Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 3, 2021, Pages. 7494 - 7504
Received 16 February 2021; Accepted 08 March 2021.

Study of Hepcidin and Many Physiological and Hematological Parameters in Women with Polycystic Ovary in Kirkuk City

Wedad Mahmood Lahmood Al-Obaidi[*1], Mohanad Hasan Mahmood Al-Izzi[2]

[1]Department of Biology, Education College (AL-Hawiga), Kirkuk University, Iraq.[2]Department of Biology, Science College, Tikrit University, Iraq.

***Corresponding author email is**: wadad.mahmud@uokirkuk.edu.iq

Abstract

Theexperimental studies suggest that Hepcidin can be a protein participating in the developmentof metabolic disorders, while its synthesis and concentration in the circulation outside of the iron metabolism parameters can be influenced by hormones. The aim of the present study wasperforming experiments to determine the concentrations of a variety of critical variables. represented by Hepcidin , erythropoietin, testosterone and many of blood parameters (hemoglobin, iron, ferritin, red blood cells and PCV) in a group of women with Poly cystic ovary syndrome (PCOs) in Kirkuk city and normal women .Materials and Methods: our study include(55) samples were collected from blood serum of women with the PCOsin age of (18-47) years with a measurement of their BMI $\leq$ 30 kg/m^2, while (25) blood samples were collected from healthy women and they were considered a control group with a BMI $\geq$22 kg/m^2, where samples were collected from volunteer women from Kirkuk hospital and external specialized clinical, after diagnosis by specialized doctor, and the study was conducted from September 2018 to March 2019, the experimental groups were divided to two groups :group 1 patients women with PCOs , group 2 healthy control group.Results: We reach to following conclusions a significant increase (P$\leq$0.01) in the concentrations of testosterone and iron with hemoglobin, red blood cells and PCV in women suffering from polycystic ovaries , but a significant decrease (P$\leq$0.05) in the concentrations of both hepcidin and ferritin in patients, in contrast there no significant change in Erythropoietin concentration for women with PCOS , all these results were compared with the healthy control group.Conclusion:Hepcidin is the essential regulator of general iron homeostasis. Dysregulation of hepcidin production effects in a variety of iron disorders. Hepcidin deficiency is the cause of iron overload in iron-loading anemias, , hepatitis C and hereditary hemochromatosis.

Key words: Polycystic ovary, Hepcidin, , Erythropoietin ,Ferritin

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Introduction

Polycystic ovary syndrome (PCOs) consider as one of the disorder in hormone at genital age affects women around the world , Women suffering PCOS have obstacle to become pregnant, and weakeness in fertility , both may be progresses to infertility, the findings and symptomsin in patients with PCOS such as Irregular menstrual durations and ovulatory dysfunction, Acne, hirsutism (growth hair on the face and body), Ovarian cysts, Mental health disorders [1]. patients with PCOS are often relcutant to the biological effects of insulin and, as a conclusion, may have high insulin concentrations; lead to diabetes type 2, obesity,disruptive sleep apnea, heart disease,mood disorder,and endometrial cancer. Other organ that may be influenced involved the pancreas,muscles,brain,liver, 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: anovulation, ovarian cysts andelevation of Testosterone level, and many other symptomsexclude Hypothyridism,adrenal hyperplasia and Hyperprolactinemia [4,5].Many studies have supported and amplified that dys-regulation of androgen secretion led to Functional Ovarian Hyperandrogenism (FOH) this result managed to oligo or anovulation, two-thirds of PCOS cases have functionally regular FOH, which distinguished by 17-hydroxy progesterone hyper-responsiveness to gonadotropin promotion. Two-thirds of the residual PCOS have FOH remarkable by testosterone elevation next adrenal androgen production put down .The many other PCOS cases have limited evidence belong toabnormalities 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 a necessary component for the development of red blood cells (RBCs). The connection between blood O2 levels and erythropoiesis was discovered by French anatomist Francois-Gilbert Viault in 1890, who discovered that a drop in tissue O2

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pressure is the basic trigger for Epo expression (Po2).The hematopoietinor Epo increased under hypoxic situationsthere is the high linkage between kidneys and liver to produce Epo by tow main factors first one isglobulinsecretion from liverlinked with Renal Erythropoeitic factor which secreted from kidney in response to cellular hypoxia; It induces erythropoiesis (the formation of red blood cells) in human bone marrow [12]. As previously discovered in previous studies, a small amount of Epo (around 10ng/ml) is necessary to stimulate the production of red blood cells. However, several causes of cellular hypoxia result in elevated Epo levels (above 1000 ng/ml)Chronic lung disease causes hypoxemia. [13].Exogenous erythropoietin and recombinant human erythropoietin (rhEPO) are erythropoiesis–stimulating agents that are produced in cell culture using recombinant DNA technology (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 myocardial infarction stroke,venous thromboembolism,the risk may be increased when EPO treatment dose causes in raises hemoglobin level over than 14g/dl to 16 g/dl [14].According to previous report had been indicated that Erythrocytosis in older men is the utmost common pernicious incidentrelated to testosterone medication, however, various research methods have revealed the mechanisms by which a high level of testosterone stimulates erythropoiesis.[15].Hepcidin is the peptide it representmain regulator of iron homeostasis in vertebrates,it was first reported as a cationic antimicrobial peptide with microbicidal properties against a variety of microbes. *In vitro*,during inflammation one of the most agents induced strongly is hepcidin, and evolvingin the development of the disease of a many cases of infections.one of previous article indicate that Hepcidin plays a role in infectious disease tolerance and susceptibility. [16].The hepcidin–ferroportin relationship is responsible for maintaining normal iron levels in both extracellular and total body tissues.Ferroportin is a functional protein that is responsible for the majority of iron export from different mammalian cells.Hepcidin regulates the amount of extracellular iron by binding to ferroportin and allowing it to degrade,As a result, intracellular iron release is prevented. Inadequate iron supply for erythropoiesis is caused by sustained hepcidin elevations,causing an iron-deficiency anemia,Hepcidin is consider among one

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of the agents influencing the cause of PCOs risk, as stated in the previous article.[17,18].The aim of this study was to determine the levels of Hepcidin in the plasma., Erythropoietinand many Physiological and Hematological parameters in patients with PCOS in Kirkuk city.

Materials and Methods

Patients and Blood collection:

This study was done in the period from September 2018 to March 2019.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. Theblood samples were collected from external laboratoriesin kirkuk city and its districts The second group includes 25 blood samples from healthywomen conceders as control group. Collection of bloodsamples blood serum was prepared from (5ml) venousblood obtained by using disposable syringe and cleandry plain tubes without any anticoagulants and left it atroom temperature to coagulate. After that centrifugedfor 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.

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].Testosteronewas determined by using its kit fromMonobind [20].Ironwas determined by using its kit from Biomaghrebcompany, ferritinVIDAS-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.

Statistical analysis:

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

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Results and discussion

The result of this study as show in (Figure1) a significant increase at concentration (P≤ 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.

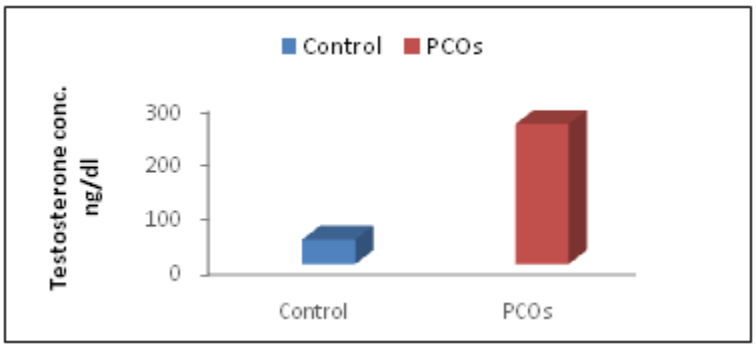

Figure (1): Concentration of Testosterone hormone (ng/dl) in studied groups

This result was agree with many previous studies showed that cysts may induce ovary to secretion a high concentration of testosterone,High testosterone levels may indicate polycystic ovary syndrome, which is one of the most common causes of female infertility. Low testosterone levels may also indicate cancer of the ovaries or adrenal glands. Although low testosterone levels are natural, extremely low levels can indicate Addison's disease, a pituitary gland disorder.[21].So the current result was agreewith 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 many of the women with PCOs were in the average range. reference range for young women ,and no increase in serum testosterone concentrations was noted even in PCOs women with hirsutism [23].The result of this studyin figure 2revealed a nosignificant difference(19.07±1.21 ng/ml) of Erythropoietin in women suffering PCOs in comparison with healthy women (24.07ng/ml).

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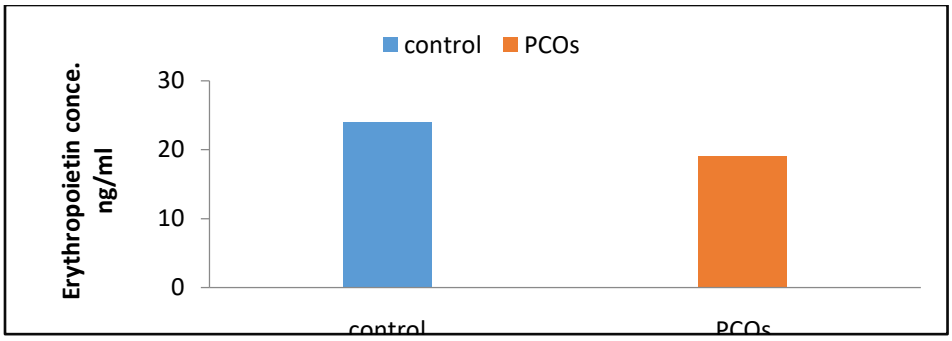

Figure:(2) concentration of Erythropoietin (ng/ml) in studied groups.

Thenormal 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 stimulate EPO transcription in Hep3B cells, an EPO-secreting cell line that is greatly sensitive to hypoxic induction [24].As a consequence, any testosterone-induced erythrocytosis process involving EPO is ruled out. Another study suggests that giving healthy men physiologic doses of testosterone suppresses the iron regulatory peptide hepcidin while leaving EPO concentrations intact after twenty weeks of treatment.This result raises the possibility that unchanged EPO concentrations represent increased iron bioavailability and thus higher biological activity of EPO.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 hepcidinFigure (3): has been shown significant decrease ($P \leq 0.01$) concentration in Hepcidin in blood concentration (6.46±0.08 ng/ml) in PCOs comparison with control group (14.13± 0.75 ng/ml).

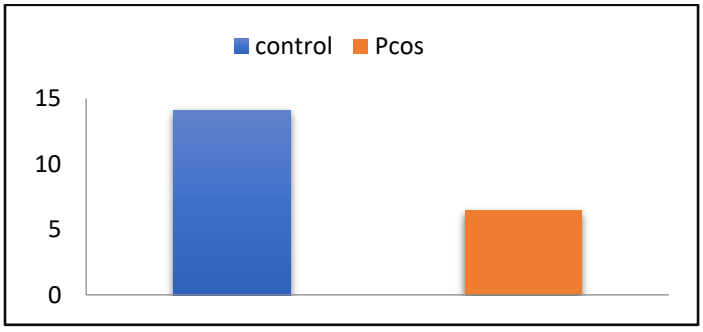

Figure: (3) concentration of hepcidin hormone (ng/ml) in studied group

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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 inliver 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 ofadministration of Testosterone,in other hand they show that hepcidin expression of mRNA was pent-up by testosterone administration [12,18].Another research shows that older men hepcidin level may be suppressedas in respond to administration 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 PCOswhen it has been compared with control group(20.21± 2.93).The result show significant decrease (P≤ 0.01) of Ferritin concentration in PCOs women (26.72±0.07) comparison with control group (40.33±1.55).

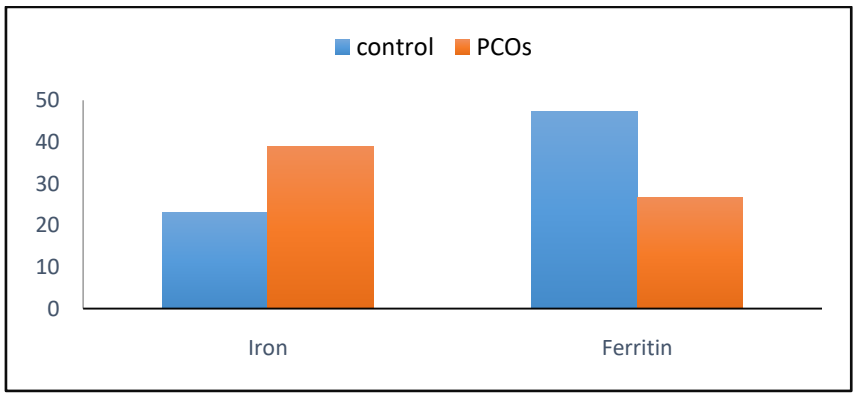

Figure:(4) Concentration of Iron and Ferritin concentration in study groups.

Figure(5):The results show a highsignificant 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).

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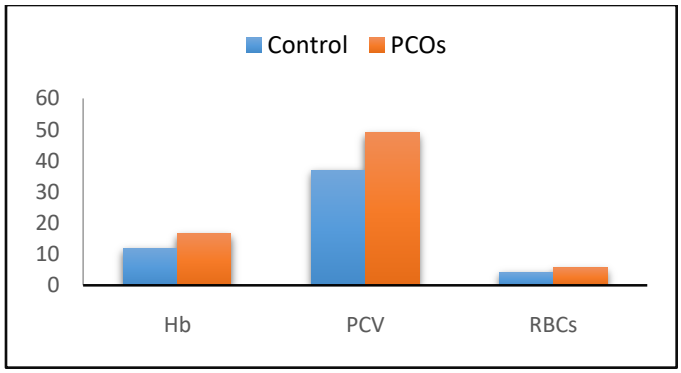

Figure:(5) Concentration of Hb, PCV and RBCs in study groups..

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 testosterone-treated mice had more ansferrin-bound 58Fe integrated into their red blood cells than control mice. [27].Addition with itraising of ferroportin secretion in the spleen was associated with excessive of Testosterone-mediated suppression of hepcidin, The Hepcidin selectively controls ferroportin expression in splenic macrophages, contrary to a previous study. [page 28] Additionally, sera from testosterone-treated mice caused substantially more hemoglobin accumulation in K562 cells induced to erythroid differentiation than sera from vehicle-treated mice. [29,30]

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.

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Recommendation

Study of Osteocalcin,Irisin and antioxidants levels in primary infertilewomen .

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