژوو:  ناوى سه‌رۆكی لیژنەى‌ زانستی به‌ش مۆری به‌ش  واژوو:  ناوى سه‌رۆكی به‌ش: | | |
| **13.**  **په‌سه‌ندكردنی پرۆپۆزەل له‌ لایه‌ن ئه‌نجومه‌نی كۆلێژ/فاکەڵتى**  ژماره‌ی كۆنوسی كۆبوونه‌وه‌:  رێكه‌وتی كۆبوونه‌وه‌:  بریار: په‌سه‌ند كرا په‌سه‌ند نه‌كرا  واژوو:  ناو راگری كۆلێژ: مۆری كۆلێژ | | |

**تێبینی:** تكایه‌ فۆرمه‌كه‌ ته‌نها به‌ یه‌ك زمان (زمانی توێژینه‌وه‌) پڕ بكرێته‌وه‌.