1

Assessment of Erythropoietin, Testosterone, hepcidin and many hematological parameters in polycystic ovary women in Kirkuk city

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Abstract: *This research was designed to assess the Testosterone, Erythropoietin and hepcidin concentrations and many hematological parameters (Hb, PCV, RBCs, Iron concentration and Ferritin concentration) in Women suffering poly cystic ovary, By which (55) blood sample were collected from women diagnosed with PCOs, at age (18-47) years with body mass index ≤ 30 kg/m^2, whereas (25) blood samples were collected from healthy women regard as control group with body mass index ≥ 22 kg/m^2,*

All samples were collected from external laboratories in Kirkuk city This study was done in the period from Sept 2016 to march 2017, so the results show significant increase at level (P≤ 0.01) in Testosterone concentration and hematological parameters (Hb, PCV, RBCs and Iron) in women suffering PCOs in comparison with control group, In other hand the study show significant decrease at level (P≤ 0.01) in Hepcidin concentration, ferritin concentration in women suffering PCOs in contrast with healthy group ,While the PCOs group showed no significant change in Erythropoietin concentration in comparison with healthy group.

Key words: PCOs, Erythropoietin, Hepcidin, Iron

I. Introduction

Polycystic ovary syndrome (PCOs) consider as one of the common hormone disorder that affects many women at reproductive age around the world, Women with PCOS have difficulty becoming pregnant, In addition to fertility weakening may be lead to infertility, a woman with PCOS may have some of the following symptoms and findings as Irregular menstrual periods in women of reproductive age ovulatory dysfunction, Acne, Excess hair growth on the face and body known hirsutism, Ovarian cysts, Mental health problems [1].

Women with PCOS are often resistant to the biological effects of insulin and, as a consequence, may have high insulin concentrations; associated conditions include diabetes type 2, obesity, disruptive sleep apnea, heart disease, mood disorder, and endometrial cancer. Other organ systems that can be affected by PCOS include the brain, pancreas, liver, muscle, blood vasculature [2,3].

This syndrome is due to a combination of environmental and genetic factors, obesity is one of risk factors, not enough physical exercise, and a family history of someone with the condition. Diagnosis is based on two of the following three results: an ovulation, ovarian cysts and elevation of Testosterone level, and many other symptoms exclude Hypothyridism, adrenal hyperplasia and Hyperprolactinemia [4,5].

Subsequent studies have supported and amplified that dys-regulation of androgen secretion led to Functional Ovarian Hyperandrogenism (FOH) this result managed to oligo or an ovulation, two-thirds of PCOS cases have functionally usual FOH, which characterized by 17-hydroxy progesterone hyper-responsiveness to gonadotropin stimulation. Two-thirds of the remaining PCOS have FOH noticeable by testosterone altitude after adrenal androgen production suppressed .The many other PCOS cases have limited evidence belong to abnormalities steroid secretory [6,7].

Testosterone is steroid hormone one the important androgens group increased during the injured with PCOs as well as the research illustration it is important role as a regulator of erythropoiesis process human [8,9].

Circulating testosterone concentrations have been related with hemoglobin concentrations in men from adult period until reached to elderly [10,11].

Erythropoietin (Epo) is required factor for red blood cell (RBC) production. The affiliation between the O_2 content of the blood and erythropoiesis was first described by the French anatomist Francois-Gilbert Viault in 1890, who detected the specific stimulus for **Epo** expression is a fall in tissue O_2 pressure (Po_2). The hematopoietin or Epo increased under hypoxic situations there is the high linkage between kidneys and liver to produce Epo by tow main factors first one is globulin secretion from liver linked with Renal Erythropoeitic factor which secreted from kidney in response to cellular hypoxia; it stimulates in human bone marrow to increase red blood cell production (erythropoiesis) [12].

as in previous studies finds that small amount of Epo (around 10ng /ml) are sufficient for stimuli product red blood cells ,many causes of cellular hypoxia resulting in raises level of Epo (above of 1000 ng/ml) hypoxemia due to chronic lung disease [13].

Exogenous erythropoietin, recombinant human erythropoietin (rhEPO) is produce by recombinant DNA technology in cell culture and are collectively called erythropoiesis–stimulating agents (ESA), it used in the treatment anemia from cancer chemotherapy , anemia in chronic kidney diseases and in case of anemia in myelodysplasia, in other wise this therapy has many side effect include myodardial infarction strocke, venous thromboembllism, the risk may be increased when EPO treatment dose causes in raises hemoglobin level over than 14 g/dl to 16 g/dl [14].

In older men Erythrocytosis is the most common adverse incident associated with testosterone therapy, however the mechanisms by which high level of testosterone stimulates erythropoiesis had revealed by different scientific approaches [15].

Hepcidin is The peptide it represent main regulator of iron homeostasis in vertebrates, the first described as a cationic antimicrobial peptide with microbicidal properties against many micro-organisms *In vitro*, During inflammation one of the most agents induced strongly is hepcidin, and evolving in the pathogenesis of a many cases of infections .one of previous article indicate that role of hepcidin in the resistance and susceptibility to infectious diseases [16].

The hepcidin–ferroportin association for controlling the normal iron level in both extracellular and total body iron levels. Ferroportin is a main protein that is the major exporter of iron from mammalian cells, Hepcidin

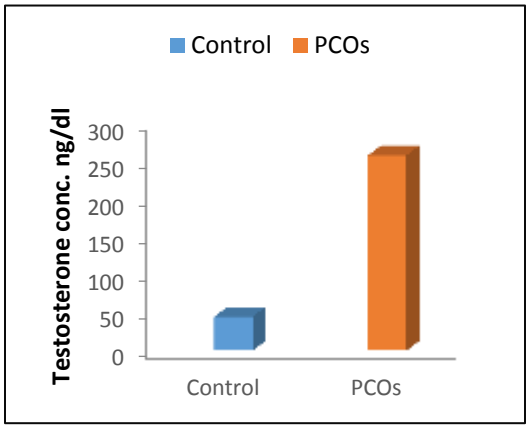
limits the extracellular iron by binding ferroportin and mediating its degradation, therefore preventing iron release from intracellular sources, Sustained raises of hepcidin result in inadequate iron availability for erythropoiesis, affecting an iron-restricted anemia, previous article mentioned to hepcidin is one of the factors affecting the pathogenesis of the PCOs disease [17,18].

The aim of this study was to evaluate plasma levels of Erythropoietin, hepcidin, Testosterone, and many Hematological parameters in patients with PCOS in Kirkuk city.

2-Materials and Methods

2-1 Patients and Blood collection:

This study was done in the period from Sept 2016 to march 2017, it involving two groups the first one includes 55 blood sample from women diagnosed with poly cystic ovary syndrome with age range (18-47) year. The blood samples were collected from external laboratories in kirkuk city and its districts The second group includes 25 blood samples from healthy women conceders as control group. Collection of blood samples blood serum was prepared from (5ml) venous blood obtained by using



disposable syringe and clean dry plain tubes without any anticoagulants and left it at room temperature to coagulate. After that centrifuged for five minutes at 3000 rpm to get serum without any hemolysis, separated serum was stored in -20 C for hormonal and

biochemical studies, Whereas (1 ml) venous blood obtained by using anticoagulant tube for hematological studies.

2-2 Determination of Parameters

Hepcidin were determined by using their kit ELISA Kit (Hep25), from (Cusabio), Erythropoietin was determined by using their Human Erythropoietin ELISA Kit (EPO) (ab119522) [19].

Testosterone was determined by using its kit from Monobind [20].

Iron was determined by using its kit from Biomaghreb company, ferritin VIDAS-Ferritin kit, was determined by using its kit from bioMerieux company, Hb, PCVs, RBCs, was determined by using its kit from HOREBA company, Hematology Analyzer.

2-3 Statistical analysis:

The data were analyzed by (SAS, 2001) software according to one way ANOVA followed by duncun range test used at a statistical concentration of ($p \leq 0.01$).

3. Results and discussion

The result of this study as show in (Figure1) a significant increase at concentration ($P \leq 0.01$) of Testosterone concentration (258 ± 1.44) ng/dl in women suffering PCOs in comparison with healthy women as a control group (45 ± 0.75) ng/dl.

Figure (1): Concentration of Testosterone hormone (ng/dl) in study groups

This result was agree with many previous studies showed that cysts may induce ovary to secretion a high concentration of testosterone, Assays of testosterone are important in the diagnosis and management of a number of clinical conditions in females including precocious puberty, androgen-secreting tumors, and polycystic ovary syndrome (PCOS) [21].

So the current result was agree with research who show that hyperandrogenism was thought to be the essential factor for PCOs and high testosterone concentrations are reported in many women with PCOs [22].

In contrast with our study found by Gomathi *et al*., 2011 serum concentrations of testosterone, Estradiol, Prolactin in women with PCOs were all within the normal reference range for young women ,and no increase in serum testosterone concentrations was noted even in PCOs women with hirsutism [23].

Figure (2): The result of this study revealed a no significant difference (19.07±1.21 ng/ml) of Erythropoietin in women suffering PCOs in comparison with healthy women (24.07ng/ml).

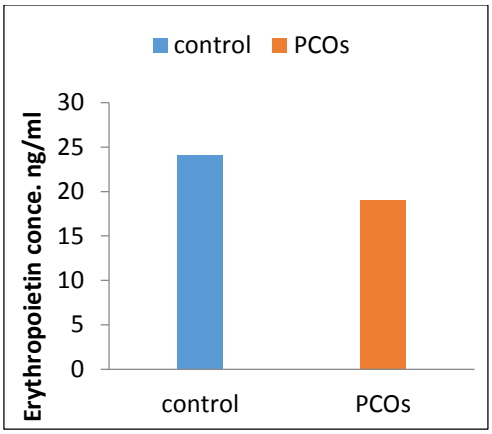


Figure: (2) concentration of Erythropoietin (ng/ml) in study groups.

The normal concentration of Erythropoietin hormone concentration evidence that even the raise of testosterone concentration did not effect on EPO in women suffering PCOs as in previous reports appointed that testosterone failure to directly activate EPO transcription in Hep3B cells, an EPO-secreting cell line that is greatly sensitive to hypoxic induction [24].

thus suggesting that any putative EPO-dependent mechanism for testosterone-induced erythrocytosis may be indirect , in another report indicate that administration of physiologic doses of testosterone in healthy men suppresses the iron regulatory peptide hepcidin while EPO concentrations remained unaffected after 20 weeks of treatment, This observation raises the possibility that unchanged EPO concentrations reflect a higher biological activity of EPO, via increased iron bioavailability, in contrast one hypothesis revealed that administration high dose of testosterone to old men or women may be stimulates EPO transiently, beside with suppression of hepcidin.

Figure (3): has been shown significant decrease (P≤ 0.01) concentration in Hepcidin in blood concentration (6.46±0.08 ng/ml) in PCOs comparison with control group (14.13 ± 0.75 ng/ml).

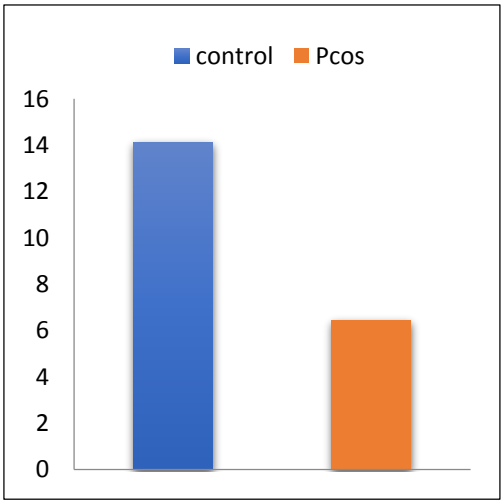


Figure: (3) concentration of hepcidin hormone (ng/ml) in study group

This result comes agree with Wen and his followers (2013) they found that administration of high dose of Testosterone to male and female mice encouraged suppress of hepcidin expression process in liver by its special effects on erythropoietin or hypoxia-sensing

mechanisms. By contrast expected increase in hemoglobin may be noticed as response to get testosterone [25]. Hossein and his followers (2017) proved in their articles there were a Fast but temporary rises in renal EPO mRNA expression and serum EPO concentrations as reflect of administration of Testosterone, in other hand they show that hepcidin mRNA expression was suppressed by testosterone administration [12,18]. Another research shows that older men hepcidin level may be suppressed as in respond to management of testosterone dependent on giving dose [26].

Figure (4): show a significant increase (P≤ 0.01) in Iron blood concentration (39.01± 4.55) in women with PCOs when it has been compared with control group (23.21 ± 2.93). The result show significant decrease (P≤ 0.01) of Ferritin concentration in PCOs women (26.72±0.07) comparison with control group (47.33±1.55).

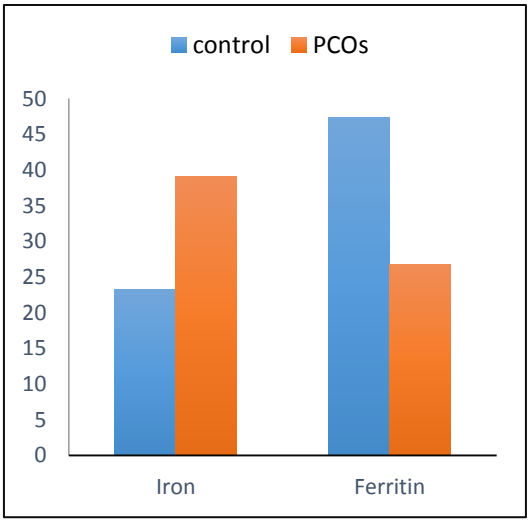


Figure: (4) Concentration of Iron and Ferritin concentration in study groups.

Figure (5): The results show a high significant at concentration (P≤ 0.01) of Hb blood concentration (16.65±1.17) in PCOs women comparison with control group (12±0.33) As well as PCV blood concentration (49±1.96) in PCOs women comparison with control

group (37±0.60) this figure has been shown high concentration in RBCs blood concentration (5.6±1.47) in PCOs women comparison with control group (4.2±1.03).

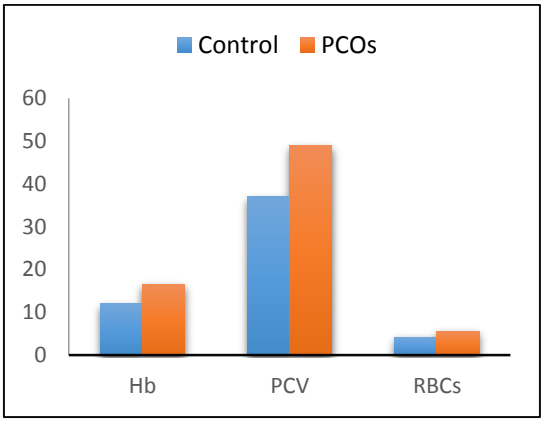


Figure: (5) Concentration of Hb, PCV and RBCs in study groups..

Previous article show that In older men and during testosterone therapy causes one of most important problem is suppress of hepcidin serum level and reflect to increases in hematocrit [26].

In other hand the decrease hepcidin level combination with up regulation of ferroportin causes in increased of iron transfer from the spleen which increase iron availability for synthesis the hemoglobin, addition to that many previous evidence indicate that erythropoiesis may stimulates by testosterone reflect in increasing of RBCs count, so Testosterone administration may be increases reticulocyte count, which regards as marker of erythropoiesis. another finding indicate that testosterone administration raises serum iron and transferrin overload; increased serum iron , reduced splenic iron stores and increased incorporation of ansferrin-bound 58Fe into the red blood cells of testosterone-treated mice compared to controls [27].

Addition with it increased ferroportin expression in the spleen was associated with excessive of Testosterone-mediated suppression of hepcidin, In contrast with a previous report that hepcidin selectively

regulates ferroportin expression in splenic macrophage. [28] Furthermore, sera get from testosterone-treated mice induced significantly greater hemoglobin accumulation in K562 cells induced toward erythroid differentiation than sera get from vehicle-treated mice. [29,30]

4. Conclusion

For our research is: serum level of hepcidin and ferritin were decreased significantly in PCOs group. Serum level of erythropoietin was no significantly differing in PCOs group compared with control group.

5. Recommendation

Study of irisin and antioxidants levels in PCOs patients.

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تقييم مستوى هرمون الاريثروبويتين والتستيرون والهيبسيدين وعدد من المتغيرات الدموية في النساء المصابات بمتلازمة تكيس المبايض في مدينة كركوك

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الخلاصة:

صمم البحث الحالي لتقييم مستوى وتركيز هرمون الايريثروبويتين والهيبسيدين والشحمون الخصوي وعدد من المتغيرات الدموية (الهيموكلوبين Hb وحجم كريات الدم الحمراء المرصوصة PCV وكريات الدم الحمراء RBCs والحديد فضلا عن تقدير تركيز الفريتين في النساء اللواتي يعانين من مرض تكيس المبايض، إذ تم جمع (55) عينة دم من نساء مصابات بتكيس المبايض واللاتي تراوحت اعمارهن ما بين (18 الى 47) سنة كما وتم قياس مؤشر كتلة الجسم لديهن اذ بلغ اكثر من 30 كغم/م2 ، بينما تم جمع (25) عينة دم من نساء سليمات اعتبرن كمجموعة سيطرة في حين اظهر مؤشر كتلة الجسم لديهن اقل من 22 كغم/م2

فقد تم جمع العينات من المختبرات الخارجية في مدينة كركوك وقد امتدت الدراسة للفترة من شهر ايلول 2016 ولغاية شهر اذار 2017 وقد اظهرت النتائج ارتفاع معنوي عند مستوى ($P\leq 0.01$) في تركيز هرمون الشحمون الخصوي والمتغيرات الدموية (Hb , PCV , RBCs و الحديد) في النساء المصابات بمتلازمة تكيس المبايض مقارن مع مجموعة السيطرة السليمات، في حين وجدت الدراسة انخفاض معنوي في تركيز الهبسيدين والفيريتين في النساء المصابات مقارنة مع مجموعة السيطرة السليمة. في حين اظهرت مجموعة النساء المصابات عدم وجود اختلافات معنوية في تركيز هرمون الاريثروبويتين مقارنة مع مجموعة السيطرة السليمة.

كلمات مفتاحية: PCOs ، ارثروبيوتين، هيبسيدين، حديد