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EVALUATION OF THE RELATIONSHIP BETWEEN SOME ANTIOXIDANT PARAMETERS AND HEAVY METALS CONCENTRATIONS IN SOME IRAQI TEENAGE PATIENTS WITH (PCOS)

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
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NOOR AHMED MOHSIN ATBINAH

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By Noor Ahmed Mohsin ATBINAH

June 2023

We certify that we have read this thesis and that in our opinion it is fully adequate, in scope and in quality, as a thesis for the degree of Master of Science

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Noor Ahmed Mohsin ATBINAH

ABSTRACT

EVALUATION OF THE RELATIONSHIP BETWEEN SOME ANTIOXIDANT PARAMETERS AND HEAVY METALS CONCENTRATIONS IN SOME IRAQI TEENAGE PATIENTS WITH (PCOS)

Noor Ahmed Mohsin ATBINAH

Master of Science in Chemistry

Advisor: Assoc. Prof. Dr. Şevki ADEM

June 2023

The aim of the study is to study the change and effect of some hormones on Iraqi girls in adolescence and how they affect the ovaries, as well as the effect of some heavy metals such as lead and cadmium on the uterus and their effect on the ovaries. This study will include two groups, the first group consisted of 40 healthy people and the second group consisted of 80 patients with (PCOS). Age and FSH, LH had significant clinical significance for women with PCOS. So was the testosterone. estradiol. AMH and Catalase are statistical, but at lower levels. For cadmium and Pb, they were not statistically significant. There was no statistical significance or differences for women with PCOS. There is a need for more study in this matter.

2023, 45 pages

Keywords: PCOS, FSH, LH, Testosterone, Estradiol, AMH, Blood glucose test, Hemoglobin A1c, Vitamın D

ÖZET

IRAKLI BAZI ERGEN (PCOS) HASTALARDA BAZI ANTİOKSİDAN PARAMETRELER İLE AĞIR METAL KONSANTRASYONLARI ARASINDAKİ İLİŞKİNİN DEĞERLENDİRİLMESİ

Noor Ahmed Mohsin ATBINAH

Kimya, Yüksek Lisans

Tez Danışmanı: Doç. Dr. Şevki ADEM

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Çalışmanın amacı Iraklı kız çocuklarında ergenlik döneminde bazı hormonların değişimi ve etkisi ile yumurtalıkları nasıl etkilediğinin yanı sıra kurşun ve kadmiyum gibi bazı ağır metallerin rahim üzerindeki etkisini ve yumurtalıklara etkisini incelemektir. . Bu çalışma, birinci grup 40 sağlıklı kişiden ve ikinci grup (PKOS) olan 80 hastadan oluşan iki grubu kapsayacaktır. Yaş ve FSH, LH, PCOS'lu kadınlar için önemli klinik öneme sahipti. Testosteron da öyleydi. östradiol. AMH ve Katalaz istatistikseldir, ancak daha düşük seviyelerdedir. Kadmiyum ve Pb için istatistiksel olarak anlamlı değildi. PCOS'lu kadınlar için istatistiksel bir anlam veya farklılık yoktu. Bu konuda daha fazla çalışmaya ihtiyaç vardır.

2023, 45 sayfa

Anahtar Kelimeler: PCOS, FSH, LH, Testosteron, Estradiol, AMH, Kan şekeri testi, Hemoglobin A1c, D Vitamini

PREFACE AND ACKNOWLEDGEMENTS

I would like to thank my thesis advisor, Assoc. Prof. Dr. Şevki ADEM, for his patience, guidance and understanding.

Noor Ahmed Mohsin ATBINAH

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CONTENTS

LIST OF SYMBOLS

-	Minus
%	Percent
**	Significant
/	Divide
+	Plus
<	Greater than
=	Equel
>	Less than
±	Plus – minus
≤	Greater or equal to
≥	Less or equal to
dL	Deciliter
g	Gram
kg	Kilogram
L	Liter
m^2	Square meter
mcg	Microgram
mg	Microgram
mIU	Milli-international units
min	Minute
mL	Milliliter
mmol	Milli mole
mol	Mole
ng	Nanogram
nm	Nanometer
NS	Non-significant
rpm	Revolutions per minute
µL	Micro liter

LIST OF ABBREVIATIONS

AMH	Anti-mullerian hormone
Ar	Arsenic
BGT	Blood glucose test
Cd	Cadimium
FSH	Follicle-stimulating hormone
IU	International units
LH	Luteinizing hormone
Pb	Lead
PCOs	Polycystic ovarian syndrome
RBS	Random bloos sugar

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1. INTRODUCTION

In women, polycystic ovarian syndrome (PCOS) is a collection of symptoms caused by a hormonal imbalance that may express itself in a variety of ways. It is the most prevalent cause of female infertility, affecting around 10% of women of reproductive age. Endocrinopathy is defined as an endocrinopathy that is marked by chronic anovulation, menstrual abnormalities. Despite this, the particular etiology of PCOS has not yet been identified and clarified. Some naturally occurring metals, such as Cadmium (Cd), Lead (Pb), Arsenic (As), and other heavy metals, have specific densities greater than 5 g/cm^3 and atomic weights greater than 40, and are therefore referred to as 'heavy metals' (Bozdag *et al*. 2016). Heavy metals such as cadmium (Cd), lead (Pb), and arsenic (As) are examples of toxic substances. This group of heavy metals has harmful effects on living organisms, leading to acute and chronic toxicities in both animals and humans as a consequence of exposure. It is necessary for the body to have other heavy metals such as zinc, iron, and copper present in order for it to operate at its normal physiological and biochemical levels. Nonetheless, when taken in significant numbers, some of the heavy metals contained in this category are harmful to the human body. Heavy metal pollution from industry happens on a continual basis, and exposure to these metals is a substantial source of public health concern since exposure to these metals has been found to cause reproductive dysfunction in females. Toxins are introduced into the environment via a variety of routes, including the soil, the air, polluted water, smoking, and food consumption (Palomba *et al*. 2015).

1.1 Aim of Study

To better understand reproductive health in Iraqi women, it is important to investigate the changing effect of some hormones on Iraqi girls during adolescence and how they affect the ovaries. Another important goal of this thesis is to investigate the effect of some heavy metals such as lead and cadmium on the uterus and their effect on the ovaries, in order to better understand reproductive health in Iraqi women.

2. LITERATURE REVIEW

In general, polycystic ovary syndrome (PCOS) is a group of symptoms caused by a hormonal imbalance that may manifest itself as a number of different symptoms. The inability to conceive affects around 10% of women of reproductive age, with polycystic ovarian syndrome (PCOS) accounting for 6.5–8% of those who are of reproductive age and being the most common cause of infertility among females1. PCOS is described as an endocrinopathy characterized by persistent anovulation and menstrual irregularities (Davis and Wahlin-Jacobsen 2015).

In spite of this, the specific aetiology of PCOS has not been determined. Some naturally occurring metals, such as Cadmium (Cd), Lead (Pb), Arsenic (As), and other heavy metals, have specific densities more than 5 g/cm3 and atomic weights greater than 40, and are hence referred to as 'heavy metals'. Cadmium (Cd), Lead (Pb), and Arsenic (As) are examples of heavy metals. These heavy metals have detrimental effects on living organisms, resulting in acute and chronic toxicities in both animals and humans. Nonetheless, several of the heavy metals included in this group are toxic when consumed in large quantities. Heavy metal pollution from industry occurs on a continuous basis, and exposure to these metals is a significant source of public health concern, particularly for women of childbearing age, since it has been shown to induce reproductive dysfunction in women. The soil, the air, contaminated water, smoking, and food are the primary pathways of exposure in the environment, respectively (Baillargeon *et al*. 2018).

An important goal of this thesis is to investigate the changing effect of some hormones on Iraqi girls during adolescence and how they affect the ovaries, as well as the effect of some heavy elements such as lead and cadmium on the uterus and their effect on the ovaries, in order to better understand reproductive health in Iraqi women. A total of 50 people infected with HIV will participate in this trial (PCOS). FSH, LH, Testosterone, Estradiol, and AMH will be measured in blood samples collected from the wounded in order to determine the ratio of FSH to LH. Hemoglobin A1c, Vitamin D, and AMH will also be measured. The patient's blood will be collected from a vein for 4 milliliters in

order to determine the ratio of FSH to LH, Testosterone, Estradiol, AMH, Blood Glucose Test, hemoglobin A1c, Vitamin D. Centrifugation at 4000 rpm for 10 minutes will separate the serum for biochemical testing, which will then be stored frozen until analysis until the results are available.

From among the patients hospitalized to Baghdad's MADINAT Al TIP hospital, a total of 120 samples will be picked for testing. The patients will then be separated into two groups: an experimental group (which will consist of 80 patients) and a control group (which will consist of 40 patients). The control group's role is to guarantee that the study's internal validity is maintained.

The following sections will discuss the main topics related to the goal of this research, foe instance, the role of testosterone in human, Vitamin D, polycystic ovary syndrome, and the main tests that will be included in the methodology of this study.

2.1 Testosterone

Testosterone is a hormone that is produced by both humans and other animals, including fish. The testicles are primarily responsible for the generation of testosterone in males. Women's ovaries also generate testosterone, but in somewhat smaller levels than men's ovaries. The amounts of testosterone in a man's body may also have an impact on his mood. Low testosterone levels, also known as low T levels, may present themselves in a number of ways in guys, including decreased sex drive, a lack of energy, weight gain, depression, and irritability, among other issues (Saad *et al*. 2017).

While testosterone production naturally drops with age, it is possible that other factors are contributing to a decrease in hormone levels as well. All cancer treatments, including chemotherapy and radiation, may have a negative impact on testosterone production, including testicular damage (Huo *et al*. 2016). The male sex hormone testosterone is beneficial to women because it increases their sex drive, increases bone density, and improves their physical strength. Women may become infertile if they have

an excess of testosterone in their bodies. Male pattern baldness can also result from an excess of testosterone. The pituitary gland and the brain are both responsible for regulating testosterone levels. In order for the hormone to carry out its many functions, it must first be produced and then circulated through the circulation (Mulhall *et al*. 2018).

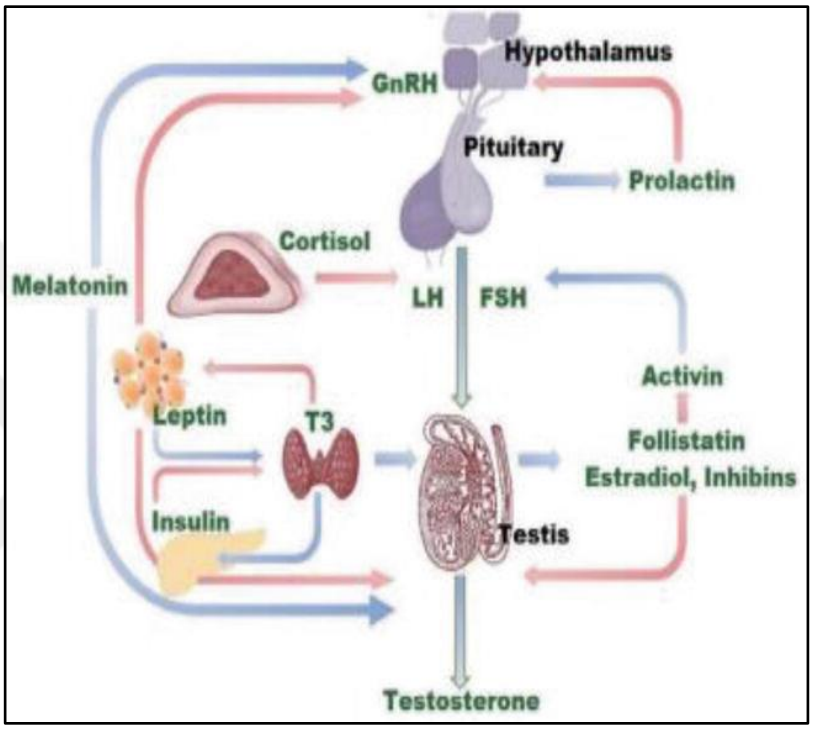

Figure 2.1 Testosterone creation (Kloner *et al*. 2016)

2.1.1 Structure

When it comes to male reproductive hormones, testosterone is the most important. It is responsible for a variety of physiological functions in the body, including the development of sex organs such as the testicles, penis, and scrotum between infancy and adolescence. Platelets must be bound by receptors in order for the appropriate production and circulation of red blood cells to be ensured. Achieving a healthy balance in testosterone levels has a number of advantages. While adequate testosterone levels in guys are beneficial to their sexual well-being, an imbalance caused by unusually low or

high testosterone levels in the body will almost always result in health complications (Gagliano-Jucá and Basaria 2019).

A drop in testosterone levels is most often caused by the effects of ageing. There are a variety of illnesses that can cause a decrease in testosterone levels in males. Excess testosterone levels may be caused by genetic abnormalities, adrenal tumors, or testicular cancer, to name a few possibilities. As a consequence of heavy steroid usage, testosterone levels in males rise significantly. In certain studies, elevated testosterone levels have been proven to impair heart function, boost hazardous LDL cholesterol levels in the blood, and even cause infertility in women (Budoff *et al*. 2017).

It is possible to lower testosterone levels using prescription medications that change the body's hormonal secretions, a well-balanced diet, regular physical exercise, a good sleep pattern, weight reduction, and adherence to a holistic lifestyle. In order to maintain optimal blood levels of testosterone, production must be carefully managed, while testosterone concentrations are normally highest in the morning and decline subsequently (Kelly and Jones 2015).

2.1.2 Role in man

Because testosterone production must be tightly controlled in order to maintain appropriate blood levels, testosterone levels are typically highest in the morning and gradually decline throughout the day. However, testosterone levels are typically highest in the morning and gradually decline throughout the day. This hormone travels from the bloodstream to the gonads, where it stimulates the production and release of testosterone (Snyder *et al*. 2016).

The impact of elevated testosterone on the body is based on the individual's age and gender. Adult men are unlikely to be affected by a disease in which they create excessive testosterone, and it might be difficult to tell whether an adult man is producing excessive testosterone in some circumstances. More specifically, young

children who have an overabundance of testosterone may have a false growth spurt as well as the signs of premature puberty, while young females may undergo aberrant modifications to their genital structures. Female infertility and precocious puberty are two consequences of high testosterone levels in both men and females (Basaria *et al*. 2010).

Additionally, there are a variety of disorders that might cause the body to produce an excessive quantity of testosterone in the first place. Males who take anabolic steroids for an extended period of time have infertility, decreased sex drive, testicular atrophy, and increased breast development. It is possible that the liver will be damaged as a consequence of the liver's prolonged efforts to detoxify the anabolic steroids. It is also possible to notice behavioral changes, for example, increased irritation (Eisenegger *et al*. 2011).

Depression, anxiety, and insomnia are all symptoms of low testosterone levels. A minority of older men (about 2 percent), according to current research, are affected by this phenomenon. In order to understand more about testosterone's effects on older men and establish whether testosterone replacement therapy may be advantageous, a big research is being done (Tyagi *et al*. 2017).

2.2 Vitamin D

It is a fat-soluble vitamin that may be found in small amounts in a few foods naturally, supplemented in others, and consumed orally. It can be obtained from the sun, food, or supplements, but it is biologically inert and must be activated by the body through two hydroxylations (Norman *et al*. 2014).

Bones may become brittle, weak, and misshapen if they do not get enough vitamin D. Vitamin D deficiency protects both neonates and adults against the diseases rickets and osteomalacia. When combined with calcium, vitamin D has been shown to be useful in the prevention of osteoporosis in the elderly (Herrick *et al*. 2019).

2.2.1 Function and sources

It has been established that vitamin D is critical in the regulation of calcium and the maintenance of phosphorus levels in the blood. These components are required for the maintenance of bone strength. A vitamin D shortage in children may result in rickets, which causes the bones to become brittle and weak, giving the child a severely bowlegged look (Fetahu *et al*. 2014). Osteomalacia, also known as bone softening, is a condition that affects people who are deficient in vitamin D. Osteomalacia is a condition that results in muscular weakness and poor bone density (Hewison *et al*. 2017).

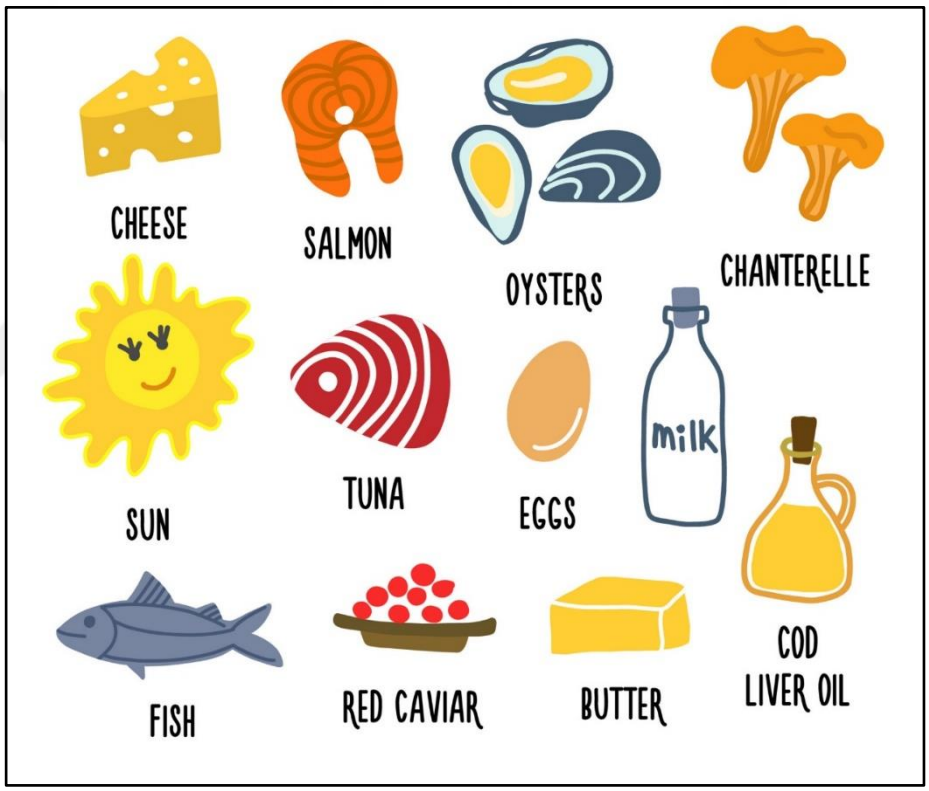

Figure 2.2 The common sources of Vitamin D (Amrein *et al*. 2020)

This ingredient can only be found in a restricted number of foods. One of the most abundant sources is fish liver oils, which may be found in the flesh of fatty fish (such as trout, salmon, tuna, and mackerel). It is the animal's diet that has an impact on the quantity of vitamin D in its tissues. Beef liver, cheese, and egg yolks all contain low quantities of vitamin D, which is mostly in the form of vitamin D3 and its metabolite

25(OH)D3. Vitamin D is also present in trace amounts in milk and cheese. Mushrooms contain various amounts of vitamin D2 depending on the kind. Some commercially available mushrooms have been exposed to ultraviolet radiation in order to increase their levels of vitamin D2 (Spiro and Buttriss 2014).

2.2.2 Deficiency

The body can produce vitamin D, but a deficit may arise for a number of causes, including exposure to the sun. For example, darker skin and sunscreen both lower the body's capacity to absorb ultraviolet B (UVB) photons emitted by the sun's ultraviolet B rays. In order for the skin to create vitamin D, it must be exposed to direct sunshine (Ebadi and Montano-Loza 2020). Using sunscreen with a sun protection factor (SPF) of 30 or higher may significantly limit the body's capacity to produce vitamin D by up to 95 percent. The use of garments to cover the skin may also inhibit the generation of vitamin D (Chang and Lee 2019).

It is recommended that those who live in northern latitudes or high-pollution regions, who work night shifts, or who are housebound get as much vitamin D as they can from dietary sources as feasible. It is necessary for babies who are exclusively breastfed, particularly if their skin is dark or they have little sun exposure. All nursing neonates, on the other hand, should get 400 international units (IU) of vitamin D orally every day while breastfeeding (Giustina *et al*. 2019).

2.3 Polycystic Ovary Syndrome (PCOS)

It is a common health condition that is caused by an imbalance of reproductive hormones. The ovaries get inflamed as a result of the hormonal imbalance. The ovaries are responsible for producing the egg that is discharged once a month as part of a normal menstrual cycle. As a result of PCOS, the egg either fails to grow properly or fails to release during ovulation in the manner that it should (Mykhalchenko *et al*. 2017).

It is a hormonal issue that is difficult to understand. 'Polycystic' is a medical term that literally means'many cysts.' These are the several partly formed follicles seen on the ovaries, each of which contains an egg, as the term suggests. These seldom mature or generate eggs that can be fertilized, and they are rarely seen in nature (Pasquali 2018).

Women with PCOS usually have high insulin levels that do not function properly, as well as high amounts of male hormones known as 'androgens,' or a combination of the two. Although the exact reason is not known, factors such as family history and genetics, hormones, and lifestyle all have a role. Insulin resistance may be seen in up to four out of every five women with PCOS, according to some estimates (Teede *et al*. 2018).

Women who have a mother, aunt, or sibling who has PCOS are 50% more likely to get the condition themselves. Women from Asian, Aboriginal and Torres Strait Islander, and African origins are more likely than other women to suffer from this illness (Rocha *et al*. 2019).

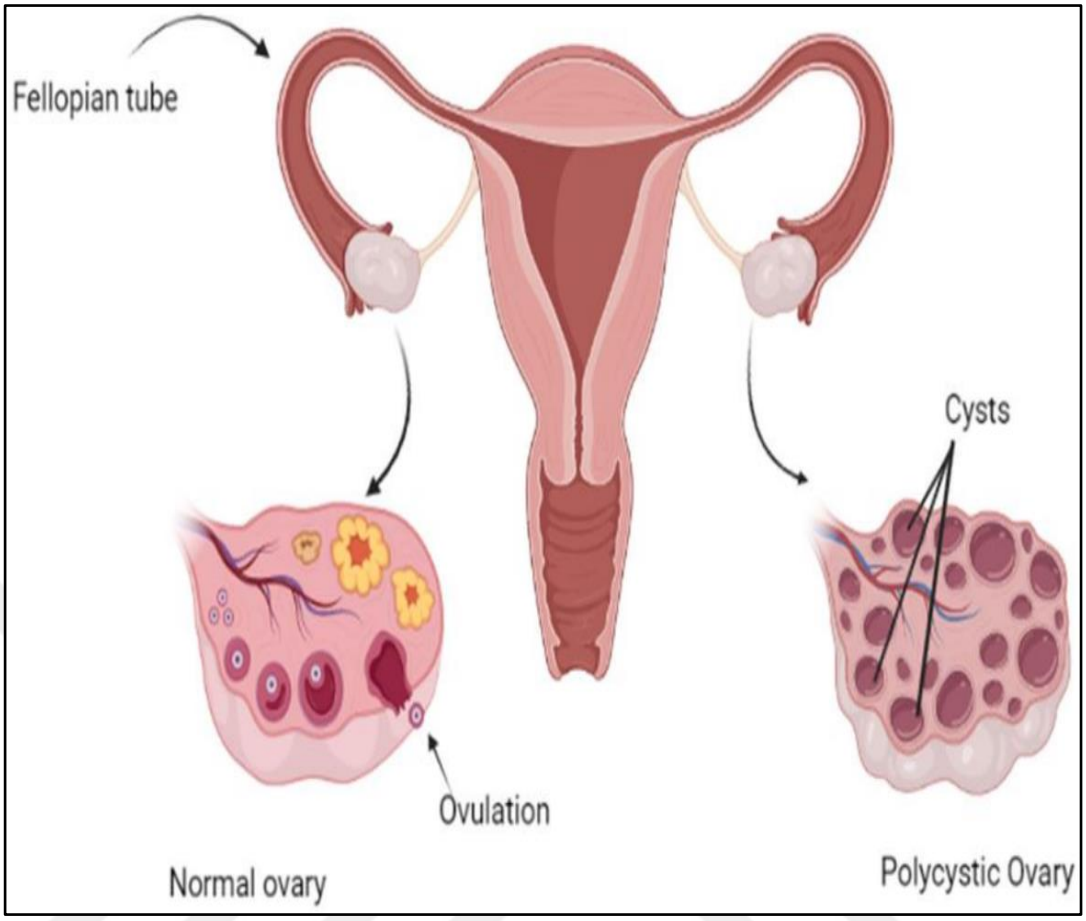

Figure 2.3 Normal versus PCOS (Macut *et al.* 2017)

Up to one-third of women may have polycystic ovaries, which may be detected by ultrasound, although not all of these women have PCOS. The three primary characteristics of PCOS are as follows (Louwers and Laven 2020):

- Irregular periods, which indicates that your ovaries do not release eggs on a regular basis (ovulation)
- High quantities of "male" hormones in your body may result in outward manifestations such as excessive face or body hair.
- Fold increase in size and number of fluid-filled sacs in ovaries is caused by polycystic ovarian syndrome.

2.3.1 Causes

Although the specific etiology of PCOS is unclear, it is known to run in families in certain cases. It is associated with aberrant hormone levels in the body, which include excessive insulin levels among other things. The insulin action in the body of many women with PCOS is inhibited, and as a result, they create greater quantities of insulin to overcome this resistance. Increased synthesis and action of hormones such as testosterone are facilitated as a result of this. Being overweight or obese also increases the quantity of insulin produced by your body, which is harmful (Bednarska and Siejka 2017).

The exact cause of PCOS is unknown at this time. The majority of scientists believe that many variables, including high levels of androgens, are responsible. Androgens are referred to as "man hormones" in certain circles, despite the fact that all women produce modest levels of androgens. Women with PCOS have higher levels of androgens than the general population (Bellver *et al*. 2018). PCOS is characterized by elevated androgen levels in females, in each and every menstrual cycle, which may prevent the ovaries from generating an egg (ovulation), as well as excessive hair growth and acne, all of which are signs of the disorder. Insulin is a hormone that controls the process by which your body transforms the fuel from the food you eat into useable form. Insulin resistance occurs when the cells of the body do not react appropriately to the hormone insulin. As a consequence, the insulin blood levels rise over what is considered normal (Lizneva *et al*. 2016).

2.3.2 Complications

If you have PCOS and your androgen levels are abnormally high, you are more likely to have a variety of issues. These might vary from woman to woman and include the following: Having difficulty becoming pregnant. Cysts in the ovaries may cause ovulation to be delayed or prevented. Each month, one of your ovaries will release an egg at this time, if you have two ovaries. You will not be able to get pregnant if a healthy egg is not accessible to be fertilized by a sperm. If having PCOS, it is possible

that the patient will be able to get pregnant. However, it is possible that you may need to take medication and work with a reproductive doctor to make it happen (Facchinetti *et al*. 2020).

Insulin resistance may lead your body to produce an excessive amount of androgens. Insulin resistance is a condition in which the cells in your muscles, organs, and other tissues are unable to take glucose from the bloodstream. Consequently, you may have an excessive amount of sugar traveling through your system. This is referred to as diabetes, and it may have negative consequences for your cardiovascular and neurological systems (Williams *et al*. 2016).

2.4 Biochemical Test

2.4.1 Follicle-stimulating hormone (FSH)

It is a hormone that plays a vital role in the development of the reproductive system. It has a role in the development of ovarian follicles in females. Women's menstrual periods are maintained by the production of estrogen and progesterone by the ovaries, which is controlled by the follicles. When it comes to males, FSH is involved in both the growth of the gonads and the generation of sperm (Hoare *et al*. 2015).

The FSH test is used to determine the amount of FSH present in the blood. During this test, your blood is drawn to determine the amount of follicle-stimulating hormone (FSH). It is produced by the pituitary gland, which is a tiny organ lying under the surface of the brain (Wang *et al*. 2016).

2.4.2 Estradiol

Estradiol is a hormone that affects the development and maintenance of feminine sex characteristics in both females and males. Because of its involvement in these activities, it is often referred to as the female hormone. Actually, estradiol is the most important

component of a triad of structurally similar estragens, which also contains estradiol and estradiol estriol, as well as estradiol and estradiol estriol. One group contains estradiol, which is the most androgenic of the three hormones, while the other two include estrone, which contains one group and estriol, which has three groups (Henderson *et al*. 2016).

In this test, however, the level of estradiol in a person's blood is measured using a simple blood test procedure. Estradiol, often known as E2, is one of the four forms of estrogen that are primarily produced by the ovaries and is one of the most important. This hormone is also produced in lower quantities by the adrenal glands, the placenta, the testicles, and other organs (Mauvais-Jarvis *et al*. 2020).

2.4.3 Blood glucose test

A blood glucose test is used to determine the amount of glucose in your blood. Glucose is a form of sugar that may be found in foods. A high or low level of glucose in the blood might indicate the presence of a potentially life-threatening medical illness. A blood glucose test is used to determine the quantity of glucose present in your blood. Glucose, which is a form of simple sugar, is the primary source of energy for your body. The carbs you consume are converted into glucose by the body. Using the test, you may determine whether or not your blood sugar levels are within normal range (Santen 2015).

2.4.4 Cadmium (Cd)

Cadmium (Cd) is resistant to corrosion and has been used to create batteries, pigments, metal coatings, and polymers, among other things. It is a chemical element that has the symbol Cd and the atomic number 48. It is found in the chemical element cadmium. This soft, silvery-white metal is chemically identical to the other two stable metals and seems to be the same color as the other two stable metals as well (Perales-Puchalt *et al*. 2017).

Exposure to cadmium occurs mostly at places of employment where cadmium-containing goods are manufactured. Inhalation of dust and fumes, as well as inadvertent ingestion of dust via contaminated hands, cigarettes, or food, are the most common routes of occupational exposure to asbestos. Cadmium exposure in the general population is mostly caused by cigarette smoke inhalation and consumption of cadmium-contaminated foods, which is the primary source of cadmium exposure for nonsmokers. Additionally, the growing nickel-cadmium (NiCd) battery recycling business may expose workers to nickel–cadmium compounds (Kim *et al*. 2017).

3. MATERIALS AND METHODS

3.1 Material

3.1.1 The study groups

A total of 120 samples will be selected from among the patients admitted to MADINAT Al TIP hospital in Baghdad. Then the patients will be divided into two groups, an experimental group (80 patiants) and a control group (40 patients), the function of control group help ensure the internal validity of the study.

3.1.2 Blood samples

The blood samples of this study were obtained from the selected patients (5 mL) by using sterile syringes from brachial vein, and placed in tubes, then left in the tube at room temperature for 30 minutes in order to coagulate the blood and centrifuge the samples centrifuge at (3000 rpm / min) for 5 minutes to separate serum from other components of blood, the serum will be separated and withdrawn serum by micropipette then place in other tubes for conduct biochemical tests.

3.2 Methods

3.2.1 Vitamin D Test

After taking a blood sample and placing it in a tube with a gold-top serum separator, we waited for it to clot. The samples are going to be centrifuged for ten minutes at a range of 1100 to 2000 g. After that, the serum will first be removed from the cells as soon as possible and then chilled between 2 and 8 degrees Celsius before being processed. Samples that were excessively haemolyzed, lipemic, or had particle matter were not accepted. An electrochemiluminescence immunoassay will be performed on an Elecsys

2010 Analyzer (Roche Diagnostics, Mannheim, Germany) in order to determine the amounts of 25-hydroxyvitamin D (25 (OH) vitamins.:

Insert the test curve card into the analyzer and click “Read Card“. Input the sample information including sample number, type and test item, then we can start the sample treatment:

A. ADD 20 μL Sample to the buffer A.
B. Use a new tipper and add 30 μL buffer B to the previos mixture.
C. Using a mixture for 5 second to mix the sample throughly.
D. Put the sample in the incubator and start dissociation for 15 minutes. The incubaoer will count down automatically as setting.
E. After the dissociation finishes, get the sample and add 80 μL sample to the cassette
F. Insert the cassette in to the incubator and start incubation for 10 minutes.
G. After the incubation finish, insert the cassette into the analyzer and click “Quick Test“. The machine will test and print the result automatically.

3.2.2 Blood glucose test (BGT)

The test that determines the amount of sugar in blood samples is the fast blood sugar assay, this test detects the quantity of glucose in samples, The blood sugar chart includes explanations of various blood sugar readings based on their representation in milligrams per deciliter. Collect 3-5 mL blood in a red top tube at any time of the day. Centrifuge the tube for 2 minutes and Use the serum for test, record the result.

3.2.3 HbA1c test

The HbA1c test were measured the average blood glucose levels by taking a samples of blood from the tested groups and analyse the results:

1. Insert the cartridge into a chamber at 30 C halfway.

2. Draw 100 µL of hemolysis buffer and dispose it into the detection buffer tube.
3. Take 5 µL of blood with the help of capillary tube from the finger prick.
4. Insert the capillary tube into the buffer tube and shake it 15 times.
5. Draw 75 µL of sample mixture and dispose it onto the cartridge sample well.
6. Take off the cartridge from i chamber and dispose the mixture onto it, i chamber temperature must be at 30 C.
7. Verify that the mixture flow well on the membrane of the cartridge (+/- 10 seconds).
8. After incubation time (12 minutes at 30 C) withdraw the cartridge and run the test on i chroma.
9. After running the test, results will bediplayed on screen.

3.2.4 FSH and LH assay

After securing the desired number of wells in the holder, transfer 50 L of the standard, specimens, and controls into each of the wells. Next, dispense 100 L of enzyme conjugate reagent into each well and admix for 30 minutes. Next, incubate the mixture at 25 °C for 45 minutes before removing it. After rinsing the wells five times with distilled water, add 100 L of TMB reagent into each After incubating the sample at 25 degrees Celsius for twenty minutes, add 100 microliters (L) of the stop solution to each well, admix for thirty seconds, and then measure the absorbance at 450 nanometers (nm) using a microtiter plate reader.

Reagents for FSH and LH assay

Mouse monoclonal anti- α – FSH antibody coated microtiter plate. Enzyme conjucate reagent 13 mL. FSH standards, LH standards, containing 0, 5, 15, 50, 100, and 200 mLU/ mL of human FSH and human LH.

3.2.5 Calcium test

Prepare a three test tube (blank, standard and specimen) and add to each tube 0.5 mL of working reagent no.1 and 0.5 mL of working reageng no.2 . Add 25 µL of distilled water to blank tube, 25 µL of standard solution to standard yube, and 25 µL of blood

sample to specimen tube. Incubate the tubes for 5 minutes under room temperature and read the absorbance at 500 nm and record the results, the Calcium test concentration can be calculated according to Equation (3.1):

$$\text{Calcium (mg / dL)} = (\text{A specimen / A standard}) \times \text{Concentration (Standard)} \quad (3.1)$$

3.2.6 Estradiol hormone test

Reagents

1. Microtiter wells that have been coated with goat anti-rabbit IgG.
2. Estradiol reference standards with concentrations of 0, 10, 30, 100, 300, and 1000 pg/mL, each in a liquid form of 0.5 milliliter and ready for application
3. Rabbit anti-estradiol reagent, 0.7 mL.
4. Estradiol-HRP conjugate reagent, 12 mL.
5. Estradiol control 1, liquid 0.5 mL, ready to use.
6. Estradiol control 2, liquid 0.5 mL, ready to use.
7. TMB reagent, 11 mL total volume
8. Stop solution, 11 milliliters.

Procedure

After securing the necessary number of wells in the holder, dispense 25 L of the standard, the specimens, and the controls into each well. Next, dispense 100 L of the estradiol-HRP conjugate reagent into each well, and then dispense 50 L of the rabbit-anti estradiol reagent to each well. Remove the mixture after it has been incubated at 25 degrees Celsius for an hour and a half, mix it for thirty seconds, and then rinse it with distilled water five times. After adding 100 L of the TMB reagent to each well, mix the contents for ten seconds. After incubating the sample at 25 degrees Celsius for twenty minutes, add 100 microliters (L) of the stop solution to each well, admix for thirty seconds, and then measure the absorbance at 450 nanometers (nm) using a microtiter plate reader.

3.2.7 Testosterone assay procedure

Secure the desired number of coated wells in the holder, dispense 10 µL of standards, specimens and controls into the wells. Dispense 100 µL of testosterone-HRP conjugate reagent into each well. Dispense 50 µL of Rabbit anti-testosterone reagent to eacg well. Incubate the wells at 37 °C for 90 minutes. Rinse and flick the microwells five times with distilled water. Dispense 100 µL of TMB reagent into each wells, mix for 10 seconds, then incubate at 25 °C for 20 minutes ,stop the reaction by adding 100 µL of stop solution mix for 10 seconds. Read the absurbance at 450 nm with a microtiter well reader immediatelly .

Reagents

1. Microtiter wells that have been coated with goat anti-rabbit IgG.
2. Testosterone reference standards consisting of 0 ng/ mL liquid, 0.1 ng/ mL liquid, 0.5 ng/ mL liquid, 2.0 ng/ mL liquid, 6.0 ng/ mL liquid, and 18.0 ng/ mL liquid, each ready to use 0.5
3. a volume of 7.0 milliliters of rabbit anti-teasosterone reagent.
4. Testosterone =HRP conjugate reagent, 12.0 mL.
5. Testosterone control 1, lquid, 0.5 mL ready to use.
6. Testosterone control 2, lquid, 0.5 mL ready to use.
7. TMB reagent, 11.0 mL total volume
8. A volume of 11.0 mL of the stop solution (1N HCL)..

3.2.8 Anti mullerian hormone (AMH) test

Reagents

R1a consists of Dynabeads particles that have been coated with monoclonal anti-AMH and are suspended in Tris buffer including surfactant, bovine protein, 0.1 percent sodium azide, and 0.1 percent proClin 300.

R1b: Anti-AMH alkaine phosphatase conjugate in MES buffer, surfactant, protein (bovine), 0.1 percent Sodium azide, 0.1 percent proClin 300.

R1c consists of Tris buffer with a surfactant, proteins derived from murine and bovine sources, 0.1 percent sodium azide, and 0.1 percent proClin.

Procedure

Collect 3-5 mL of blood sample in gel tube or green top tube.

Centrifuge for five minutes at 3000 rpm and use the serum to calculate the AMH concetration and print the results. The results will be determined automatically (ng / mL) the amount of AMH in the sample.

3.2.9 Lead test

1. Label a treatment reagent tube tube “level 1 control“, mix in the white top vial, then place top on clean surface.
2. Insert capillary tube with the green band out, fill to the black line.
3. Wipe out side of tube to remove excess, and place capillary in reagent tubeand insert black plunger, then dispense entire volume .
4. Replace top ofreagent tube, invert tube eight times to mix completelly.
5. Remove sensor from the container, be sure to close the container, and insert sensor into the analyzer until it beeps.
6. Open reagent, take dropper from blue container, squeeze the sides and insert into the tube, release to fill.
7. After the beep, sample analysis starts automatically, the display counts down from 180 seconds, results will displayed in microgram per deciliter.
8. Repeat the same process for level 2 control.

Blood sample testing

Prepare clean finger, use fleshy part and collect blood sample, place capillary tube in tratment reagent vial.

Insert plunger into capillary tube, push down and analyzed the sample and then record the results, the results are displayed in microgram of lead per deciliter (µg / dL) of whole blood.

4. RESULTS AND DISCUSSION

4.1 Results

4.1.1 Age

For the patients with PCOs, age was measured in two groups of women (patient and controls) to discover the effect the poly cystic ovarian syndrome on women. The age mean and standard deviation of these studied groups were a significant difference when compared to control group (29.0000 ± 3.11677) and (33.0000 ± 4.70976) respectively, the results of mean of age for all studied group and the comparsion are show clearly in Figure 4.1 and Table 4.1.

Describtive statstics of age that recorded in women with PCOs and without PCOs in Iraq including 95% Confidence Interval for Mean upper and lower bound, Median, Variance, Minimum and Maximum, Range, and other statistics with Std. Error were tabulated in Table 4.2.

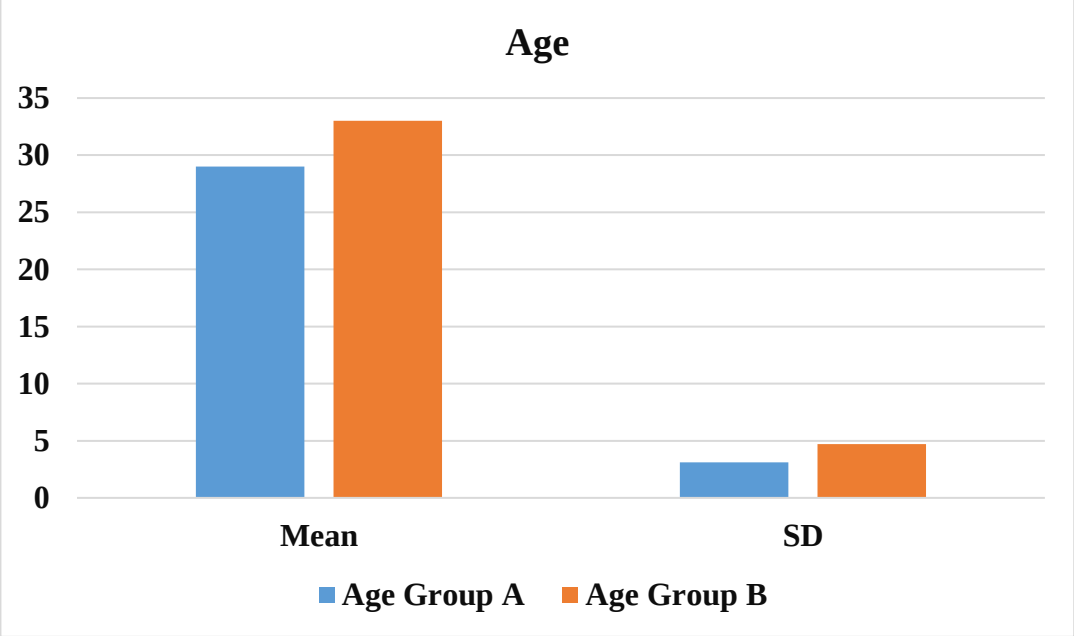

Figure 4.1 The mean and standard deviation of age in women with PCOs and without PCOs

Table 4.1 Mean and standard deviation of women with pregnant and without pregnant in Iraq

Group Statistics						
	Group	**N**	**Mean**	**Std. Deviation**	**Std. Error Mean**	**P-value**
Age	Group A	40	29.0000	3.11677	1.10195	0.035
	Group B	80	33.0000	4.70976	1.35959	0.035
FSH	Group A	40	5.3891	2.04107	0.72163	0.013
	Group B	80	8.5425	2.85202	.82331	0.013
LH	Group A	40	9.5924	3.25027	1.14914	0.000
	Group B	80	20.8248	5.87530	1.69605	0.000
Testeo	Group A	40	137.7832	43.0434	15.21817	0.000
	Group B	80	66.5872	20.5763	5.93988	0.002
E2	Group A	40	162.1239	90.7284	32.07736	0.032
	Group B	80	97.8834	27.5308	7.94747	0.088
AMH	Group A	40	1.2123	1.06033	0.37488	0.048
	Group B	80	2.4574	1.41096	0.40731	0.038
RBS	Group A	40	90.1945	12.9350	4.57322	0.756
	Group B	80	91.9367	11.5542	3.33542	0.763
HbA1c	Group A	40	4.0416	0.91883	0.32486	0.840
	Group B	80	4.1418	1.15614	0.33375	0.832
Ca	Group A	40	8.7071	0.56587	0.20007	0.201
	Group B	80	8.2066	0.95639	0.27609	0.159
cadmium	Group A	40	0.3389	0.00918	0.14467	0.752
	Group B	80	0.3926	0.03575	0.09692	0.763
Pb	Group A	40	0.6852	0.31949	0.11296	0.089
	Group B	80	1.3800	1.05401	0.30427	0.089
Catalase	Group A	40	83.3379	11.6686	4.12551	0.001
	Group B	80	104.7204	6.34792	1.83249	0.001
Vit.D	Group A	40	13.9166	3.98424	1.40864	0.945
	Group B	80	14.0453	4.06037	1.17213	0.945

Table 4.2 Describtive statstics of age recorded in women with PCOs and without PCOs in Iraq

		F	t	df	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
							Lower	Uper
Age	EVA	2.276	-2.105	18	-4.0000	1.9002	-7.9923	-.00763
	EVnA		-2.286	17.996	-4.00000	1.75008	-7.67684	-.32316
FSH	EVA	1.308	-1.838	18	-2.15336	1.17180	-4.61521	.30849
	EVnA		-1.967	17.844	-2.15336	1.09480	-4.45488	.14817
LH	EVA	2.826	-4.902	18	-11.2324	2.29144	-16.0465	-6.41831
	EVnA		-5.483	17.592	-11.2324	2.04869	-15.5437	-6.92114
Testeo	EVA	2.352	4.985	18	71.1960	14.28320	41.1881	101.2039
	EVnA		4.358	9.160	71.1960	16.33631	34.3389	108.0531
E2	EVA	24.26	2.325	18	64.2405	27.62996	6.19211	122.2888
	EVnA		1.944	7.867	64.2405	33.04723	-12.1915	140.6725
AMH	EVA	1.150	-2.121	18	-1.24510	.58698	-2.47830	-.01190
	EVnA		-2.249	17.639	-1.24510	.55357	-2.40981	-.08039
RBS	EVA	.053	-.315	18	-1.74225	5.52740	-13.3548	9.87039
	EVnA		-.308	13.921	-1.74225	5.66033	-13.8889	10.40441
HbA1c	EVA	.599	-.205	18	-.10016	.48844	-1.12634	.92602
	EVnA		-.215	17.306	-.10016	.46575	-1.08148	.88116
Ca	EVA	3.849	1.327	18	.50058	.37735	-.29221	1.29337
	EVnA		1.468	17.851	.50058	.34095	-.21617	1.21733
Cd	EVA	1.486	-.321	18	-.05369	.16708	-.40471	.29734
	EVnA		-.308	13.025	-.05369	.17413	-.42981	.32243
Pb	EVA	7.903	-1.796	18	-.69487	.38692	-1.50776	.11802
	EVnA		-2.141	13.828	-.69487	.32456	-1.39179	.00205
Catalase	EVA	5.190	-5.319	18	-21.3825	4.02016	-29.8286	-12.93656
	EVnA		-4.737	9.792	-21.3825	4.51418	-31.4698	-11.29535
Vit.D	EVA	.001	-.070	18	-.12873	1.83986	-3.99414	3.73668
	EVnA		-.070	15.363	-.12873	1.83253	-4.02666	3.76920

EVA= Equal variances assumed
EVnA= Equal variances not assumed

4.1.2 Follicle-stimulating hormone (FSH)

For the patients with PCOs, FSH was measured in two groups of women (patient and controls) to discover the effect the poly cystic ovarian syndrome on women. The FSH mean and standard deviation of these studied groups were a high significant difference when compared to control group (5.3891 ± 2.04107) and (7.5425 ± 2.85202) respectively, the results for the mean and SD level of follicle-stimulating hormone (FSH) related for selected studied groups are show clearly in Table 4.1 and were confirmed Figure 4.2.

Describtive statstics of FSH that recorded in women with PCOs and without PCOs in Iraq including 95% Confidence Interval for Mean upper and lower bound, Median, Variance, Minimum and Maximum, Range, and other statistics with Std. Error were tabulated in Table 4.2.

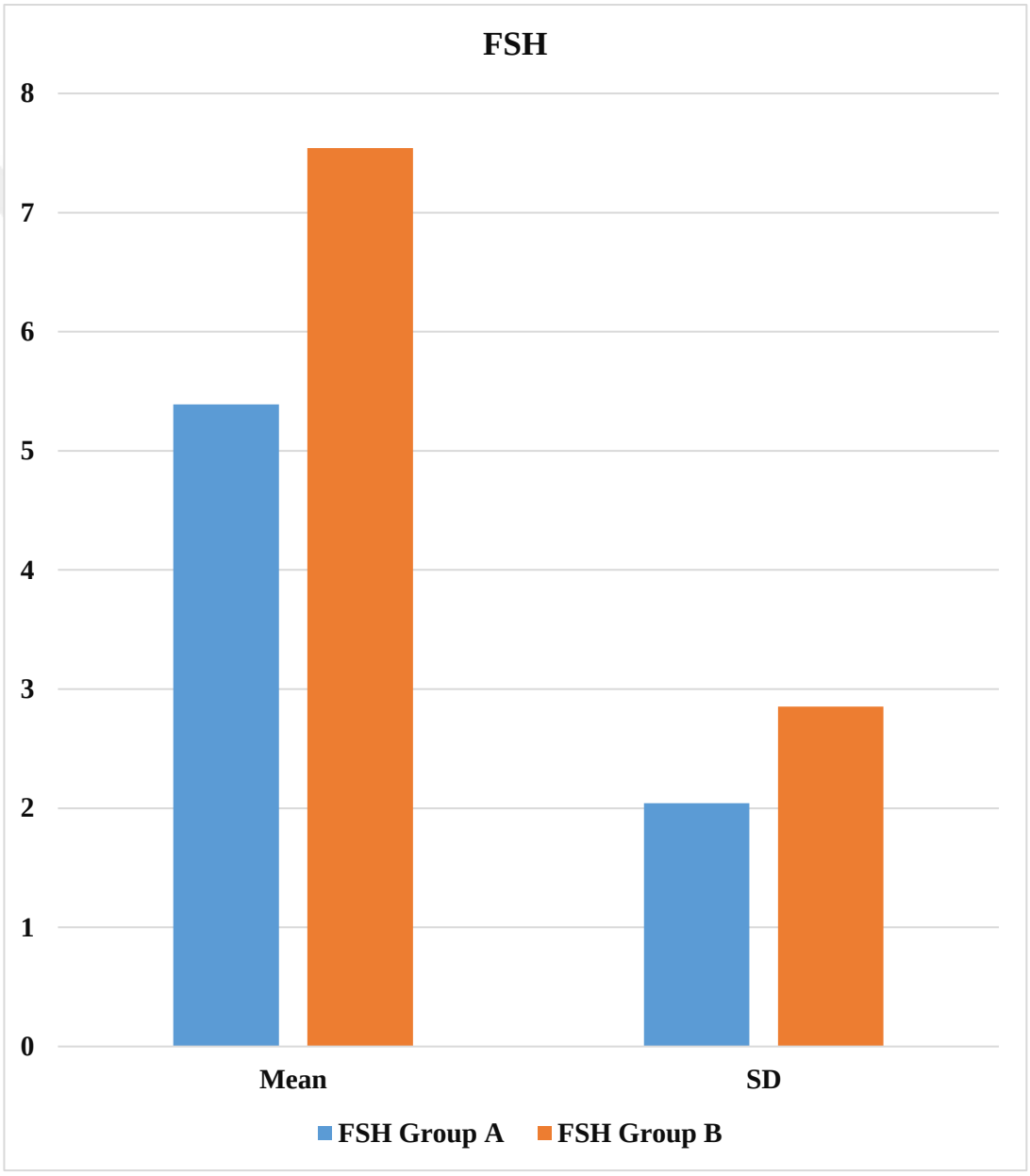

Figure 4.2 The mean and standard deviation of FSH in women with PCOs and without PCOs

4.1.3 Luteinizing hormone (LH)

For the patients with PCOs, LH was measured in two groups of women (patient and controls) to discover the effect the poly cystic ovarian syndrome on women. The LH mean and standard deviation of these studied groups were a significant difference when compared to control group (9.5924 ± 3.25027) and (20.8248 ± 5.87530) respectively, the results for the mean and SD level of luteinizing hormone (LH) related for selected studied groups are show clearly in Table 4.1 and were confirmed Figure 4.3.

Describtive statstics of LH that recorded in women with PCOs and without PCOs in Iraq including 95% Confidence Interval for Mean upper and lower bound, Median, Variance, Minimum and Maximum, Range, and other statistics with Std. Error were tabulated in Table 4.2.

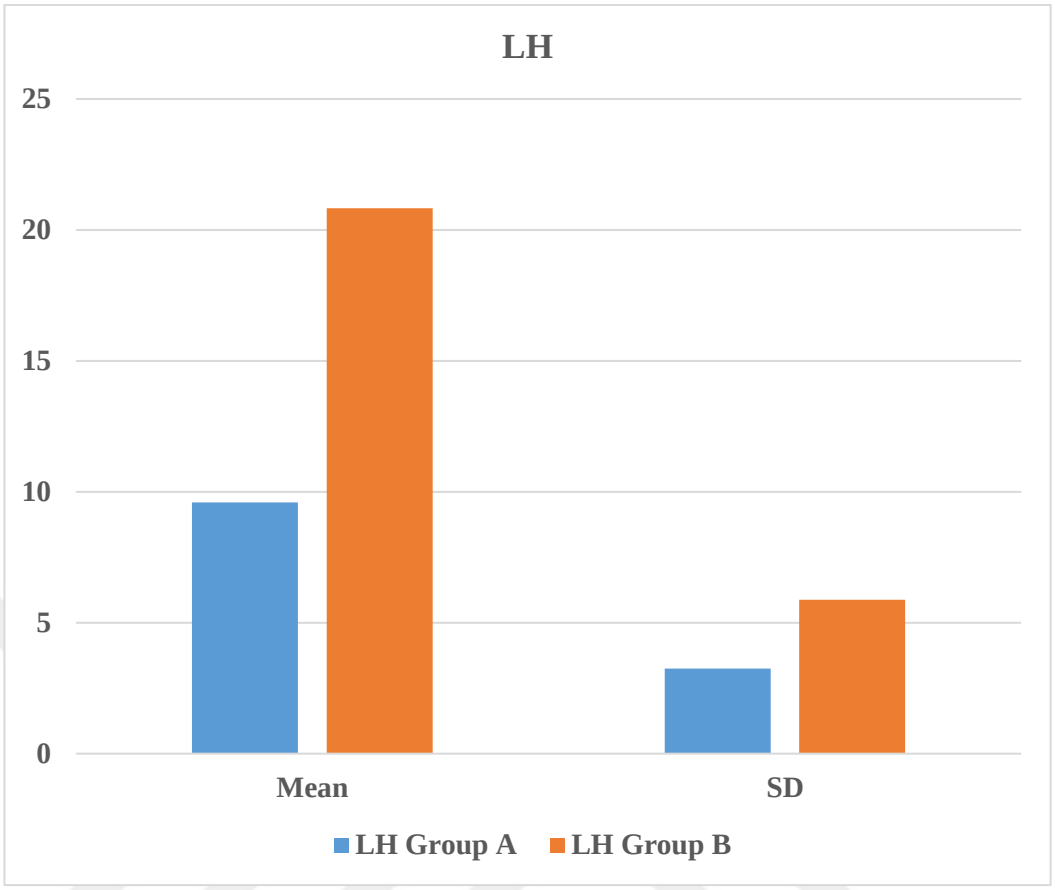

Figure 4.3 The mean and standard deviation of LH in women with PCOs and without PCOs

4.1.4 Testeosterone

The testosterone in women with PCOs and without PCOs groups were recorded as (137.7832 ± 43.04349) and (66.5872 ± 20.57636) and was significant difference as a shown in Table 4.1. Describtive statstics of testeosteron that recorded in women with PCOs and without PCOs in Iraq including 95% Confidence Interval for Mean upper and lower bound, Median, Variance, Minimum and Maximum, Range, and other statistics with Std. Error were tabulated in Table 4.2, the results for the mean and SD level of testosterone in women with PCOs and without PCOs groups related for selected studied groups are show clearly in Table 4.1 and were confirmed Figure 4.4.

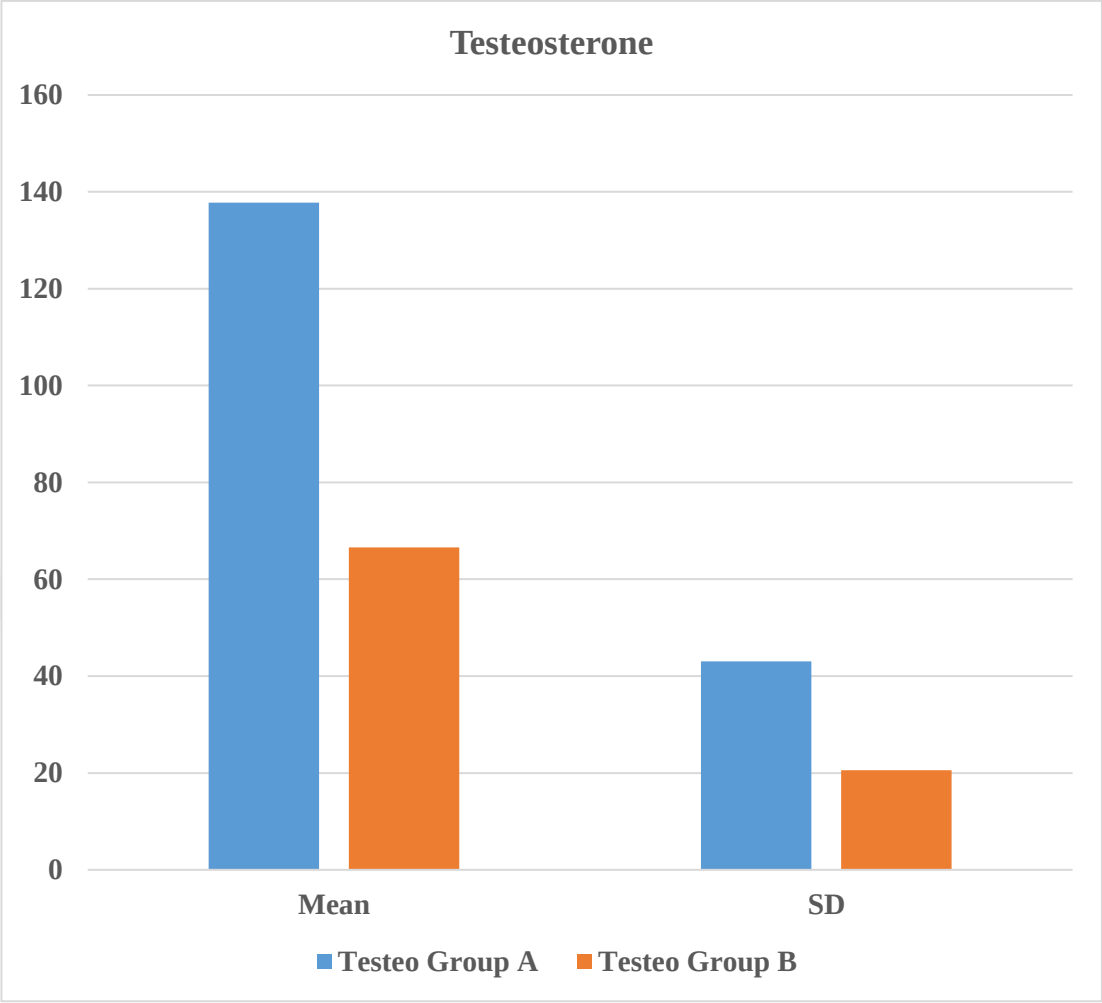

Figure 4.4 The mean and standard deviation of Testeosterone in women with PCOs and without PCOs

4.1.5 E2

The E2 in women with PCOs and without PCOs groups were recorded as (162.1239 ± 90.7284) and (97.8834 ± 27.5308) and was significant difference as a shown in Table 4.1, the results for the mean and SD level of E2 in women with PCOs and without PCOs groups are show clearly in Table 4.1 and were confirmed Figure 4.5.

Describtive statstics of E2 that recorded in women with PCOs and without PCOs in Iraq including 95% Confidence Interval for Mean upper and lower bound, Median, Variance, Minimum and Maximum, Range, and other statistics with Std. Error were tabulated in Table 4.2.

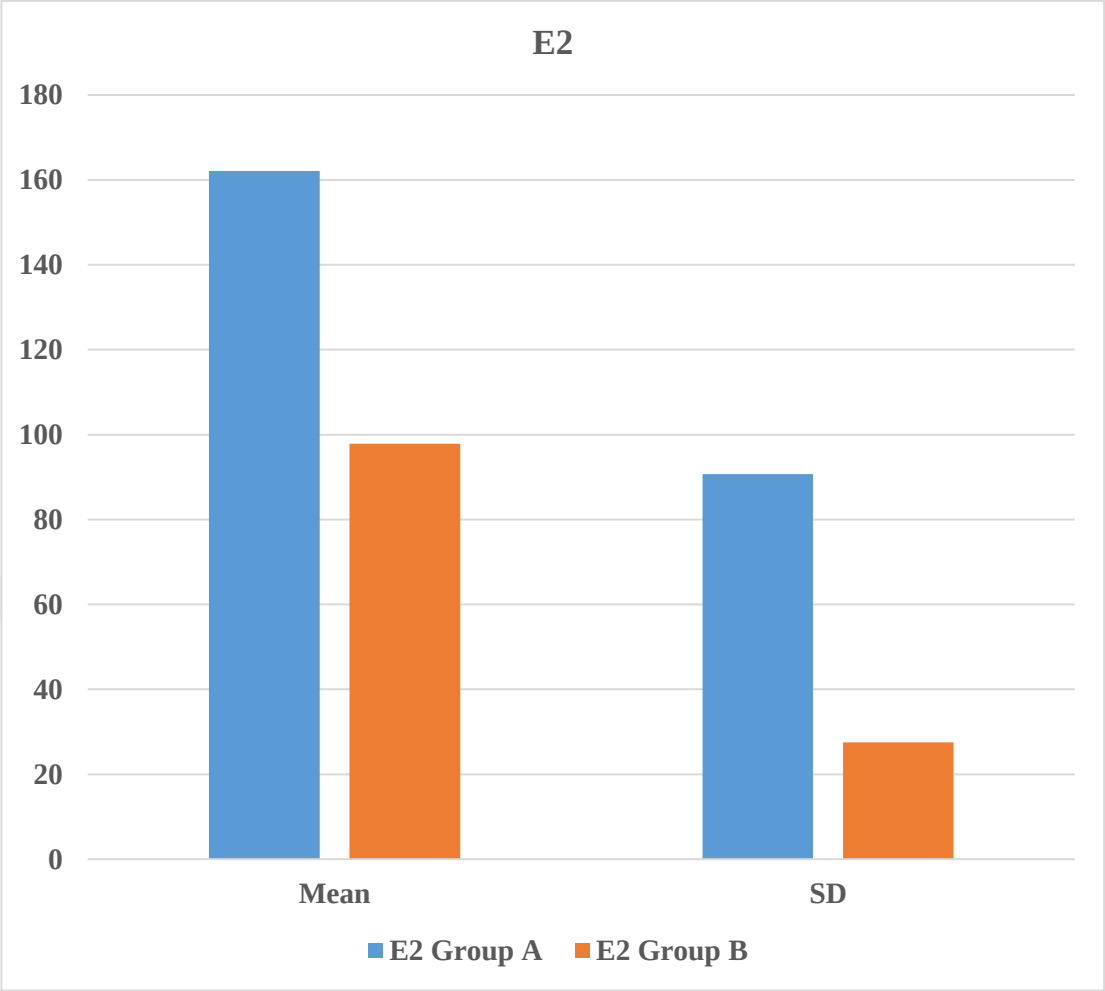

Figure 4.5 The mean and standard deviation of E2 in women with PCOs and without PCOs

4.1.6 Anti-Mullerian hormone (AMH)

AMH levels were recorded in women with PCOs and without PCOs groups groups as (1.2123 ± 1.06033) and (2.4574 ± 1.41096), this results was low significant statistic when compared with control group as in Table 4.1, these results were confiremed in Figure 4.6 for the levels of AMH to the all studied groups.

Describtive statstics of AMH that recorded in women with PCOs and without PCOs in Iraq including 95% Confidence Interval for Mean upper and lower bound, Median, Variance, Minimum and Maximum, Range, and other statistics with Std. Error were tabulated in Table 4.2.

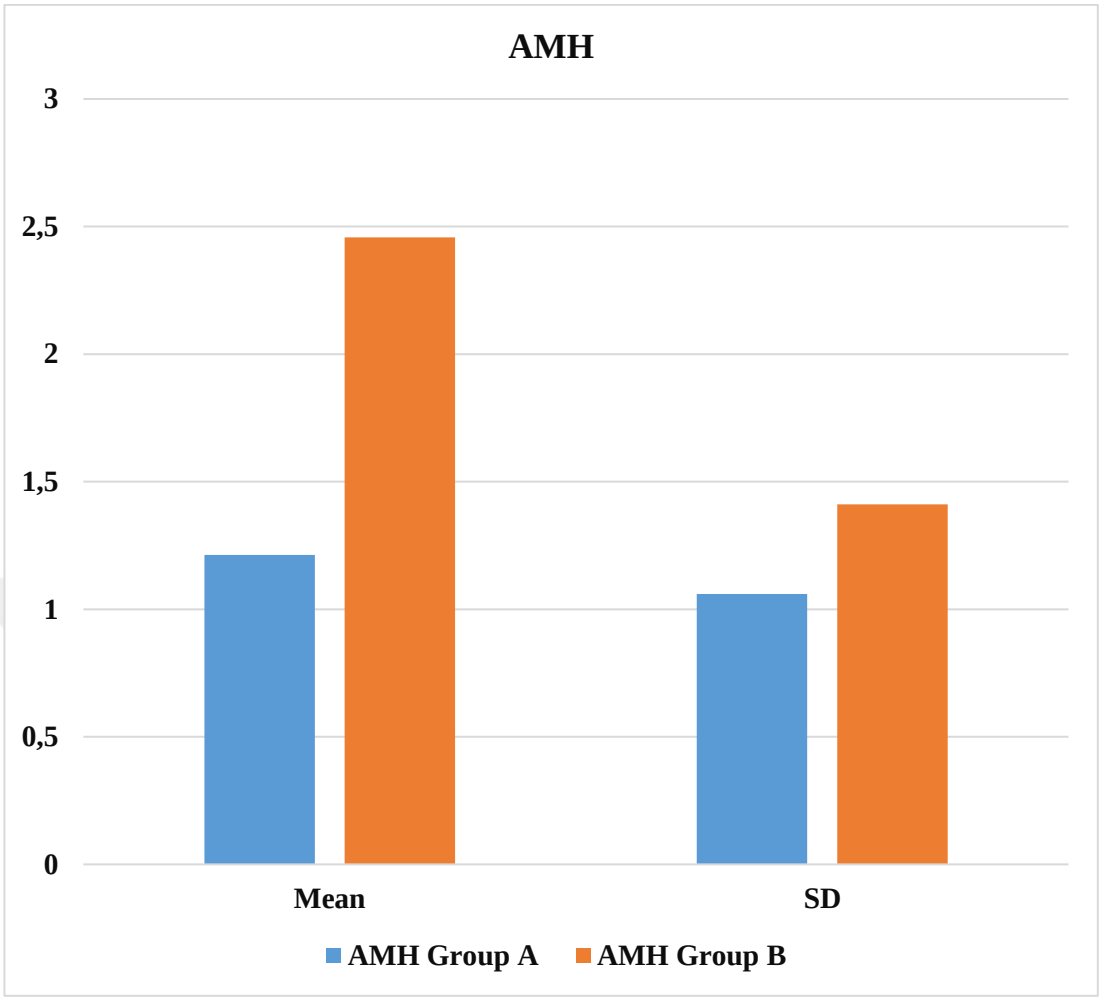

Figure 4.6 The mean and standard deviation of AMH in women with PCOs and without PCOs

4.1.7 Random bloos sugar RBS

Glucose levels were recorded in women with PCOs and without PCOs groups groups as (90.1945 ± 12.9350) and (91.9367 ± 11.5542), this result indicated to non-significant difference as in Table 4.1, these results were confiremed in Figure 4.7.

Describtive statstics of glucose that recorded in women with PCOs and without PCOs in Iraq including 95% Confidence Interval for Mean upper and lower bound, Median, Variance, Minimum and Maximum, Range, and other statistics with Std. Error were tabulated in Table 4.2.

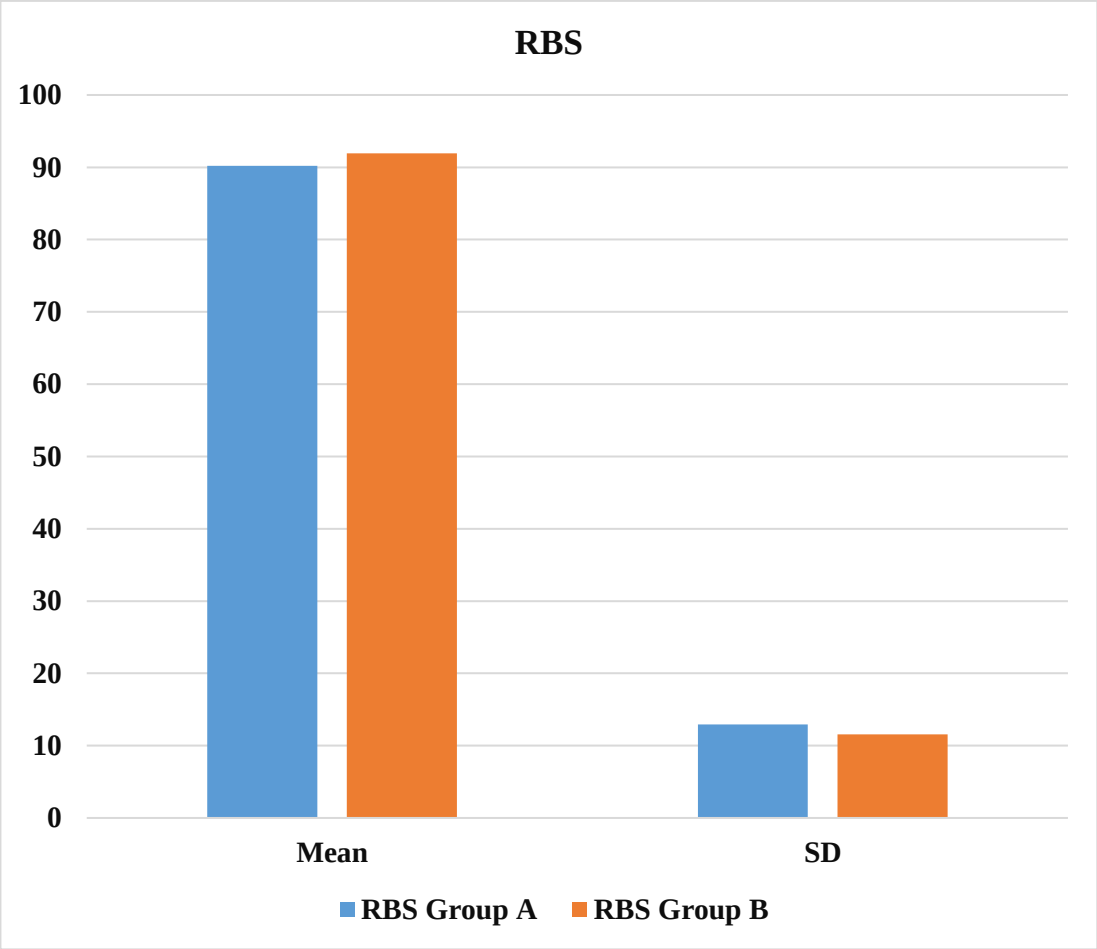

Figure 4.7 The mean and standard deviation of RBS in women with PCOs and without PCOs

4.1.8 HbA1c

HbA1c levels were recorded in women with PCOs and without PCOs groups groups as (4.0416 ± 0.91883) and (4.1418 ± 1.15614), was non-significant difference as in Table 4.1, and were confiremed in according to Figure 4.8

Describtive statstics of HbA1c that recorded in women with PCOs and without PCOs in Iraq including 95% Confidence Interval for Mean upper and lower bound, Median, Variance, Minimum and Maximum, Range, and other statistics with Std. Error were tabulated in Table 4.2.

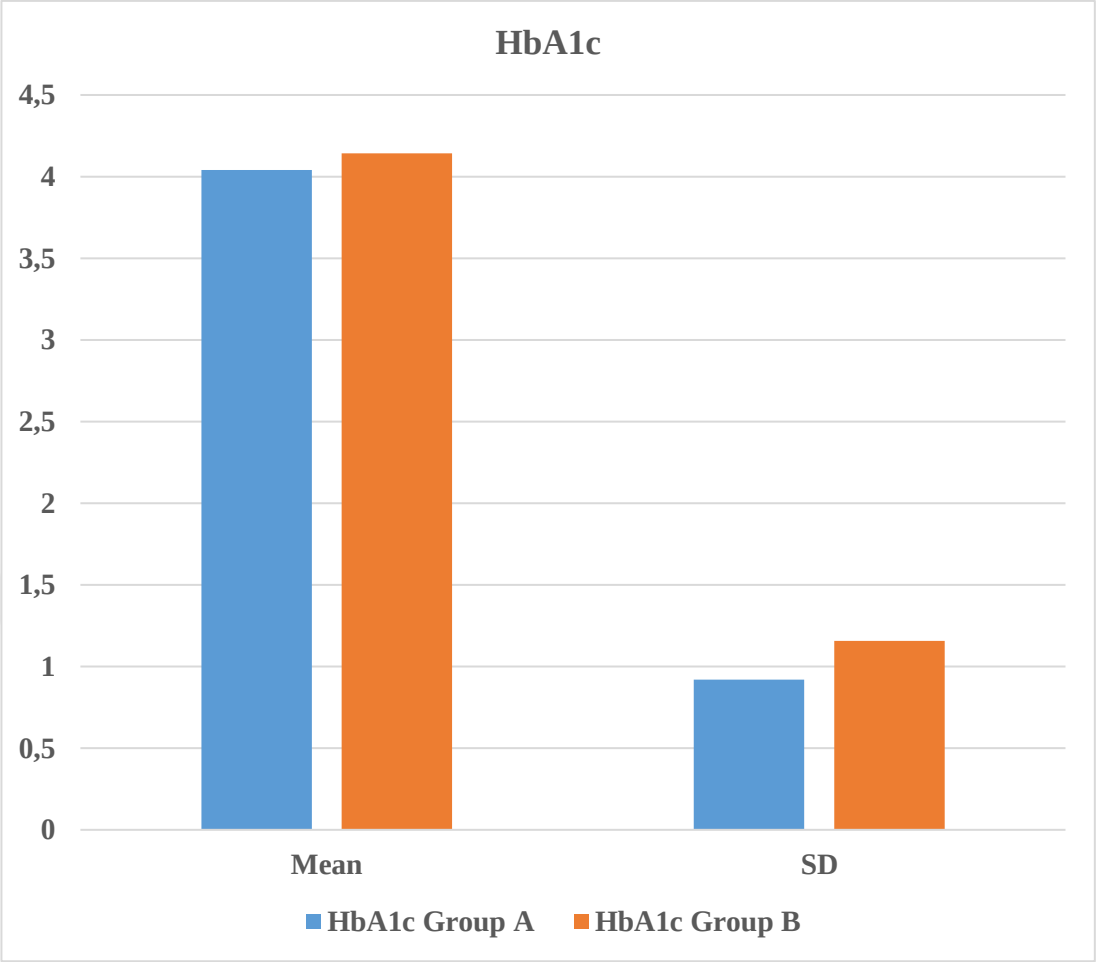

Figure 4.8 The mean and standard deviation of HbA1c in women with PCOs and without PCOs

4.1.9 Calcium (Ca)

Calcium levels were recorded in women with PCOs and without PCOs groups groups as (0.3389 ± 0.00918) and (0.3926 ± 0.03575), was a non-significant difference when compared to control group as in Table 4.1, these results were confirmed according to Figure 4.9.

Describtive statstics of calcium that recorded in women with PCOs and without PCOs in Iraq including 95% Confidence Interval for Mean upper and lower bound, Median, Variance, Minimum and Maximum, Range, and other statistics with Std. Error were tabulated in Table 4.2.

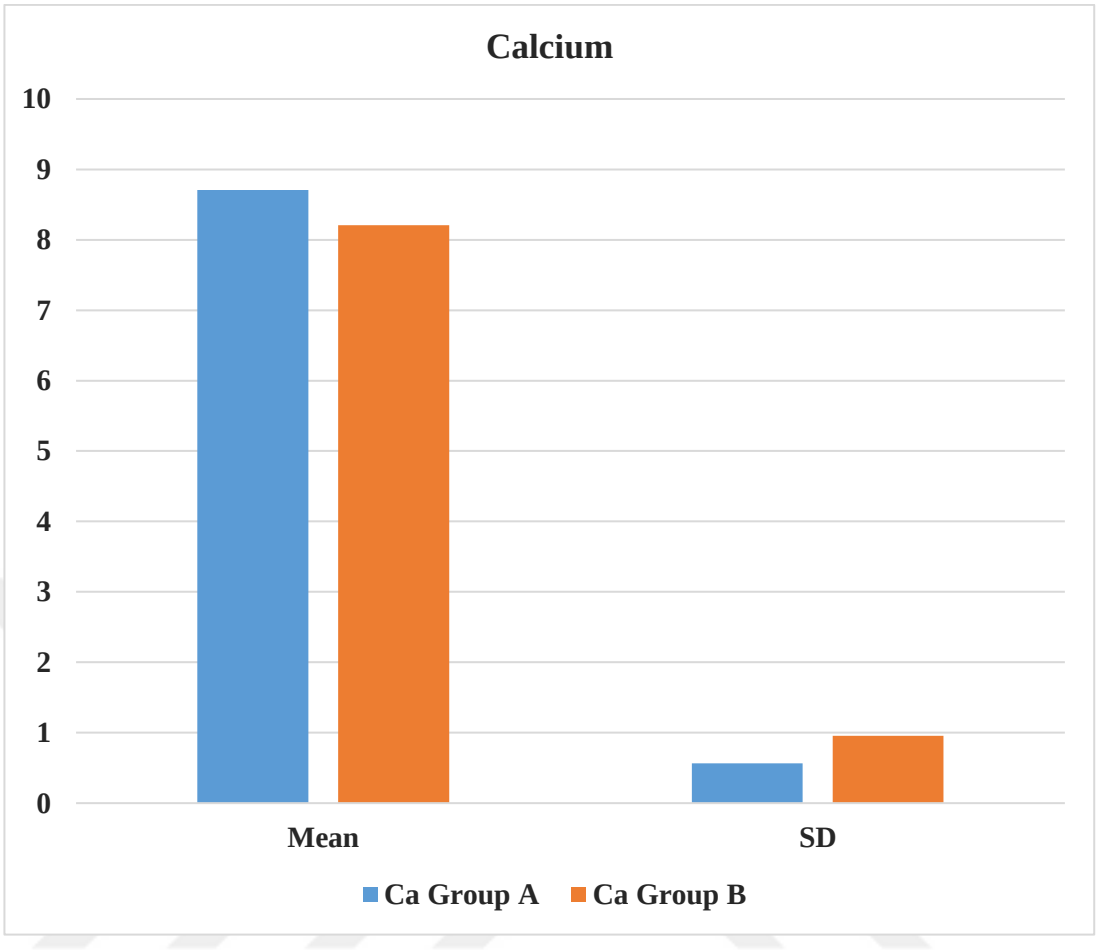

Figure 4.9 The mean and standard deviation of Calcium (Ca) in women with PCOs and without PCOs

4.1.10 Cadmium (Cd)

cadmium levels recorded in women with PCOs and without PCOs groups as (4.8 ± 2.39) and (3.1 ± 1.58), these results were non-significant difference as shown in Table 4.1, and were confiremed according to Figure 4.10.

Describtive statstics of cadmium that recorded in women with PCOs and without PCOs in Iraq including 95% Confidence Interval for Mean upper and lower bound, Median, Variance, Minimum and Maximum, Range, and other statistics with Std. Error were tabulated in Table 4.2.

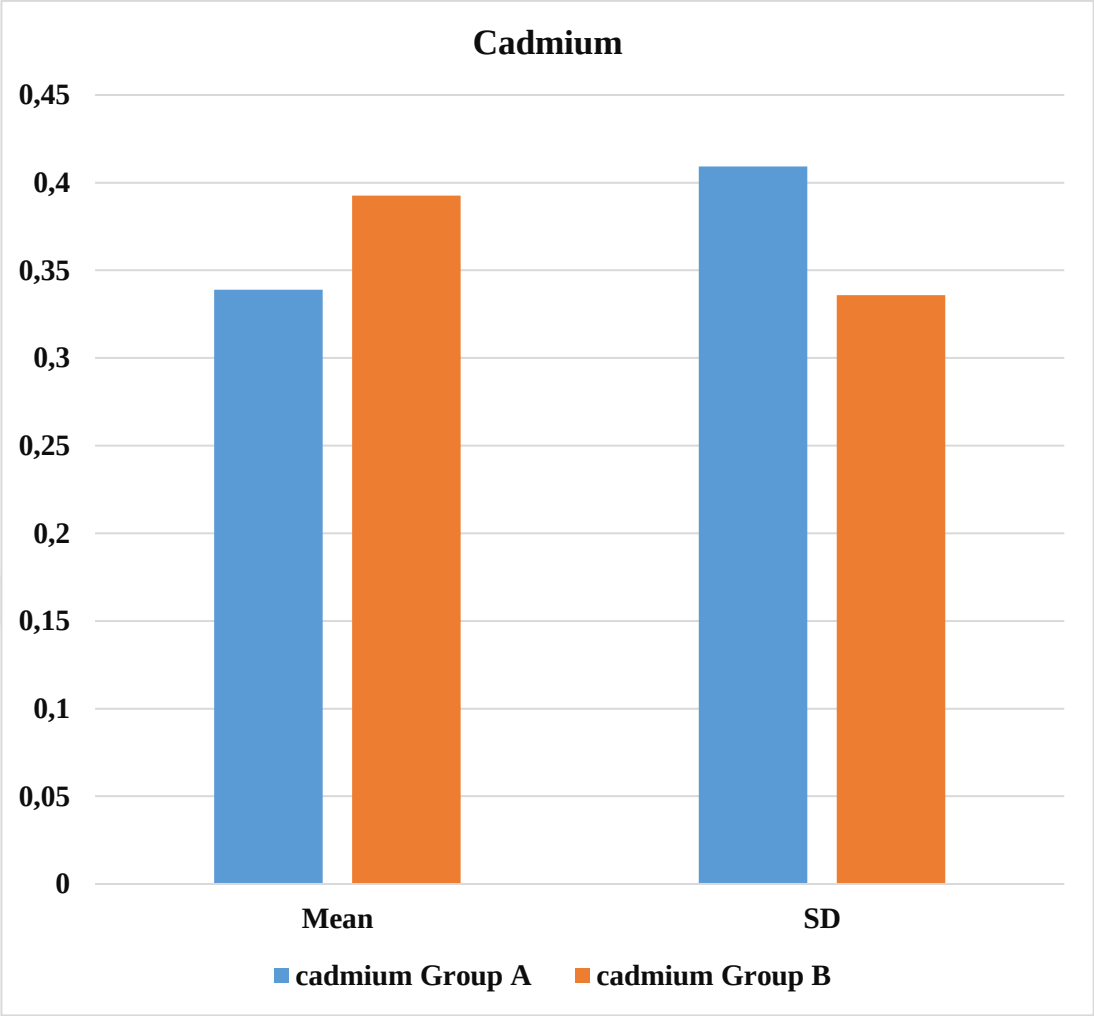

Figure 4.10 The mean and standard deviation of cadmium in women with PCOs and without PCOs

4.1.11 Lead (Pb)

Pb levels were recorded in women with PCOs and without PCOs groups groups as (0.6852 ± 0.31949) and (1.3800 ± 1.05401), was a non-significant statistic when compared to control group as in Table 4.1, and these results were confirmed according to Figure 4.11.

Describtive statstics of Pb that recorded in women with PCOs and without PCOs in Iraq including 95% Confidence Interval for Mean upper and lower bound, Median, Variance,

Minimum and Maximum, Range, and other statistics with Std. Error were tabulated in Table 4.2.

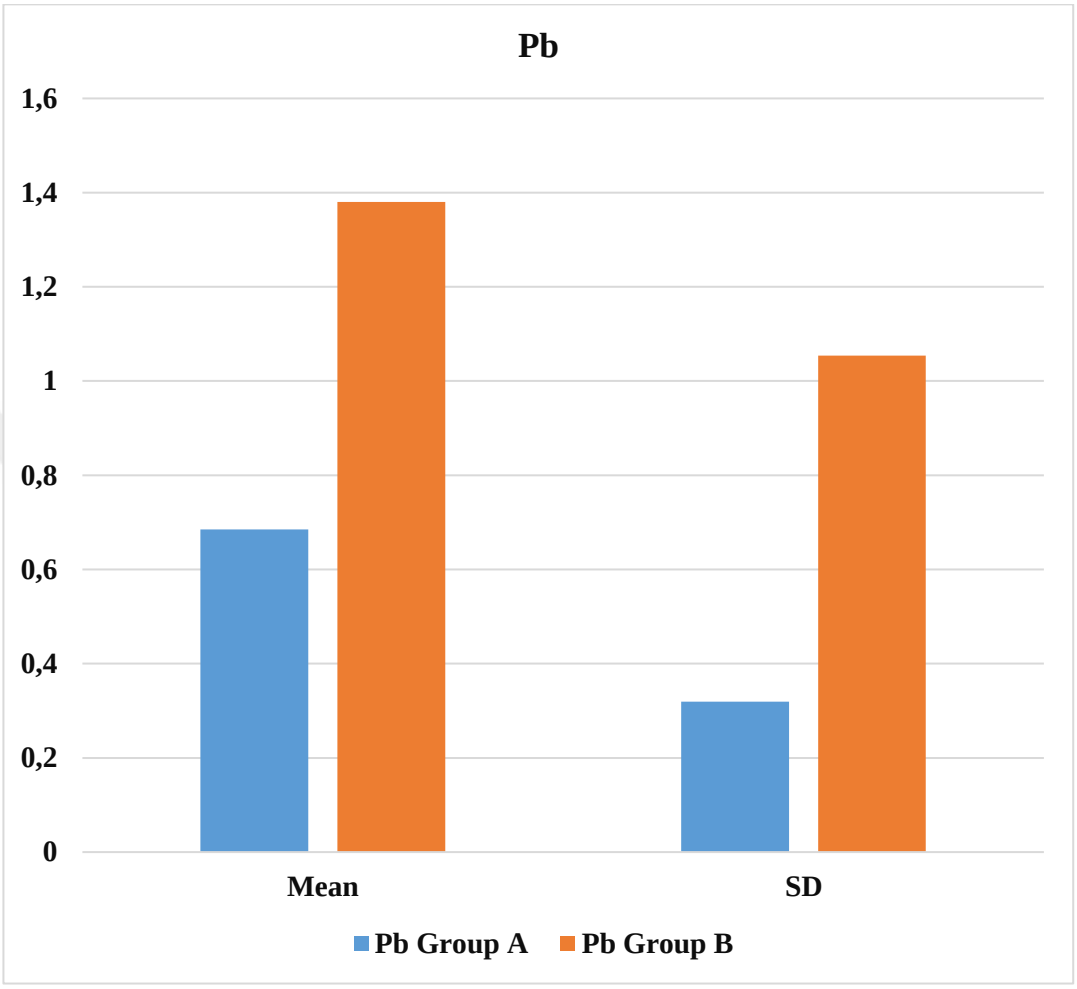

Figure 4.11 The mean and standard deviation of Pb in women with PCOs and without PCOs

4.1.12 Catalase

Catalase levels were recorded in women with PCOs and without PCOs groups groups as (83.3379 ±11.6686) and (104.7204 ± 6.34792), this result was a high significant difference when compared to control group as in Table 4.1, and these results were confirmed according to Figure 4.12.

Describtive statstics of catalase that recorded in women with PCOs and without PCOs in Iraq including 95% Confidence Interval for Mean upper and lower bound, Median, Variance, Minimum and Maximum, Range, and other statistics with Std. Error were tabulated in Table 4.2.

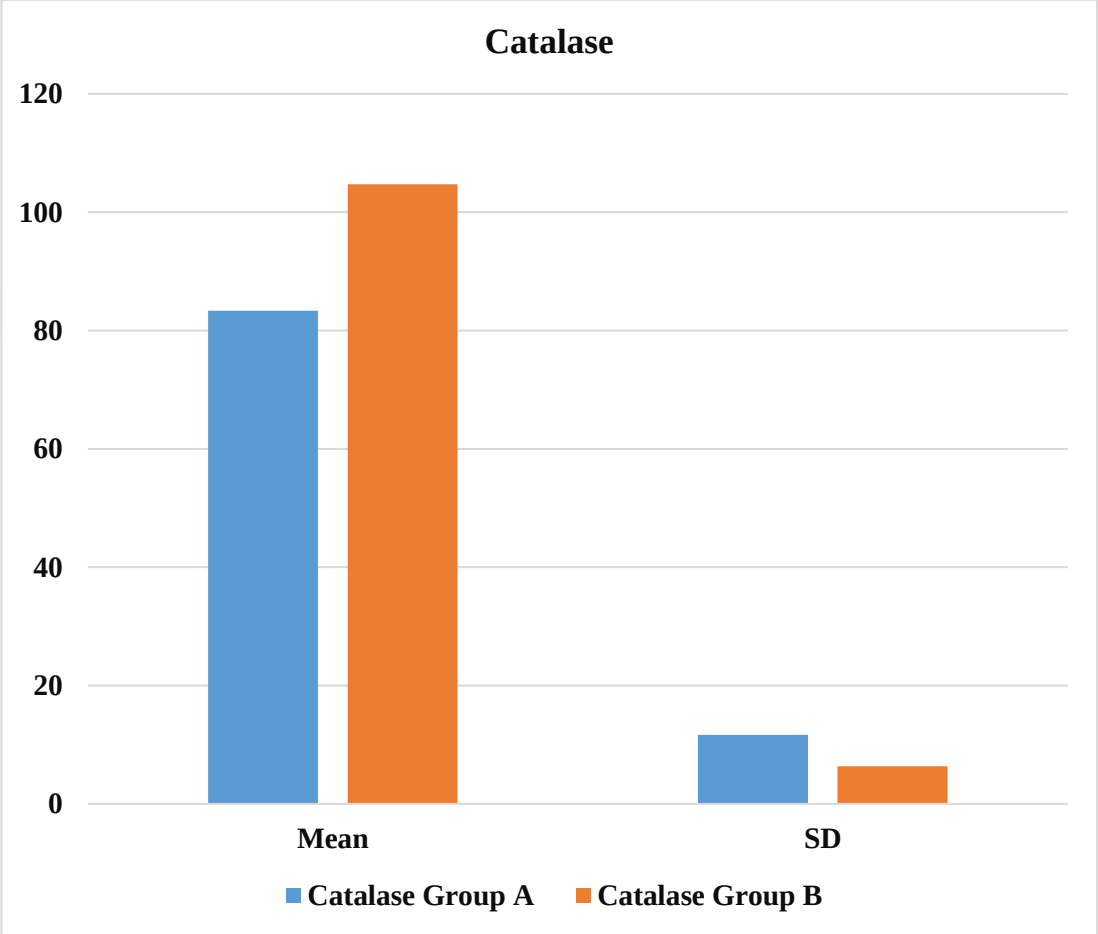

Figure 4.12 The mean and standard deviation of catalase in women with PCOs and without PCOs

4.1.13 Vitamin D

Vitamin D levels were recorded in women with PCOs and without PCOs groups groups as (13.9166 ± 3.98424) and (14.0453 ± 4.06037), vitamin D result was a non-significant difference as a shown in Table 4.1, and these rsults were confirmed according to Figure 4.13.

Describtive statstics of Vitamin D that recorded in women with PCOs and without PCOs in Iraq including 95% Confidence Interval for Mean upper and lower bound, Median, Variance, Minimum and Maximum, Range, and other statistics with Std. Error were tabulated in Table 4.2.

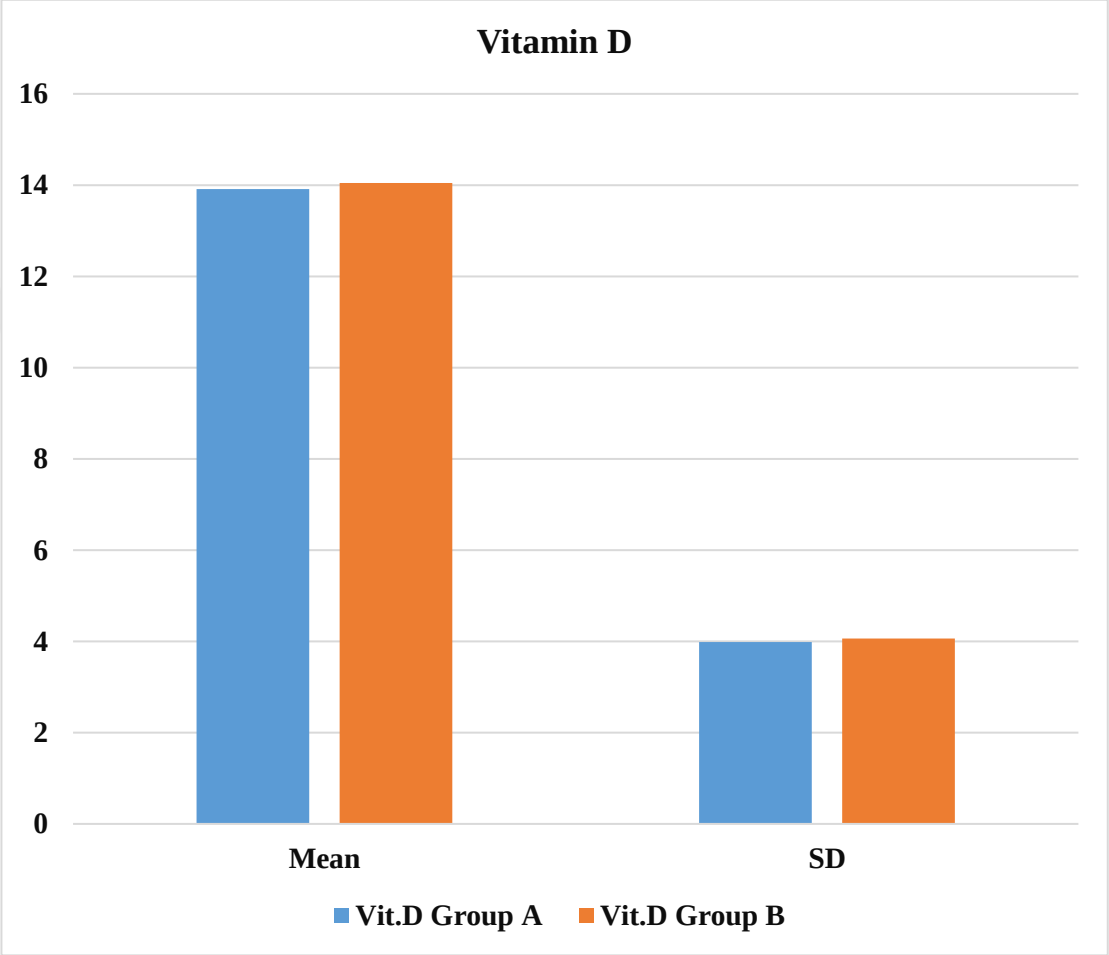

Figure 4.13 The mean and standard deviation of vitamin D in women with PCOs and without PCOs

4.1.14 Parameter correlations

The results of this study showed that there is positive correlation between catalase with age, FSH, LH and Pb. The negative correlation between catalase with testosterone in PCOs women in Iraq Table 4.3.

Table 4.3 Correlation between catalase level and studied parameters in groups

Correlations		
		Result
Catalase - Age	Pearson Correlation	0.514*
	Sig. (2-tailed)	0.021
Catalase - FSH	Pearson Correlation	0.499*
	Sig. (2-tailed)	0.025
Catalase - LH	Pearson Correlation	0.598**
	Sig. (2-tailed)	0.005
Catalase - Testeo	Pearson Correlation	-0.694**
	Sig. (2-tailed)	0.001
Catalase - E2	Pearson Correlation	-0.104
	Sig. (2-tailed)	0.661
Catalase - AMH	Pearson Correlation	0.347
	Sig. (2-tailed)	0.134
Catalase - RBS	Pearson Correlation	-0.009
	Sig. (2-tailed)	0.969
Catalase - HbA1c	Pearson Correlation	0.049
	Sig. (2-tailed)	0.838
Catalase - Ca	Pearson Correlation	-0.092
	Sig. (2-tailed)	0.699
Catalase - cd	Pearson Correlation	-0.166
	Sig. (2-tailed)	0.485
Catalase - Pb	Pearson Correlation	0.452*
	Sig. (2-tailed)	0.046
Catalase - Vit.D	Pearson Correlation	0.186
	Sig. (2-tailed)	0.432

*. Correlation is significant at the 0.05 level (2-tailed)., **. Correlation is significant at the 0.01 level (2-tailed).

5. CONCLUSION AND DISCUSSION

The aim of the study is to study the change and effect of some hormones on Iraqi girls in adolescence and how they affect the ovaries, as well as the effect of some heavy metals such as lead and cadmium on the uterus and their effect on the ovaries. Age and FSH, LH had significant clinical significance for women with PCOS. So was the testosterone. estradiol. AMH is statistical, but at lower levels. There was no statistical significance or differences for women with PCOS.

PCOS is a condition that may manifest in a wide variety of ways, and its underlying pathophysiological processes are not fully understood. Anti-Müllerian hormone (AMH) levels in the blood are markedly elevated in patients with polycystic ovary syndrome (PCOS). Growing follicles in the ovaries are responsible for the production of the hormone known as AMH, which is a member of the transforming growth factor family. The transcriptional regulation of AMH and AMHR2 is changed in patients with PCOS, which results in an increase in the duration of the temporal expression pattern. In addition, the extragonadal effects of AMH that have just lately been found give the impression that there may be some kind of communication going on between the ovary, the placenta, and the brain. This review provides a concise summary of the most current research about AMH and its involvement in the pathogenesis of PCOS (Moolhuijsen *et al*. 2020).

Based on the findings, it seems that a greater LH/FSH ratio is not associated with a higher body mass index. Since the LH/FSH ratio was the same in women with a normal BMI, those working in the medical field need to think about other approaches to correct this ratio other just losing weight (Saadia 2020).

It is possible that the levels of oxidized low-density lipoprotein (oxLDL) and enzyme antioxidants in the intrafollicular fluid of obese and infertile women are factors in the development of reproductive problems. Our findings lead us to the conclusion that greater amounts of antioxidants in the follicular fluid of obese women are linked to higher levels of catalase activity. Both of these parameters are unrelated to PCOS. The

amounts of oxidized low-density lipoprotein and the activity of catalase seem to represent various degrees of oxidative stress (Bausenwein *et al*. 2009).

Cadmium is a substance that has the ability to alter endocrine function and has an effect on the hypothalamic-pituitary-gonadal axis. In spite of the fact that there is evidence to indicate that it might have a function in modifying the androgen production and metabolic pathways that are typical of polycystic ovarian syndrome (PCOS), its relevance in healthy women of reproductive age is still largely unclear. Because women with moderate PCOS subclinical characteristics who may not satisfy the diagnostic criteria for PCOS may nevertheless have diminished fecundability while not having PCOS (Kim *et al*. 2021).

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