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Effect of vitamin D Supplementation on Insulin Sensitivity and testosterone level in Vitamin D Deficient polycystic ovary syndrome Patients

A thesis

Submitted to The Council of the of the college of Medicine at the University of AL- Qadisiyah in partial fulfillment of the requirements for the Degree of Higher Diploma Equivalent to Master Degree in Family Medicine

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بِسْمِ اللَّهِ الرَّحْمَٰنِ الرَّحِيم

قَالُوا سُبْحَانَكَ لَا عِلْمَ لَنَا إِلَّا مَا عَلَّمْتَنَا ۖ إِنَّكَ أَنتَ الْعَلِيمُ الْحَكِيمُ

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Dedication

To …

My father and mother

My husband

My lovely children

(Maryam and Noor)

Amaal

2018

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All Gratefulness and thanks are due to you who gave me life and guided me to the right way , my God .

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Abstract

Background

Polycystic ovary syndrome is the worldwide women endocrine disorder characterized by insulin resistance ,ovarian dysfunction and hyperandroginism. It affects women in reproductive age and causes many metabolic disorders. decreased level of vitamin D is common in women with PCOS. It has been reported that vitamin D status may contribute to the progress of the metabolic disorders associated with PCOS, chiefly hyperinsulinemia and impaired glucose tolerance test. There is a limited evidence that giving vitamin D supplementation had beneficial effects on insulin resistance and dysfunction of menstrual cycle in females with PCOS.

Aim of the study :

To study the effect of vitamin D supplementation on insulin sensitivity and testosterone level in Iraqi females with polycystic ovary syndrome and deficiency of vitamin D level.

Material and method

A randomized observational clinical trial design , studied 60 Iraqi females with PCOS referring to the private clinic, women' s consultant unit and outpatients at Maternity and Pediatrics teaching hospital in AL-Diwanyia city, Iraq. Sampling involved women aged from 18 to 45year established on inclusion criteria .The patient's basic data have been recorded. Then we measured vitamin D ,testosterone level and impaired glucose tolerance test to all females who agreed to participate in this study. The study was conducted at single private laboratory to increase reliability and to minimize the variance between laboratory . After the diagnosis of vitamin D deficiency has been done vitamin D was administering at 50000 units weekly for 8weeks each women in this study .All data were re-measured after two months.

RESULT :

Sixty patients with poly cystic ovary syndrome were enrolled in study, age range from 18-39 years, mean age 27.48±5.95 years, Vitamin D level 16.1±5.6,impaired glucose tolerance test reading 8.8± 0.7 and testosterone level4.5 ±0.64. There were 31.7% normal body mass index , 40% over weight, 28.3% obese. In the beginning of the study level of vitamin D was16.11 ±5.6, after 2 month became 35.9 ±4.3 which was of significant difference. After 2 month of supplementation 83.7% of patients reached the normal level of vitamin D and 16.3% still had low level of vitamin D (p value = 0.002) between two groups. Impaired glucose tolerance test after 2 month of supplementation, there were 51.6% of patients still had impaired test while 48.4% reached the normal reading (p value = 0.001). The other 75% of patients reached the normal level of testosterone and 25% had abnormal levels.

Conclusions

Our study concluded that women with PCOS have statically significant low levels of vitamin D,impaired glucose tolerance test and high levels of testosterone .Inverse Correlations between vitamin D with IGT and testosterone, as the increment in vitamin D level after supplementation leads to decrease in serum level of testosterone and also decrease in reading of impaired glucose tolerance test..

List of Contents

List of Tables

List of Figures

List of Abbreviations

ACOG	American college of obstetrician and gynecologist
AI	Adequate Intake
FSH	Follicles _Stimulating Hormone
FNB	Food and nutrition board
GNRH	Gonadotropin releasing hormone
IR	Insulin Resistance
LH	Luteinizing hormone
PCOS	Polycystic ovary syndrome
RDAS	Recommended Dietary allowances
SOGC	Society of obstetrician and gynecologist of Canada
TSH	Thyroid stimulating hormone
Up	Upper limit
CVD	Cardiovascular Disease
Vit D	Vitamin D

DPP	**Diabetes Prevention Program**
SHBG	**Se Hormone Binding Globulin**
IGTT	**Impaired glucose tolerance test**
NIH	**National Institutes of health**
NICHD	**National Institute of child Health and human development**
BMI	**Body mass index**
HDL-C	**High -density lipoprotein cholesterol**
HOMA-IR	**Homeostatic model assessment**
VDR	**Vitamin D receptor**
NHANES	**National Health and Nutritional examination survey**
COPD	**Chronic obstructive lung disease**

SPSS	**Statistical package of social science**
MFG	**Modified ferryman –Gallewy scoring system**
FIA	**Fluorescence immunoassay method**
FAI	**Free androgen index**
DHEAS	**Dehydroepiandrosterone sulfate**
T2DM	Type 2diabetes mellitus

Chapter one

Introduction and Review of Literature

Introduction

Polycystic ovary syndrome (PCOS) is the most common ovarian disorder associated with disturbances of reproductive function , hyperinsulinemia and androgen excess in women [**1**] .

Definition and diagnosis of PCOS is based on criteria including clinical evidence of hyper androgenism and ovarian dysfunction such as oligo-ovulation, afterexclusion of other causes of hyperandrogenism such as adrenal hyperplasia, hyperprolactinemia, and thyroid disorders .[**2**]

There are 3 available criteria used to diagnose PCOS: (i) the1990 National Institutes of Health (NIH) and National Institute of Child Health and Human Development (NICHD) criteria; (ii) the 2003 Rotterdam criteria; and (iii) the 2006 Androgen Excess Society criteria. The first set of criteria states that diagnosis is confirmed if a woman presents hyperandrogenism and ovulatory dysfunction; while the Rotterdam criteria, composed by the American Society of Reproductive Medicine and the European Society of Human Reproduction and Embryology ,affirm that diagnosis is confirmed if a woman presents two out of the following three manifestations :hyperandrogenism ,ovulatory dysfunction, and polycystic ovaries by ultrasound . The Androgen Excess Society criteria state that PCOS women should present ovarian dysfunction,polycystic ovaries, or both complications as well as hyperandrogenis. [**3**]

PCOS may be associated with insulin resistance, hypertension, and central obesity , all of which are risk factors for metabolic disorder , T2DM., diabetes, and coronary artery disease **[4].**

It is known that vitamin D affects glucose metabolism and may play a role in the development of symptoms of PCOS. [**5**]

Metabolic disturbances are common in PCOS women: 30–40% have glucose tolerance disorder, 60–80% are resistant to insulin, and 10% have T2DM in their thirties or forties. Evidence suggests the pivotal role of insulin resistance in PCOS pathogenicity **[5]** Dyslipidemia is common in PCOS and includes high levels of total cholesterol and LDL, triglycerides and low HDL. Lipid disorders are seen in about 65–81% of these women[**6**]. the increased production of reactive oxygen species (ROS) in PCOS is related to vascular complications **[7]**. Decrease level of vitamin D is common in women with PCOS [**8].** It has been reported that vitamin D status may contribute to the progress of the metabolic disorders associated with PCOS, chiefly hyperinsulinemia and impaired glucose tolerance states **[9]** . Because of changes in insulin receptor expression and cytokine suppression. Such process may be related to insulin resistance during vitamin D deficiency, consequently resulting in obesity**[10,11,12].**

Vitamin D deficiency in PCOS women was associated with a reduced likelihood of these women becoming pregnant and delivering babies, regardless of BMI, race, age, markers of metabolic functioning, or fertility treatment[**13**]. Infertile PCOS women had a higher number of dominant follicles and improvements in menstrual regularity when taking metformin + Vitamin D (100,000 IU/month for 6 months) compared with metformin alone.[**13,14**]
Infertile women with PCOS who underwent clomiphene citrate stimulation had more mature follicles (57%) and were more likely to get pregnant (26.4%) with higher vitamin D levels**. [15].** Vitamin D supplementation has been associated with reducing androgen levels in women with PCOS **[16].**

Hyperandrogenism, one of the primary symptoms of PCOS,is characterized by excessive levels of androgens in the body. It is reported that approximately 75% of PCOS patients have hyperandrogenism [**17**].

There is a direct relation between hyperandrogenism and vitamin D deficiency, revealing a possible interaction between androgens and vitamin D balance, mediated by a not yet completely known mechanism .[**18**]

Aim of the study:

To study the effect of vitamin D supplementation on insulin sensitivity and testosterone level in Iraqi females with polycystic ovary syndrome and deficiency of vitamin D level.

Review of Literature

1.1 POLYCYSTIC OVARY SYNDROM (PCOS)

PCOS is the most common cause of ovarian dysfunction in women with an ovulation, hyperandrogenism, and /or the presence of polycystic ovary morphology by ultrasound examination **[19]**.

The clinical manifestation of this syndrome is associated with various degrees of gonadotropic and metabolic abnormalities determined by interaction of multiple environmental and genetic factors **[20,21]**.

A polycystic ovary by ultrasound is defined as an ovary containing of an estimate of 10 small cysts of a diameter ranging between 2 and 9 mm develop on one or both ovaries and/or the ovarian volume in at least one ovary exceeds 10 ml [**22**,**23**].

1.2 Pathogenesis of PCOS

Polycystic ovaries develop when the ovaries are stimulated to produce excessive amounts of androgen hormone and high levels of insulin in the blood (hyperinsulinaemia) in women whose ovaries are sensitive to this stimulus or reduced levels of sex-hormone binding globulin (SHBG) resulting inincreased free androgens [**24**].

The disorder acquired its name due to the common sign on ultrasound examination of multiple ovarian cysts which represent Immature follicles .The follicles have developed from primordial follicles but the development has stopped at an early stage due to the disturbed ovarian function. The follicles may be oriented along the ovarian periphery appearing as a ‘string of pearls’ on ultrasound examination [**25**].

Patients with PCOS have high level of gonadotrophin releasing hormone (GnRH) so, that results in an increase in Luteinizing hormone/follicles-stimulating hormone LH/FSH ratio in females with PCOS. Large numbers of patients with PCOS have insulin resistance and/or obesity[**26**]. Their elevated insulin levels contribute to or cause the abnormalities seen in the hypothalamic-pituitary-ovarian axis that lead to PCOS. Hyperinsulinemia increases GnRH

pulse frequency, LH over FSH dominance, increased ovarian androgen production, decreased follicular maturation and decreased sex hormone binding globulin (SHBG) binding. All these factors contribute to the development of PCOS **[27].**

PCOS is characterized by a complex positive feedback of insulin resistance and hyperandrogenism.. In most cases, it cannot be determined which of those two should be regarded to be the causative agent. Experimental treatment with either anti-androgens or insulin sensitizing agents improves both hyper-androgenism and insulin resistance **[28**].

Adipose tissue possesses aromatase, an enzyme that converts androstenedione to estrone and testosterone to estradiol. The excess of adipose tissue in obese patients causes them to have both excess androgens (which are responsible for hirsutism and virilization) and estrogens (which inhibit FSH via negative feedback**) [29]**.

1.3 Classical features of PCOS:-

The classical features of PCOS includes but are not limited to; infertility , hyperandrogenism , truncal obesity, abnormal glucose metabolism ,insulin resistance (IR), hirsutism , acne vulgaris and acanthosis nigricans. Symptoms typically manifest in early puberty and worsen with maturation. Nearly 80% of patients affected present with more than one symptom **[30].**

However, there is a considerable intra individual variation in presentation. Although not required for diagnosis, the presence of insulin resistance and hyperinsulinemia is common and places those affected at increased risk of diabetes and cardiovascular disease **[31].**

1.3.A Menstrual abnormalities

Patients with PCOS have abnormal menstruation patterns attributed to chronic anovulation. (The patient usually has a history of menstrual irregularity dating

back to menarche.) Some women have oligomenorrhea (ie, menstrual bleeding that occurs at intervals of 35 days to 6 months, with < 9 menstrual periods per year) or secondary amenorrhea (an absence of menstruation for 6 months). Dysfunctional uterine bleeding and infertility are the other consequences of ovulatory menstrual cycles. The menstrual irregularities in PCOS usually present around the time of menarche[**32,33**] .

1.3.B.Increased androgen level

Hyperandrogenism clinically manifests as excess terminal body hair in a male distribution pattern. Hair is commonly seen on the upper lip, on the chin, around the nipples, and along the linea alba of the lower abdomen. Some patients have acne and/or male-pattern hair loss (androgenic alopecia). Other signs of hyperandrogenism (eg. Clitoromegaly, increased muscle mass, voice deepening) are more characteristic of an extreme form of PCOS termed hyperthecosis. These signs and symptoms could also be consistent with androgen-producing tumors, exogenous androgen administration, or virilizing congenital adrenal hyperplasia. Premature adrenarche is a common occurrence and, in some cases, may represent a precursor to PCOS. Hirsutism and obesity may be present in premenarchal adolescent girls with PCOS. The American College of Obstetricians and Gynecologists (ACOG) recommends screening with 17-hydroxyprogesterone levels in women suspected of having PCOS who are at an increased risk for non classical congenital adrenal hyperplasia **[34].**

1.4 Associated Morbidities

1.4.A Obesity and metabolic syndrome

Nearly half of all women with PCOS are clinically obese. A study comparing the body mass index BMI in American and Italian women with PCOS showed that American women had a BMI higher than that of their Italian counterparts**.[35]**

Women with PCOS should be assessed for their cardiovascular risk by evaluating their BMI , fasting lipid and lipoprotein levels, and risk factors for metabolic syndrome**. [36]**

Many patients with PCOS have characteristics of metabolic disorder; one study showed a 43% prevalence of metabolic syndrome in women with PCOs.[**35**] In women, metabolic syndrome is characterized by abdominal obesity (waist circumference >35 in), fasting blood sugar more than 110mg/dl (6.1mmol/l),dyslipidemia (triglyceride level >150 mg/dL, high-density lipoprotein cholesterol [HDL-C] level < 50 mg/dL), elevated blood pressure, a proinflammatory state characterized by an elevated C-reactive protein level, and a pro thrombotic state characterized by elevated plasminogen activator inhibitor-1 (PAI-1) and fibrinogen level**. [37,38]**

1.4.B.Insulin Resistance

A lot of attention has been given to the metabolic disturbances that accompany PCOS, as well as to the consequences of these disturbances later in life. Today, insulin resistance is considered the main pathogenic factor in the background of increased metabolic disturbances in women with PCOS **.**which can explain hyperandrogenism, menstrual irregularity, and other metabolic manifestations seen in this disease**.[39,40**]

Multiple studies supported a correlation between diabetes and PCOS and showed that insulin-sensitizing drugs and dietary/lifestyle modifications improve hyperandrogenism in patients suffering from PCOS **[41,42].**

Increased insulin levels in patients with PCOS may, along with the high levels of luteinizing hormone, trigger the arrest of follicular growth which contributes to anovulation **[43]**.

Hyperinsulinemia also alters the gonadotropin-releasing hormone (GnRH) pulse secretion pattern, suppresses the sex hormone-binding globulin (SHBG) and potentiates ovarian androgen production in women with PCOS**) [44,45]**.

When the hormone leptin is used as insulin-sensitizing agent, it decreases androgen levels and induces menstruation in affected lean women **[46]**.

This is one of the critical junctures in the treatment of PCOS, which led to the consideration of insulin-mimetic or insulin-sensitizing agents as part of the management of the disease. These agents, as mentioned later in the review, include metformin, myo-inositol supplements, and thiazolidinedione.

PCOS patients should be tested for insulin resistance according to the diabetes prevention program **[47].**

A few biomarkers have been used to detect insulin resistance in PCOS women. For instance, insulin restrains the release of sex hormone binding globulin (SHBG) from the liver and the production of insulin-like growth factor binding protein 1 (IGFBP-1**) [48,49]**.

1.4.C .Diabetes mellitus

ACOG recommends screening for type 2 diabetes and impaired glucose tolerance in women with PCOS by obtaining a fasting glucose level and then a 2-hour glucose level after a 75-g glucose load.[55]Approximately 10% of women with PCOS have type 2 diabetes mellitus, and 30-40% of women with PCOS have impaired glucose tolerance by 40 years of age**.[50,51]**

1.4.D.Cardiovascular Disease

Data showed that patients with PCOS have significantly elevated levels of circulating biomarkers of CVD, including C-reactive protein. and lipoprotein A, in comparison to matched controls. Other studies demonstrated a higher burden of indicators of atherosclerosis with early onset cardiovascular dysfunction, i.e.,

arterial stiffness, endothelial dysfunction, and coronary artery calcification **[52,53].**

In 2010, the Androgen Excess-PCOS society provided a consensus statement about increased risk of CVD in women with PCOS and developed a guideline to prevent such complication **[54].**

Yet, despite the increased cardiovascular risk markers and the indubitable presence of CVD risk factors in this population, uncertainty remains regarding the increased cardiovascular morbidity and mortality in patients with PCOS **[55]**

.1.4.E. Cancer

Females suffering from PCOS show a lot of risk factors related to the development of endometrial cancer, such as obesity, insulin resistance, type II diabetes mellitus, and anovulation. **[56]**

Anovulation triggers an unopposed uterine estrogen exposure. This can subsequently trigger the development of endometrial hyperplasia and ultimately endometrial cancer. **[57]**

As a matter of fact, studies show that women with PCOS have a three-fold increased risk of developing endometrial cancer[**58,59**] which is mostly well differentiated with a good prognosis.[**60**] Regardless, no data support ultrasound screening for endometrial thickness in women with PCOS, which comes in agreement with the American Cancer Society against screening for endometrial cancer in patients with average or increased risk. Yet women should be advised to notify their healthcare provider for any spotting or unexpected bleeding**.[61]** On the other hand, there are limited data to support any association between PCOS and breast and ovarian cancer **.[62]**

1.4.F. Infertility

Women with PCOS may have reduced fertility**[63,64].**due to the associated endocrine and gynecologic abnormalities that impact ovarian quality and function. **[65]**

Accounting for up to 90% of ovulatory disorders[**66**], newly in 2015, a study by Hart and Doherty showed that infertility is 10 times more common among women with PCOS in comparison to healthy controls.**[67]**

On the other hand, some studies suggested that females with PCOS who conceive might suffer from pregnancy-related complications such as gestational diabetes**,** pregnancy induced hypertension and preeclampsia **[68,69]**

The influence of PCOS on female fertility remains poorly comprehended. There is increased negative pregnancy outcomes in this group of women. Concerning the effects on the embryo, women with PCOS are 2.5 times at a higher risk of giving birth to small for gestational age children in comparison to healthy females and offspring show an increased morbidity and mortality compared to control [**70**,**71**] .

1.5. Diagnosis

Patients must have two out of the three features below:

Amenorrhea or oligomenorrhea

Clinical or biochemical hyperandrogenism

Polycystic ovaries on ultrasound **[72,73]**

The conditions that have the same features of PCOS should be ruled out as adrenal hyperplasia ,and cushing syndrome [**74,75**].

A Karyotype studies should be done to exclude mosaic turner syndrome as a cause of the primary amenorrhea.

The Royal College of Obstetricians and Gynaecologists has been (RCOG) recommends the following baseline screening tests for the women with suspected polycystic ovarian syndrome (PCOS) : thyroid function tests, serum prolactin levels, and a free androgen index (defined as total testosterone divided by sex hormone binding globulin [SHBG] × 100,to give a calculated free testosterone level **[76]**

Patient should be in a fasting state and samples for laboratory studies should be drawn early in the morning in women with regular menses, samples should be taken between days 5 and 9 of the menstrual cycle.A serum human chorionic gonadotropin (hCG) level should be checked to rule out pregnancy in women with oligomenorrhea or amenorrhea .[**77**]

1.5.1 Hormone Level

1.5.A. Androgens

Androgen excess can be tested by measuring total and free testosterone levels or a free androgen index. An elevated free testosterone level is a sensitive indicator of androgen excess. Other androgens, such as dehydroepiandrosterone sulfate (DHEA-S), may be normal or slightly above the normal range in patients with polycystic ovarian syndrome (PCOS). levels of sex hormone–binding globulin (SHBG) are usually low in patients with PCOS. Androstenedione levels are also elevated in women with PCOS. This androgen precursor is 60% ovarian and 40% adrenal in derivation .[**78,79**]

Patients with androgen-secreting ovarian or adrenal tumors can present with hirsutism , amenorrhea, and signs of virilization. Although the clinical picture of symptom onset and progression is more predictive than androgen levels, their

testosterone level may be greater than 150 ng/dL and their DHEA-S level may be above 800 mcg/dL. DHEA-S is derived from the adrenal gland, and therefore, elevation of DHEA-S would be suggestive of an adrenal origin.[**80**]

1.5.B. Follicle-stimulating hormone and luteinizing hormone levels

The follicle-stimulating hormone (FSH) level should be checked to rule out primary ovarian failure. In patients with PCOS, FSH levels are within the reference range or low. Luteinizing hormone (LH) levels are elevated for Tanners stage, sex, and age. The LH-to-FSH ratio is usually greater than 3.Stimulation testing with a long-acting gonadotropin-releasing hormone (GnRH) agonist induces a characteristic rise in ovarian-derived 17-hydroxyprogesterone after 24 hours. This is thought to be a result of excessive 17-hydroxylase activity [**81**]

1.5.C . Thyroid-stimulating hormone and free thyroxin levels

Thyroid dysfunction, rather than PCOS, may be the source of amenorrhea and hirsutism. (In patients with PCOS, thyroid function tests are within the reference range.)Long-standing primary hypothyroidism can be associated with a markedly elevated circulating thyroid-stimulating hormone (TSH) level. Elevated alpha subunit delivery (from one half of the diametric TSH molecule) can then cross-react with FSH and LH receptors on breast tissue, leading to premature thelarche and, on ovarian tissue, resulting in a PCOS–like picture. These physical findings of the van Wyk-Grumbach syndrome (ie, juvenile hypothyroidism, precocious puberty, and ovarian enlargement) resolve upon thyroxine replacement therapy.[**82**]

1.5.D. Glucose, Insulin, and Lipids

Because the prevalence of impaired glucose tolerance and type two diabetes mellitus is high in women with polycystic ovarian syndrome (PCOS)—particularly those who have a body mass index (BMI) greater than 30 kg/m^2, have a strong family history of type 2 diabetes mellitus(T2D.M), or are older than 40 years—a 75-g oral glucose-tolerance test (OGTT) should be performed. A 2-hour post load glucose value of less than 140 mg/dL indicates normal glucose tolerance; a value of 140-199 mg/dL indicates impaired glucose tolerance; and a value of 200 mg/dL or higher indicates diabetes mellitus.[**83**]

Women diagnosed with pre pregnancy PCOS should be screened for gestational diabetes before 20 weeks' gestation.**[84]**

Some women with PCOS have insulin resistance and an abnormal lipid profile (cholesterol >200 mg/dL; LDL >160 mg/dL). Approximately one third of women with PCOS who are overweight have impaired glucose tolerance or type 2 diabetes mellitus by 30 years of age.[**85**]

study by Barber TM at el (2007)established that inflammatory marker and resistance insulin might be help to identify adolescent girls with polycystic ovarian syndrome whom at increased of developing the metabolic syndrome . [**86**]

According to phenotypic subgroup , metabolic heterogeneity also found in females with PCOS, metabolic disorder confined to the subgroup with both hyperandrogenic features and oligomenorrhea [**87**] .

Imaging study for PCOS

1.6.1:Ultrasonography

Current recommended sonographic criteria for multifollicular ovarian morphology:

- 25 or more follicles per ovary (superseding the earlier Rotterdam criteria of 12 or more follicles) [**88**]
- increased ovarian size (>10ml): less sensitive than the follicle number criteria, but has a role when image resolution does not allow accurate follicle count,e.g. trans abdominal scanning ,older equipment Other morphological features include:
- hyperechoic central stroma
- peripheral location of follicles: which can give a string of pearl appearance
- follicles of similar size measuring 2-9 mm

Ovaries may be normal in PCOS, and conversely, polycystic ovaries may be seen in women without the syndrome. Diagnosis requires correlation with features of hyperandrogenism and oligo-anovulation.[**89**,**87**]

1.6.2 MRI

MRI is not routinely warranted in the investigation of PCOS, nonetheless pelvic MRI may show most or all of the above sonographic features. Signal characteristics include:

- **T1:** small uniform follicles are low in signal while the central stroma is of intermediate signal (vs normal myometrium)
- **T2:** follicles have high T2 signal while the central stroma is of comparatively low T2 signal .[**88**]

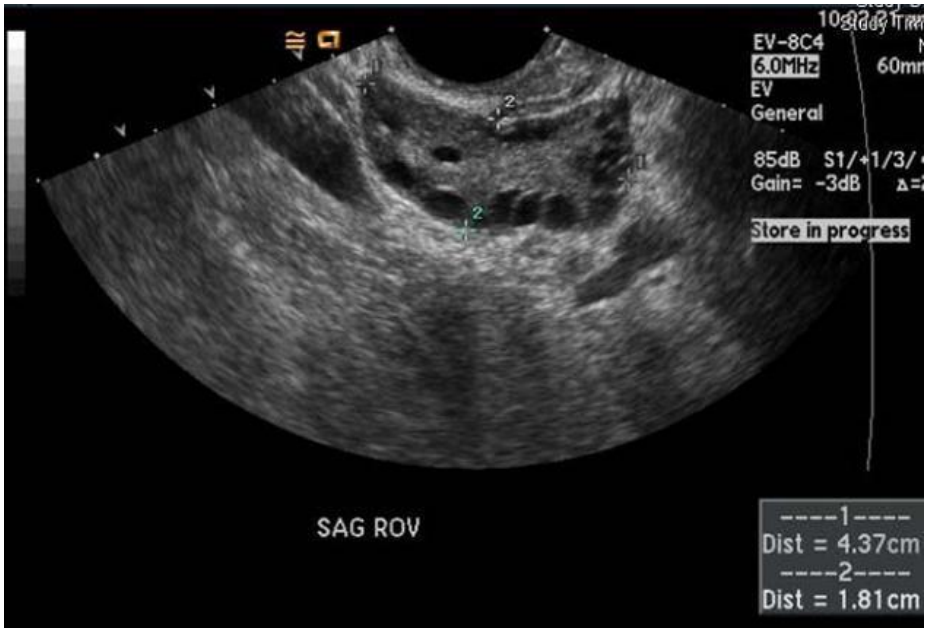

Figure (1) Imaging for PCOS

1.7. Treatment :

1.7.A. Lifestyle modification

The American College of Obstetricians and Gynecologists (ACOG) and the Society of Obstetricians and Gynaecologists of Canada (SOGC) indicate that lifestyle modifications such as weight loss and increased exercise in conjunction with a change in diet consistently reduce the risk of diabetes. This approach has been found to be comparable to or better than treatment with medication and should therefore be considered first-line treatment in managing women with polycystic ovarian syndrome .These modifications have been effective in restoring ovulatory cycles and achieving pregnancy in obese women with PCOS. Weight loss in obese women with PCOS also improves hyperandrogenic features. **.[89,90]**

1.7.B. Drug Treatment:

Management of Polycystic ovary syndrome is designed at the treatment of metabolic instabilities, anovulation, hirsutism, and irregularity of menstrual cycle. The use of insulin-sensitizing drugs to improve insulin sensitivity is

associated with a reduction in circulating androgen levels, as well as improvement in both the ovulation rate and glucose tolerance .[**91**]

The Endocrine Society has issued A clinical practice guideline on hirsutism assessment and treatment in premenopausal women.[**92**]

ACOG notes that eflornithine in conjunction with laser treatment is superior to laser therapy alone in treatment of hirsutism.[**93**]

First-line medical therapy usually consists of an oral contraceptive to induce regular menses. The contraceptive not only inhibits ovarian androgen production but besides that rise sex hormone-binding globulin (SHBG) production. ACOG recommends use of combination low-dose hormonal contraceptive agents for long-term management of menstrual irregularity[**94**] . If symptoms such as hirsutism are not sufficiently alleviated, an androgen-blocking agent may be added. Pregnancy should be excluded before therapy with oral contraceptives or androgen-blocking agent .[**95**]

First-line treatment for ovulation induction when fertility is desired is clomiphene citrate drug [**96,97**]

A randomized study suggested that combined metformin/letrozole and bilateral ovarian drilling are similarly effective as second-line treatment in infertile women with clomiphene citrate–resistant PCOS [**98**].

1.7.B. Metformin

For the patient who develops T2DM., is consider as a treatment with oral hypoglycemic agent , such as metformin. Metformin can also be considered in other women with PCOS who are insulin resistant and therefore at risk of developing cardiovascular disease, even women without type 2 diabetes [**99**].

Clinical trials have shown that metformin can effectively reduce androgen levels, improve insulin sensitivity, and facilitate weight loss in patients with PCOS as early as adolescence**.[100,101**].

One study by Begum MR at el (2009) concluded that the use of metformin throughout pregnancy was associated with a 9-fold decrease in gestational diabetes in women with PCOS [**102**].

In addition to having the potential to reduce gestational diabetes in pregnant women with PCOS, metformin may also reduce the risk of preeclampsia in this population. [**103**].

A long-term study suggested that metformin continued to improve the metabolic profile of women with PCOS over a 36-month treatment course, particularly improving circulating high-density lipoprotein cholesterol (HDL-C), diastolic blood pressure, and BMI [**104**].

1.7.C. Other agents

If the patient has concomitant adrenal hyperandrogenism, treatment with low-dose prednisone or dexamethasone may be considered.Depot leuprolide acetate (Lupron) is effective in suppressing ovarian hormone production, which effectively induces menopause; therefore, this drug must be accompanied by hormone replacement therapy. This treatment approach has not gained widespread favor.[**105**]

1.8. VITAMIN D

Vitamin D is a fat-soluble vitamin that is naturally present in very few foods, added to others, and available as a dietary supplement. It is also produced endogenously when ultraviolet rays from sunlight strike the skin and trigger vitamin D synthesis. Vitamin D obtained from sun exposure, food, and supplements is biologically inert and must undergo two hydroxylations in the body for activation. The first occurs in the liver and converts vitamin D to 25-hydroxyvitamin D [25(OH)D], also known as calcidiol. The second occurs

primarily in the kidney and forms the physiologically active 1,25-dihydroxyvitamin D [1,25$(OH)_2$D], also known as calcitriol [**106**]**.**

Vitamin D promotes calcium absorption in the gut and maintains adequate serum calcium and phosphate concentrations to enable normal mineralization of bone and to prevent hypocalcemic tetany. It is also needed for bone growth and bone remodeling by osteoblasts and osteoclasts [**107,108**].

Without sufficient vitamin D, bones can become thin, brittle, or misshapen. Vitamin D sufficiency prevents rickets in children and osteomalacia in adults [**109**].

Together with calcium, vitamin D also helps protect older adults from osteoporosis.Vitamin D has other roles in the body, including modulation of cell growth, neuromuscular and immune function, and reduction of inflammation [**110**,**111**] . Many genes encoding proteins that regulate cell proliferation, differentiation, and apoptosis are modulated in part by vitamin D [**112**].

Many cells have vitamin D receptors, and some convert 25(OH)D to 1,25$(OH)_2$D.Serum concentration of 25(OH)D is the best indicator of vitamin D status. It reflects vitamin D produced cutaneously and that obtained from food and supplements [1] and has a fairly long circulating half-life of 15 days [**113**].

25(OH)D functions as a biomarker of exposure, but it is not clear to what extent 25(OH)D levels also serve as a biomarker of effect (i.e., relating to health status or outcomes) [**114**]

Serum 25(OH)D levels do not indicate the amount of vitamin D stored in body tissues. In contrast to 25(OH)D, circulating 1,25$(OH)_2$D is generally not a good indicator of vitamin D status because it has a short half-life of 15 hours and

serum concentrations are closely regulated by parathyroid hormone, calcium, and phosphate [**115**]

Levels of 1,25$(OH)_2$D do not typically decrease until vitamin D deficiency is severe [**116**,**117**].There is considerable discussion of the serum concentrations of 25(OH)D associated with deficiency (e.g., rickets) , adequacy for bone health , and optimal overall health , and cut points have not been developed by a scientific consensus process. Based on its review of data of vitamin D needs, a committee of the Institute of Medicine concluded that persons are at risk of vitamin D deficiency at serum 25(OH)D concentrations <30 nmol/L (<12 ng/mL). Some are potentially at risk for inadequacy at levels ranging from 30–50 nmol/L (12–20 ng/mL). Practically all people are sufficient at levels ≥50 nmol/L (≥20 ng/mL); the committee stated that 50 nmol/L is the serum 25(OH)D level that covers the needs of 97.5% of the population. Serum concentrations >125 nmol/L (>50 ng/mL) are associated with potential adverse effects [**118**] [table].

Table show Serum 25-Hydroxyvitamin D [25(OH)D] Concentrations and Health* [1]

nmol/L**	**ng/mL***	**Health status**
<30	<12	Associated with vitamin D deficiency, leading to rickets in infants and children and osteomalacia in adults
30 to <50	12 to <20	Generally considered inadequate for bone and overall health in healthy individuals
≥50	≥20	Generally considered adequate for bone and overall

Table show Serum 25-Hydroxyvitamin D [25(OH)D] Concentrations and Health* [1]		
nmol/L**	**ng/mL***	**Health status**
		health in healthy individuals
>125	>50	Emerging evidence links potential adverse effects to such high levels, particularly >150 nmol/L (>60 ng/mL)

* Serum concentrations of 25(OH)D are reported in both nanomoles per liter (nmol/L) and nanograms per milliliter (ng/mL).
** 1 nmol/L = 0.4 ng/mL

An additional complication in assessing vitamin D status is in the actual measurement of serum 25(OH)D concentrations. Considerable variability exists among the various assays available (the two most common methods being antibody based and liquid chromatography based) and among laboratories that conduct the analyses **[119,120**].

This means that compared with the actual concentration of 25(OH)D in a sample of blood serum, a falsely low or falsely high value may be obtained depending on the assay or laboratory used [**121**]. A standard reference material for 25(OH)D became available in July 2009 that permits standardization of values across laboratories and may improve method-related variability **[122,123**].

The Food and Nutrition board(FNB) established an Recommended dietry allowances (RDAS) for vitamin D representing a daily intake that is sufficient to maintain bone health and normal calcium metabolism in healthy people. RDAs

for vitamin D are listed in both International Units (IUs) and micrograms (mcg); the biological activity of 40 IU is equal to 1 mcg (Table 2). Even though sunlight may be a major source of vitamin D for some, the vitamin D RDAs are set on the basis of minimal sun exposure .[**124**]

Table show Recommended Dietary Allowances (RDAs) for Vitamin D [1]

Age	Male	Female	Pregnancy	Lactation
0–12 months*	400 IU (10 mcg)	400 IU (10 mcg)		
1–13 years	600 IU (15 mcg)	600 IU (15 mcg)		
14–18 years	600 IU (15 mcg)	600 IU (15 mcg)	600 IU (15 mcg)	600 IU (15 mcg)
19–50 years	600 IU (15 mcg)	600 IU (15 mcg)	600 IU (15 mcg)	600 IU (15 mcg)
51–70 years	600 IU (15 mcg)	600 IU (15 mcg)		
>70 years	800 IU (20 mcg)	800 IU (20 mcg)		

* Adequate Intake (AI)

1:8:1:Classical Action of Vitamin D: Regulation of Calcium and Phosphate Homeostasis.

Calcitriol participates in the regulation of plasma ionized calcium and phosphate levels by acting on their intestinal absorption, renal excretion, and calcium bone mobilization as described below (Fig. 2). When serum calcium levels decrease,

PTH secretion is stimulated and activates calcitriol synthesis. Both PHT and calcitriol stimulate calcium renal reabsorption and mobilization from bones (bone resorption). In contrast, if serum calcium levels rise, PTH secretion drops, leading to a decrease of calcitriol and calcium mobilization. Indeed, if serum calcium levels become too high, the parafollicular cells of the thyroid secrete calcitonin, which block calcium mobilization from the bone and stimulate calcium and phosphorous excretion.[**125]**contribute to keep calcium levels within the normal range.

Calcitriol acts directly on 3 target tissues with the aim of maintaining optimal serum calcium levels. In addition, through Vitamin D Receptor, calcitriol suppresses parathyroid gene expression and parathyroid cell proliferation, reinforcing its direct action on increasing levels of serum calcium.[**126**]

The first target organ is the intestine (without PTH mediation); here calcitriol stimulates intestinal calcium absorption that depends on its presence in the diet, intestinal solubility, and intestinal absorption capacity, which is the result of the balance between transcellular and paracellular intestinal absorption **[127**].

The second organ are the kidneys; calcitriol with PTH encourages the renal distal tubule reabsorption of calcium. Calcitriol influences (1) calcium entrance through the apical membrane; (2) calbamicin-mediated calcium diffusion; and (3) active transport thought the basolateral membrane.[**126**,**127**]

Vitamin D inhibits phosphate reabsorption indirectly by increasing FGF-23 osteocytes expression, and directly by inducing α-klotho (FGF-23 co-receptor).[**128**]

The third target tissue is the bone. Calcitriol mobilizes calcium from bone, a process requiring PTH.[**133**] When serum calcium levels decrease, PTH-dependent calcitriol activation prompts the formation and VDR-mediated

differentiation of osteoclasts. This activation induces the mobilization of calcium from the bone by stimulating the secretion of the receptor activator for nuclear factor kappa-B ligand, which, in turn, is responsible for osteoclastogenesis and bone resorption.[**129**]

At the same time, vitamin D inhibits mineralization through the increase of pyrophosphate levels and osteopontin.[**130**]

Calcitriol promotes bone formation and growth, by activating chondrocyte differentiation, and increasing serum calcium and phosphate levels. Thus, vitamin D deficiency results in inadequate mineralization of the skeleton, and when low vitamin D levels are maintained, bone growth plates cannot be mineralized due to calcium and phosphate depletion.[**131**,**132**]

1.8.2: non classical function of vitamin D

1.8.2:A: vitamin d and PCOS

A high rate of vitamin D deficiency has been found in women with polycystic ovarian syndrome (PCOS). There have been cross-sectional studies showing a possible association between low vitamin D and menstrual dysfunction ,infertility ,obesity, insulin resistance in patients with PCOS**.[133]**
the American college of obstetrics and gynecology has recommended as the institute of medicine of600 IU of vitamin d daily for pregnant women ,but ,if deficiency is identified ,most experts agree that 1000 to2000 IU per day is safe. [**134**]

1.8.2..A:1: Vitamin D and insulin resistance

Accumulating evidence suggests that vitamin D is associated with a lot of metabolic and reproductive features of PCOS and thus may be involved in the pathogenesis of the syndrome. It is noticeable that hyperinsulinemia and insulin resistance have an important role in the pathogenesis of PCOS,

affecting the severity of clinical features independently of the presence of obesity. The following potential mechanisms linking vitaminD with IR have been proposed: (i) vitamin D improves insulin action by up regulating the expression of the insulin receptor and enhancing insulin responsiveness for glucose Transport **. [135]**

(ii) 1,25(OH)2D3 activates the transcription of the VDRE of the human insulin gene which it has in its promoter**;[136]**

(iii) vitamin D regulates intracellular and extracellular calcium, which is crucial for insulin-mediated actions in insulin-responsive tissues;[**137**]

(iv) vitamin D exerts anti-inflammatory actions.2,9 However, most PCOS women are either overweight or obese. Obesity is associated with lower 25(OH)VitD levels, mainly due to the sequestration of the lipophilic vitamin in adipose tissue as well as due to lower sunlight exposure of obese subjects.

1.8.2.A:2: VDR gene polymorphisms and PCOS

The effect of VDR in the regulation of the human genome has motivated researchers to examine the contribution of the vitamin D receptor (*VDR*)gene polymorphisms in metabolic and endocrine disturbances of PCOS [**138**].The results reflect an influence of *VDR* gene variants in PCOS features; however, because they are as yet controversial,it is difficult to establish a clear association of VDR polymorphisms with the development of PCOS Vitamin D Receptor **[139,140]**. polymorphisms were associated with an increased risk of PCOS. also there is an association of the VDR gene polymorphism with testosterone levels in PCOS women, whereas VDR *Cdx2* variants were associated with insulin sensitivity **[141].**

1.8.2.A.3:(c)25(OH)Vitamin D status and hyperandrogenism markers

A study of 120 PCOS women (median age 28years), 25(OH)Vit D levels were significantly correlated with free androgen index (FAI) and SHBG but not with testosterone, DHEA-S, androstendione and LH/FSH ratio**.[142]**

In subsequent studies 25(OH)Vitamin D levels were positively associated with SHBG.[**143**,**144**]and negatively associated with FAI**. [145,146]** furthermore, a study of 100 PCOS women demonstrated that 25(OH)Vit D levels were negatively correlated with testosterone and DHEA-S levels in obese PCOS subjects**.[147]**However, a recent study failed to observe any association between 25(OH)Vitamin D levels and hyperandrogenism markers**.[148]**

finally recent retrospective cohort study reported that PCOS infertile women with adequate 25(OH)VitD levels (>30 ng/ml) were more likely to achieve ovulation compared to those with 25(OH)VitD levels <20 ng/ml. Moreover, women achieving live births had higher 25(OH)Vitamin D levels compared to those failing to carry out a live birth. Thus, an adequate 25(OH)VitD status could be a determining factor for a successful ovulation and pregnancy outcome for infertile PCOS women **[149]**

1.8.2.B: CANCER

a 17% reduction in cancer mortality with each 20 ng/mL (50 nmol/L) increase in circulating 25-hydroxyvitamin D concentrations. Yet, a sex-based subgroup analysis of eight studies found an inverse association between circulating vitamin D and cancer mortality in women, but not in men **[150,151,152**].

In addition, increasing evidence suggests that a few variations in the gene coding for the vitamin D receptor (VDR) might influence individual vitamin D

status and subsequently modify the susceptibility to site-specific cancers and influence cancer survival **[153,154]**.

Finally, many malignant tumors have been found to express the VDR, including breast,lung, skin (melanoma), colon and bone , suggesting that they might be susceptible to the effects of vitamin D [**155**].

1.8.2.C:Acute respiratory infections

More than 200 viruses are responsible for causing familiar infections of the upper respiratory tract (URT), known as the common cold, resulting in symptoms of nasal congestion and discharge, cough, sore throat, and sneezing [**156**].

The analysis of cross-sectional data from 18,883 participants (ages 12 years and older) of the Third US National Health and Nutrition Examination Survey (NHANES III) reported an inverse relationship between serum 25-hydroxyvitamin D concentrations and recent (self-reported) URT infection (URTI). [**157**]Compared to levels of circulating vitamin D of 30 ng/mL or above, the risk of URTI was 24% higher in individuals with concentrations between 10 and 29 ng/mL and 36% higher in those with levels below 10 ng/mL [**158**].

A subgroup analysis indicated that low concentrations of serum 25-hydroxyvitamin D in subjects with asthma and chronic obstructive pulmonary disease(COPD) were linked to a greater susceptibility to URTI when compared to people without pulmonary disease [**159**]**.**

1.8.2.D: Cardiovascular Disease

Living at higher altitudes increases the risk of hypertension and cardiovascular disease [**160**].

In a study of patients with hypertension who were exposed to ultraviolet B radiation three times a week for 3months,25-hydroxyvitamin D levels increased by approximately 180%, and blood pressure became normal (both systolic and diastolic blood pressure reduced by 6 mm Hg) [**161**] .

Vitamin D deficiency is associated with congestive heart and blood levels of inflammatory factors, including C-reactive protein and interleukin-10 [**162**].

Chapter Two

Patients and Methods

2: patients and Methods:

2.1:Study design

After having permission from Ethics Committee of university of AL–Qadisiyah of medical sciences, this study was conducted as clinical trial design from March 2018 TO July 2018 we studied 60 Iraqi females with polycystic ovary syndrome referring to private clinic, women' s consultant unit, outpatients clinics at Maternity and Pediatrics Teaching Hospital in AL-Diwaniyah city, Iraq. Sampling involved women aged from 18 to 45year established on inclusion criteria.. In our study, the aim of the project was explained to all females , and if they agreed, informed consent was obtained . then ,the patient's basic data including : age, blood pressure , body mass index ,history of regularity of period , marital status, previous history of miscarriage and infertility, number of parities, vitamin D level ,testosterone level ,and the glucose tolerance test have been recorded .

2.1: Inclusion criteria

1. the age of women range from 18 to45 years.

2. serum Vitamin D below 30 ng/ml .

3. Rotterdam criteria for PCOS diagnosis have been use {3} so patient should encounter at least 2 out of these criteria including:

A: oligo-ovulation or anovulation characterized by oligomenorrhea or amenorrhea.

B: Hyperndrogenesis with a clinical or laboratory diagnosis characterized by hirsutism or alopecia ,acne assessed by dermaloogist or increased blood testosterone levels .

C: Polycystic ovary characterized by ultrasound that means at least 12 follicles per ovary, or 9–2 mm in size, or ovarian enlargement of more than 10 ml obtained from the formula [0.5 _ length _ width _ thickness]in Ultrasound **[90]**

5.all women which included in this study have testosterone level between 3.5 to 5nmol/L (normal level of testosterone in females between 0.5 to 3.5 nmol/L)

6. screening for impaired glucose tolerance test had been done for 80 women and only 60 women was included in this study who have IGTT (two hour plasma glucose level of 140 t0 199mg /dl (7.8 to 11.o mmol/L)).

2.1.3Exclusion criteria

Women suffering from Diseases which are chronic for example chronic kidney disease, liver cirrhosis, pancreatitis, nephrotic syndrome , tumors and Diabetes mellitus, Cushing's syndrome ,hyper prolactenemia , congenital adrenal hyperplasia(adult onset) , and androgen secretion tumors. Drugs which affecting metabolic parameters such as metformin, corticosteroid stopped three months prior to the study. Calcium and multivitamin should not be used six months before the experiment. women should not be pregnant or lactating.

2.2: Measurement methods

Calculation of body mass index(BMI): By measuring body weight in kilograms using a digital scale and dividing it in to squared height in meter (Kg/m2).Quantitative test of total 25 (OH)D2/D3 level in human serum /plasma was measured by ICHROMA device using immunofluorescence method using vitamin D kit .This test kit is standardized to detect vitamin D concentrations as the follow:

Less than 10 ng /ml this indicate vitamin D deficiency

Between(10-30)ng /ml this indicate vitamin d insufficiency.

Between 30 -100 ng /ml this indicate vitamin d sufficiency

Testosterone level was measured by using quantitative test of testosterone in human serum/plasma by ICHROMA device using Fluorescence immunoassay method (FIA). The lowest testosterone reading was 3.7nmol/Land highest 5nmol /L.

Impaired glucose tolerance is done to all females included in this study. women being tested should be in a fasting state [having no food or drink except water for at least 10 hours but not greater than 16 hours], then patient giving 75 g of oral glucose then after 2hour post prandial plasma samples were collected in 10 ml tubes , plasma glucose concentrations were measured in certified clinical laboratory using photometric method .After diagnosis of impaired glucose tolerance test depend on if blood glucose between 140-199mg /dl(7.8 to 11.o mmol/L) about 20 women was excluded because they have normal IGTT and the other 60 women was included in our study .

2.2.1:clinical Assessment :include determination of hirsutism by dermatologist using modified Ferriman-Gallewy scoring system (mfG) . **[162]**

Blood pressure was measured for all the patients, ask them about any skin change like acne and counseling of dermatologist in order to made the diagnosis of acne. It should be noted that all test were conducted at single private laboratory to increase reliability and minimize the variance between laboratories .after the diagnosis of vitamin d deficiency has been done vitamin d was administering at 5000 units daily or 50000 unit weekly for 8weeks according to patients wish and availability of the drug for each women in this study . all data were also re-measured two months after the start of the treatment. In the end, descriptive statistics were used to provide statistical indices, tables and charts .

2.3: Statistical Analysis

Data were collected and included in a data based system and analyzed by statistical package of social sciences((SPSS, Inc., Chicago, IL, USA)) version 20. Parametric data were expressed as mean ± standard deviation (SD).It was analyzed statistically using student t-test while non-parametric data were expressed as percentages and were analyzed using chi square. Significance was set at the $P \leq 0.05$ level in all analyses.

Chapter Three

Results

3.1:Result

Sixty patients with poly cystic syndrome enrolled in study, age range from 18-39 years, mean age 27.48±5.95 years as in table 1. With metabolic parameter at start of study ,Vitamin D level 16.1±5.6, impaired glucose test reading 8.8± 0.7 and testosterone level 4.5 ±0.64. sixty five percent of patient were married and 35% were unmarried as in table 2.

Table 1: age distribution.

Age (Mean ±Std. Deviation)	**27.48 ±5.95**
Minimum	18
Maximum	39

Table 2: marital status of study sample.

	Frequency	Percent
married	39	65
unmarried	21	35
Total	60	100

Out of 39 married patient there are 25.6% had no pregnancy else, 25.6% had one pregnancy, 33.4% 2 pregnancy and 2.5% for patients had 4 and 5 pregnancy as in table 3.

Another result shows in table 4 period of infertility, 46% had no period infertility, 20.5% had 4 years infertility, 12.5% of patient had 3 years infertility, patient had period of infertility 7 years constitute 5%.

Table 3: parity of patients

	parity	**Frequency**	**Percent**
	0	**10**	**25.6**
	1	**10**	**25.6**
	2	**13**	**33.4**
	3	**4**	**10.4**
	4	**1**	**2.5**
	5	**1**	**2.5**
	Total	**39**	

Table 4: period of infertility for patient

	infertility	Frequency	Percent
	2 year	1	2.5
	2.5	2	5
	3	5	12.5
	3.5	2	5
	4	8	20.5
	5	1	2.5
	7	2	5
	no	18	46
	Total	39	100

body mass index(BMI = Weight [in Kg] / Height^2 [in meters]) , Out of 60 patient there were 31.7% normal weight, 40% over weight, 28.3% obese as in table 5.

Table 5: body mass index of patient.

	BMI	number	Percent
BMI	normal weight	19	31.7
	Overweight	24	40
	Obese	17	28.3
	Total	60	100

At the start of the study there were ninety percent of patient had menstrual irregularity,10% had regular menstrual cycle without any hormonal therapy, as in table 6**.** Sixty percent of patient had skin changes, appearance of acne and 40% had no such problem as in table 7. Seventy percent had manifestation of hirsutism, while 30% of patient give no history of hair changes, as in table8.

Table 6: regularity of menstrual cycle.

	Mc	Frequency	Percent
Menstrual cycle (mc)	irregular	54	90
	Regular	6	10
	Total	60	100

Table7: skin changes in sample.

	acne	Frequency	Percent
Acne	yes	36	60
	no	24	40
	Total	60	100

Table8: distribution of hair changes in patients.

		Frequency	Percent
Hirsutism	No	18	30
	Yes	42	70
	Total	60	100

At the beginning of the study mean level of vitamin D was16.11 ±5.6, after 2 month became 35.9 ±4.3 which is significant difference (p value = 0.002) as in table 9. after 2 month of supplementation there were 83.7% of patients reach the normal level of vitamin D and 16.3% still had low level of vitamin D (p value = 0.002) between two groups, as in table10.

Table9: level of serum vitamin D through the study.

	No.	Vitamin D	p-value
At baseline	60	16.11 ±5.6 ng/l	0.002
After 2 months	50	35.9 ±4.3ng/l	

Table 10: serum level of vitamin D between groups.

		No. (%)	Mean ±SD	p-value
Vitamin D after 2 month	Normal	50 (83.7)	35.1±4.3ng/ml	0.001
	deficiency	10 (16.3)	17.8±7.4ng/ml	
	Total	60		

Impaired glucose tolerance test after 2 month of supplementation there were 51.6% of patients still had impaired test while 48.4% reach the normal reading (p value = 0.001), as in table 12. On another hand 75% of patients reach the normal level of testosterone and 25% had abnormal level (p value = 0.001), as in table 14 .

Table 11: IGT reading before and after vitamin supplementation.

	No.	IGT	p-value
At baseline	60	8.8 ±0.9mmol/l	0.02
After 2 months	60	7.4 ±1.08mmol/l	

Table 12: IGT after 2 months.

		No. (%)	Mean ± SD	p-value
IGT after 2 months	Impaired	31 (51.6)	8.2±0.4mmol/l	0.001
	Normal	29 (48.4)	6.4± 0.8mmol/l	
	Total	60		

Table 13: testosterone level before and after vitamin supplementation.

	No.	Testosterone	p-value
At baseline	**60**	**4.5±0.6nmol/l**	**0.01**
After 2 months	**60**	**2.8 ±1.02nmol/l**	

Table 14: testosterone level after 2 months.

		No. (%)	Mean ± SD	p-value
Testosterone after 2 months	**Normal**	**45 (75)**	**2.2±0.4nmol/l**	**0.001**
	Abnormal	**15 (25)**	**4.02±0.8 nmol/l**	
	Total	**60**		

After 2 month of supplementation of vitamin D there were 77.7% without hormonal therapy became regular menstrual cycle, while 22.3% still irregular cycle as in table 15. About acne which assessed by dermatologist there were 47.3% had improvement from acne, and 52.7% had no changes in appearance of acne from start of study, as in table 16. Hirsutism became better

in 66.9% of patients and 33.1% same hirsutism in comparism to beginning of study as in table 17.

Table 15: menstrual cycle after vitamin D.

		Frequency	Percent
Menstrual cycle after vitamin D	**Yes**	**42**	**77.7**
	No	**12**	**22.3**
	Total	**54**	

Table 16: distribution of Acne after vit D.

		Frequency	Percent
improvement of Acne after vit D	**Yes**	**17**	**47.3**
	No	**19**	**52.7**
	Total	**36**	**100**

Table 17 : hair changes after vitamin D

		Frequency	Percent
hirsutism after vit D improvement	**Better**	**28**	**66.9**
	Same	**14**	**33.1**
	Total	**42**	**100**

In table 18 show inverse Correlations between vitamin D with IGT and testosterone, which mean an increase in vitamin D level after supplementation lead to decrease in serum level of testosterone (p value =0.02) and also decrease in reading of glucose in patient who had a history of impaired tolerance test(p value = 0.04).

Table 18: correlation between vitamin D and IGT and testosterone level.

	r	p-value
IGT	**-0.39**	**0.02**
Testosterone	**-0.15**	**0.04**

Chapter Four
Discussion

4.1: DISCUSSION

Polycystic ovarian syndrome (PCOS) is one of the most common endocrine disorders among women of reproductive age. Its worldwide prevalence has been estimated between2.2% and 26%.[**163,164**]

In our study demonstrate mean age of sample 27.48 ±5.95,consist with study by Homeira Rashidi at el (2016) [**165**], while other study by Nashwa E. Hassan at el (2012)reported mean of age much less than our study23.4±71.[**166**].

other study in Iran M, at el (2017) recorded age at higher than our study 31.35 ± 6.57 [1**76**]

Our result indicated women with PCOS have significant low level of vitamin D in mean 16.11± 5.6 ng/ml , impaired glucose tolerance test in mean 8.8±0.8 mmol/l, and high level of testosterone 4.5±0.6 nmol/L. consistent with the findings of previous studies that reported lower vitamin D levels in PCOS patients [**169**,**170**].These indicate that low vitamin D levels are associated with insulin resistance in women with PCOS [**171**].

Hahn et al.(2013) reported in 120 PCOS women an association of low vitamin D levels with insulin resistance.[**172**]

Other studies suggest that low vitamin D levels are related to impaired glucose clearance, insulin secretion, and insulin resistance.[**173**]

The mechanisms underlying the association of low vitamin D levels and insulin resistance First , vitamin D may have a beneficial effect on insulin action by stimulating the expression of insulin receptor and thereby enhancing insulin responsiveness for glucose transport .[**174,175**]

Secondly, vitamin D regulates extracellular and intracellular calcium, which is essential for insulin-mediated intracellular processes in insulin-responsive tissues such as skeletal muscleand adipose tissue.[**176**]

Finally, as vitamin D has a modulating effect on the immune system, hypovitaminosis D might induce a higher inflammatory response, which is associated with insulin resistance .[**128**] In turn, an additional mechanism might be seen in impaired b-cell function in PCOS women.[**128**]

There is evidence suggesting that low serum vitamin D levels are associated with abnormalities in markers of hyperandrogenism. For instance, some studies report inverse associations between serum vitamin D levels and testosterone, with PCOS[**172**,**176**] consistent with results were obtained from our study.

In our result there were 90% of patients had menstrual irregularity at start of study, these figure coincide with study by Li hw et al(2011)which reported 89% menstrual irregularity[**169**],while other studies by Gallea el at. (2014) reported 83% and 66.7% [**170**] .

After vitamin D supplementation for 2 month 42 out of 54 PCOS Women previously affected by menstrual disturbances reported improvement of menstrual regularity .[**171**]

Rashidi et al.(2017) reported a beneficial effect of vitamin D therapy in regulating the menstrual cycle with possible increase in pregnancy rates in PCOS patients. Thus, vitamin D supplementation might be an element in the complex treatment of PCOS women.[**168**]

A study by Norman at el.(2006) in Netherlands demonstrated an improvement in menstrual regularity and acne, and two women became pregnant in a follow-up period of 6 months.[**116**] In addition, a more recent study carried out in Iran including 60 infertile PCOS women has found an improvement in menstrual regularity after 3 months of supplementation with 1000 mg/day calcium and 400 IU/day vitamin D .[**167**]

In another study by Irani et al. in 68 vitamin D deficient PCOS women who were randomized to either 50,000 IU of cholecalciferol vs. placebo over 8 weeks, a significant decrease in transforming growth factor-β1 (TFG-β1) bioavailability, correlating with a possible improvement in several adverse parameters associated with PCOS was reported.[**172**]

Another finding of that study by Raja khann et al.(2014) was a significant reduction of interval between menstrual periods. Further, a positive effect on menstrual cycle regulation as observed when metformin plus vitamin D was compared with metformin alone .[**173**]

Our result demonstrate 28.3% of patient were obese, 40% were overweight, coincide with other study by Rashidi et al (2017)[**168**] Moreover, obesity is highly prevalent in women affected by PCOS, with the highest prevalence being reported in studies conducted in the USA and Australia, with 61–76% of women with PCOS being considered obese and 85% considered overweight or obese .[**128**]

Our data suggest a relationship of vitamin D and BMI in PCOS women , which is in agreement with many studies Gunjan Gargl et al.(2015), Li hw et al (2011)and Raja Khann et al(20143).[**167**,**169**,**175**]

However it is not clear whether vitamin D insufficiency results from obesity and/or whether obesity is a consequence of vitamin D insufficiency. On the one hand, obesity may contribute to low circulating vitamin D levels by trapping vitamin D in fat tissues.[**167**]

It has also been shown that obesity rates are higher in PCOS patients than in healthy individuals .[**166**]

It is known that obesity is the main risk factor for developing insulin resistance, T2 DM., and cardiovascular diseases .One explanation may be that, inflammatory mediators released from adipose tissue immune cells contribute to metabolic disorders in such cases .[**176**]

However, this fat-soluble vitamin is stored in the body's adipose tissue in terms of increased total fat mass, which explains the reason of 35% increase in vitamin D deficiency in obese patients compared to normal weight subjects.[**175**]

While several mechanisms have been proposed for the impact of vitamin D supplementations on glucose metabolism, suppression of pro-inflammatory cytokines and increased insulin sensitivity are considered the most important factors for this event . [**177**]

It therefore appears that obesity is the main cause of both vitamin D deficiency and insulin resistance in PCOS patients .[**166**]

Krul-Poel (2013) [**150**] demonstrated inverse relationship between BMI and vitamin D status has been established . [**178**]

It has been shown that in obese individuals a higher proportion of vitamin D, which is fat soluble, is sequestered in adipose tissue and there by the bioavailability of vitamin D is low..[**174**]

Alternatively, obese individuals tend to spend less time outdoors exposed to sunlight, leading to insufficient biosynthesis of vitamin D generated through the skin . [**179**]

Another study found that in obese women with PCOS and insufficient vitamin D levels, vitamin D replacement therapy significantly decreased impaired glucose tolerance test. [**173**]

Regarding clinical signs of hyperandrogenism, 60% of PCOS subjects presented with acne, similar result by Gunjan Garg[**166**] , while Nashwa E. Hassan (2012) [**167**] reported 35% of cases had acne, after supplemented of vitamin D there were 47.3% became improved.

Our result reveal 70% of patient had hirsutism , while 30% had no such symptoms, other study reported Sixty-one women had Hirsutism [**167**],, and study by Gunjan Garg (2015)[**168**] reported 37% only. After vitamin D

supplemented there were 66.9% of hirsutism patient became better. These coincide with study by Nashwa E. Hassan (2012) .[**167**]

Some studies also reported a significant effect of vitamin D supplementation on androgen concentrations: The study by Jamilian et al (2017)found a significant reduction in testosterone level and hirsutism after cholecalciferol supplementation. [**180**]

In a study by Razavi et al.(2016) supplementation of 2000 IU vitamin D lead to a significant decrease in testosterone level .[**181**]

Vitamin D levels in women 16.11± 5.6 and 35.9 ±4.3 before and after treatment, respectively. 83.7% reach the normal level t-test showed significant difference between vitamin D levels before and after treatment ($P < .05$). study by Homeira Rashidi (2017) showed no significant difference between vitamin D levels before and after treatment ($P > .05$).[**165**]

The IGTT level at baseline was 8.8 ± 0.9mmol/l and after treatment, 48.4% became normal reading (6.4± 0.8)mmol/l , t-test showed a significant difference between the level of IGT before and after treatment ($P < .05$).

On the other hand, the level of testosterone were4.5 ± 0.6 nmol/l before treatment , while 75% after treatment decrease to normal level (2.2±0.4) ($P < .05$). a significant fall in serum testosterone was observed in 2 months in comparison with the baseline in the same group. A similar result has also been described in a study by Pal et al. .[**170**]

However study by Chunla He et al.(2015) have observed no effect on serum testosterone levels. A direct effect of vitamin D on the steroidogenesis pathway (ovarian and/or adrenal) has been proposed to explain the observed reduction in circulating androgens . **[170**]

Other study by Nashwa E. Hassan et al.(**2012**) results revealed that after receiving vitamin D supplementation, there were a changes in impaired glucose

tolerance test and reduction in concentration of testosterone, but these changes not reach to normal value.[**166**]

Several factors may be able to explain the conflicting results, including the different characteristics of the research subjects, the length of study and the various vitamin D forms used for supplementation.[**179,181**]

If a causal relationship exists, intervention of vitamin D is supposed to result in mitigation of metabolic and hormonal features in PCOS. In this regard, it is questionable that there is a cause-effect relationship between Vitamin D deficiency and PCOs.[**123**]

Shoelson SE et al.(2007) found that serum vitamin D levels were not significantly associated with insulin resistance. On the other hand, conflicting results have been reported for such variables, as vitamin D deficiency was positively associated with insulin resistance among PCOS women .[**172**]

Lack of significant changes in the studied factors in the study population has led us to reconsider the hypothesis of vitamin D administration with the aim of preventing PCOS since if vitamin D only changes serum levels of IGT and testosterone, when these markers are not changed to normal as found in the present study, it will not be a good solution for preventing the disease or assisting women. [**123**]

This finding suggests that administration of vitamin D has not succeeded in stabilizing the women' serum profile and helping them to reduce the complications of the disease .[**175**]

A study by Selimoglu et al. in (2010) in Turkey conducted on 11 PCOS women. They reported that only two women were within the normal range of vitamin D. By administering single oral doses of 300,000 units of vitamin D3, they found that plasma glucose and insulin levels did not change significantly while no change was observed in testosterone and DHEAS levels.[**182**]

Also, Raja Khan et al. (2014) evaluated the effect of high dose of vitamin D in form of a daily dose of 12,000 IU of vitamin D3 along with a daily placebo administration for 12 weeks on Insulin Sensitivity Check Index.Compared with the placebo group , the level of vitamin D increased in the group treated, but insulin sensitivity remained unchanged in women receiving high doses of vitamin d.[**173**]

These conflicting result of studies explained by the differences in study population sizes and study design (e.g. study durations of 8 weeks vs. 12 weeks vs. 6 months) or by different dosing regimens (e.g. daily administration vs. weekly administration vs. one-time administration at study start) .. [**174**]

Additionally, study by Gallea M, at el (2014) recruited PCOS patients regardless of their vitamin D status while others included only vitamin D-deficient participants.[**170**]

Chapter Five

Conclusion and Recommendations

5.1:Conclusion :

1. from Our study we concluded that women with PCOS have statically significant low level of vitamin D in mean ,impaired glucose tolerance test in mean and high level of testosterone .consistent with the findings of previous studies that reported lower vitamin D levels in PCOS patients.
2. After vitamin D supplementation for 2 month women previously affected by menstrual disturbances reported improvement of menstrual frequency.
3. Our data suggest a relationship of vitamin D and BMI in PCOS females .
4. Marked reduce in the level of glucose in patient with IGTT after treatment with vitamin d but not significant enough to reach the normal .
5. a significant fall in serum testosterone was observed in 2 months in comparison with the baseline in the same group.
6. Inverse correlations between vitamin D with IGT and testosterone, which mean an increase in vitamin D level after supplementation lead to decrease in serum level of testosterone .
7. There is an improvement in symptom of hyperandrogenism like hirsutism and acne but not significant enough to reach the normal after supplementation of vitamin D.

5.2: Recommendations:

1. Studying the effect of vitamin D supplementation on insulin sensitivity and androgen level in vitamin D deficient polycystic ovary syndrome patient with larger sample size .
2. Long-term randomized clinical study to assess the effect of vitamin D supplementation on polycystic ovary syndrome females .
3. Assessing the effect of vitamin D on testosterone using more specific hormone like free androgen ,androstenedione hormone .
4. Using insulin sensitivity test instead of IGTT.

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Appendices

موافقه لاشتراك فى البحث العلمى

اسم الباحث :

عنوان البحث:

انت مدعوة للمشاركه ببحث علمي سريري سيجرى في .....

الرجاء ان تأخذي الوقت الكافي لقراءة المعلومات التاليه بتأن قبل ان تقرري اذا كنت تريدين المشاركه ام لا. بامكانك طلب ايضاحات او معلومات اضافيه عن اي شي مذكور في هذه الاستماره عن هذه الدراسه ككل من طبيبتك .

في حال وافقت على المشاركه في الدراسه ،سيبقى اسمك طي الكتمان ولن يكون لاي شخص ،مالم ينص عليه القانون على ذلك ،حق الاطلاع على ملفك الطبي بأستثناء الطبيب المسؤول عن الدراسه ومعاونيه .

موافقة المشترك :

لقد قرأت استمارة القبول هذه وفهمت مضمونها .تمت الاجابه على اسئلتي جميعها .وبناءا عليه

فأنني حر مختار ،اجيز اجراء هذا البحث واوافق على الأشتراك فيه ،واني اعلم ان الباحث الدكتور ..... وزملائه ومعاونيه او مساعديه سيكونون مستعدين لاجابه عن اسئلتي وانه بأستطاعتي الاتصال بهم على الهاتف .....

واذاشعرت لاحقا ان الاجوبه تحتاج المزيد من الايضاح فسوف اتصل بأحد اعضاء لجنة الاخلاقيات كما اعرف تمام المعرفه بأنني حر في الانسحاب من هذا البحث متى شئت حتى بعد التوقيع على الموافقه دون ان يأثر ذلك على العنايه الطبيه المقدمه لي .

اسم المشترك :

توقيع المشترك :

Name?

Age?

married single Marital status?

History of miscarriage ?

Number of parity?

DO you have irregular periods?

YES ONE of the following is true

My period com about every 2-3mouths or more

I get my period every 2-3 weeks

N0 my period is regular

History of infertility and duration of infertility ?

DO you have acne or other skin problem example: darker patches on the back of the axilla or the back of the neck?

YES NO

Do you have any hair changes or extra hair growth? if the answer was yes one or more of the following true

Dark hair above lip like moustache

I have dark hair on the other places chin ,neck, or chest area

The hair on my head feels like getting thin

Body mass index=weight in kg/(height in meter)2?

DO you have grave for carbohydrate and sugar ?

Yes NO

BLOOD Pressure monitoring?

Testosterone Level : before treatment and after treatment

Vitamin D level before and after treatment

Impaired glucose tolerance before and after treatment

١. الاسم :
٢. العمر:
٣. الحاله الأجتماعيه:
٤. عدد الأطفال:
٥. هل الدوره الشهريه منتظمه اذا كان الجواب نعم احدى هذه النقاط صحيحه

١)دورتي تأتي كل ٢الى ٣ اشهر او اكثر

٢)دورتي تأتي كل ٢الى ٣ اسبوع

الجواب لا يعني ان الدوره منتظمه

٦)هل هناك تاريخ عقم وكم استمرت مدة العقم ؟

٧)هل هناك مشكلة ظهور حب الشباب في الوجه او تغيرات في الجلد مثلا وجود بقع غامقة اللون في الجزء الخلفي من الرقبه ؟

نعم لا

٨)هل هناك تغيرات في شعر الرأس او نمو شعر جديد في اماكن غير مألوفه اذا كانت الأجابه نعم احدى هذه النقاط صحيحه

١)نمو شعر كثيف وغامق اللون فوق الشفه يشبه اللحيه

٢)لديك شعر داكن في اماكن متفرقه من الذقن والرقبه والصدر

٣)الشعر في الرأس يبدو اصبح اقل كثافه٠

٩)قياس مؤشر كتلة الجسم =الوزن بال كغم /الطول ب المتر؟

١٠)هل لديك شهيه قويه وغير مسيطرعليها للحلويات او الكاربوهيدرات؟

نعم لا

١١)قياس ضغط الدم =الضغط العالي /الضغط الواطئ

١٢)هل هناك تاريخ سابق للأجهاض ؟

١٣)قياس الهرمون الذكري قبل العلاج وبعد العلاج

١٤)قياس فيتامين دي في الدم قبل العلاج وبعد العلاج

١٥)قياس تحمل السكر في الدم قبل العلاج وبعد العلاج

المقدمه

متلازمة تكيس المبايض هو اكثر امراض الغدد الصماء الخاصه بالنساء انتشارا في العالم يتميز بوجود خلل في عمل المبايض الانثويه ومقاومة الجسم لعمل الانسولين وزيادة الهرمون الذكري في الاناث .عادة يصيب الاناث في سن الخصوبه ويسسبب العديد من التغيرات الايضيه الغير طبيعيه .حديثا وجد ان لمستويات فيتامين دي في الدم لها علاقه مباشره في التغيرات الغير طبيعيه التي تحدث في هذه المتلازمه اهمها زياده مستويات الانسولين في الدم وضعف تحمل الكلكوز في الدم .هناك ابحاث قليله اثبتت ان اعطاء فيتامين دي لهؤلاء النساء له آثار ايجابيه كبيره على مقاومة الأنسولين وعلى اضطراب العاده الشهريه لدى هؤلاء النسوة لذلك من المحتمل مستقبلا ان يكون لفيتامين دي الدور الأهم في علاج متلازمة تكيس المبايض وهذا بدوره يحتاج الى المزيد من الأبحاث مستقبلا .

الهدف من الدراسه

قياس مدى تأثير اعطاء فيتامين دي على مقاومة الانسولين والهرمون الذكري في النساء اللواتي يعانون من نقص مستويات فيتامين دي ومتلازمة تكيس المبايض

الهدف والطريقة

هذه الدراسه اجريت على ٦٠امرأه عراقيه اعمارهم تتراوح بين (١٨الى ٤٥سنه) يعانون من متلازمة تكيس المبايض كانوا قد قاموا بزيارة الاستشاريه النسائيه والعيادات الخارجيه في المستشفى التعليمي للولاده والأطفال في مدينة الديوانيه في العراق .جميع البينات للمرضى سجلت وتم اجراء جميع التحاليل المختبريه في مختبر خاص واحد لكي نقلل من نسبة اللأختلافات في النتائج وبعد التأكد من تشخيص نقص مستويات فيتامين دي في هؤلآء النسوه الموافقات على المشاركه في التجربه تم اعطاء فيتامين دي بجرعة ٥٠٠٠٠ وحده اسبوعيا لمدة ثمان اسابيع متتاليه ومن ثم تم اعادة اجراء كافة الفحوصات المختبريه بعد اعطاء العلاج .

النتيجه

أجريت الدراسه على ستين مريضه مصابه بمرض متلازمة تكيس المبايض العمر يتراوح بين ١٨الى ٣٩سنه وكانت نتائج المتغيرات الايضيه مدى العمر من ١٨ال٣٩سنه ،معدل الاعمار من ٢٧.٤٨ -+٥.٩٥وكانت المتغيرات الايضيه في بداية الدراسه معدل قياسات فيتامين د ٥.٦+-١٦.١وكانت معدل قياس اختبار تحمل السكر في الدم ٨.٨+-٠.٧،معدل قياسات الهرمون الذكري للنساء المشاركات كان ١٤.٥+-٠.٦٤ز اما بالنسبه لقياس كتلة الجسم للنساء المشاركات من ٦٠مريضه كانت نسبة النساء ذوات الوزن الطبيعي ٣١.٧% وكانت صاحبات الوزن الزائد ٤٠%وكانت نسبة النساء البدينات حوالي ٢٨.٣%.كان نسبه النساء اللاواتي يعانين من التغيرات في الجلد (حب الشباب) حوالي ٦٠%واللواتي يعنين من ظهور الشعر في اماكن غير طبيعيه حوالي ٧٠%. في بداية الدراسه قياس فيتامين د كان ١٦.١١+-٥.٦وبعد شهرين من اعطاء العلاج اصبح المعدل ٣٥.٩الى ٤.٣ وهذا يعتبر تغير ملحوظ .بعد شهرين من العلاج كانت نسبه النساء اللواتي وصلن للمعدلات الطبيعيه ٨٣.٧%وكانت نسبه اللواتي لم يحدث تحسن في قرأتهن حوالي ١٦.٣%عند المقارنه بين هاتين المجموعتين كان نسبه

النساء اللاتي وصلن للقراءات الطبيعيه لفحص تحمل السكر حوالي ٥١.٦%وكانت نسبه النساء اللاتي وصلن للمعدلات الطبيعيه لقياس الهرمون الذكري حوالي ٧٥%وكانت القراءات الغير طبيعيه حوالي ٢٥%.

الأستنتاج :

من خلال هذه الدراسه نجد ان هناك علاقه وثيقه بين معدل نقص مستويات فيتامين د مع متلازمة تكيس المبايض ومع ضعف تحمل اختبار السكر بالدم والمعدلات العاليه لأرتفاع الهرمون الذكري في دم النساء المصابات بمتلازمه تكيس المبايض وهذا جاء متناسبا مع استنتاجات البحوث السابقه التي وجدت علاقه عكسيه بين فيتامين د واختبار تحمل السكر في الدم ومع قياسات الهرمون الذكري

أقرار المشرف

اني الاستاذ المساعد الدكتور صبا مطشر سوادي الثويني المشرف على رسالة طالبة الدبلوم العالي (المعادل للماجستير)امال رعد احمد ،قد اطلعت على رسالة الطالبه المذكوره والتي انجزت تحت اشرافي ،اقر واؤيد صلاحيتها للمناقشه لأستيفائها كافة المتطلبات العلميه لدرجة الدبلوم العالي .

التوقيع:

المشرف: **الاستاذ المساعد الدكتوره صبا مطشر الثويني**

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تأثيرات إعطاء فيتامين (د) على مستويات هرمون التيستوستيرون ومقاومة الانسولين في النساء المصابات بمتلازمة تكيس المبايض اللواتي لديهن نقص فيتامين (د)

رسالة مقدمة الى مجلس كلية الطب في جامعة القادسية كجزء من متطلبات نيل درجة الدبلوم العالي المعادل للماجستير في طب الأسرة

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