PhysioGynecological study of the effects of Metformin, Diane, and Androcur in treatment of infertile women with Polycystic Ovary Syndrome in Babylon province

A thesis

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دراسة فسلجية نسائية لتأثيرات كل من عقار المتفورمين والديان و الاندروكيور عند النساء العقيمات المصابات بمتلازمة تكيس المبايض المتكرر في محافظة بابل

رسالة مقدمة

الى مجلس كلية الطب /جامعة بابل
كجزء من متطلبات نيل درجة الماجستير في علوم الفسلجة الطبية

من قبل
بان ظاهر ذباح الشمري
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٢٠٠٩م ١٤٣٠هـ

الخلاصة:

إن العقم هو احد أهم المشاكل التي تصيب النساء في مجتمعنا ,وهناك أسباب عديدة لعقم النساء ,وأحد أهم هذه ألأسباب هو زيادة مستوى هرمون الاندروجين في الجسم خصوصا لدى النساء المصابات بمتلازمة تكيس المبايض المتكرر.

وأن هدف هذه الدراسة هو وصف التأثيرات المفيدة والمضرة لنوعين من الأنظمة العلاجية المعطاة للنساء العقيمات والمصابات بمتلازمة تكيس المبايض المتكرر(PCOS) في محافظة بابل.وقد تم جمع العينات في الفترة من تشرين الثاني- ٢٠٠٨ولغاية نيسان .٢٠٠٩ والهدف الأخر هو تقييم بعض العوامل الفسيولوجية النسائية المتعلقة بتكيس المبايض المتكرر وكذلك مقارنة تأثير المتفورمين وحده مع المجموعة الأخرى التي تتعاطى المتفورمين وحبوب منع الحمل والانتي اندروجين (Metformin+Diane+Androcur)على مرضى متلازمة تكيس المبايض المتكرر .إن عوامل الدراسة التي تم قياسها تقسم إلى عوامل سريريه وعوامل متعلقة بأمراض الدم وعوامل كيميائية:

العوامل السريرية عادة تؤخذ عن طريق التاريخ الطبي للمريض و الفحص ألسريري وتشمل :عمر المريض ونوع العقم ,محل الإقامة والحالة الاقتصادية ,تاريخ العائلة ,عمر البلوغ ,عدد أيام الدورة الشهرية,والفترة بين دورة وأخرى.أما فيما يخص الفحص الطبي فيشمل قياس ضغط الدم ومعامل كتلة الجسم ,قياس الخصر(WC) ونسبة الخصر إلى الحوض .

العوامل الكيميائية وتشمل الهرمونات (الهرمون المحفز لحويصلات المبيضFSH ,والهرمون الليوتيني LH , والهرمون الذكري التستوستيرون Testosterone وهرمون الحليب Prolactin)وكذلك نسبة البروتين الكلية في المصل ونسبة الكلوبيولين والالبومين في المصل وقياس إنزيمات الكبد.

أما العوامل المتعلقة بأمراض الدم فتشمل قياس نسبة الدم (كريات الدم المضغوطة),قياس عدد كريات الدم البيضاء وقياس الأنواع المميزة لهذه الكريات(Differential WBC s) ومعرفة صنف الدم .وأخيرا يتم إرسال المريض لفحص السونار.

إن هذه المجاميع الثلاثة يتم قياسها كعوامل أولية لمجاميع العينات الثلاثة المعتمدة في الدراسة(٣٠ مريضة تعالج بالمتفورمين وحده و٣٠ مريضة تعالج بالمتفورمين والدايان والانتي اندروجين,٣٠ امرأة كمجموعة سيطرة)ويتم إعادة قياس العوامل السابقة بعد 6 أشهر .

اما فيما يخص النتائج المتوخاة من هذه الدراسة فقد سجلت ارتفاع نسبة التوزيع الجغرافي للنساء العقيمات المصابات بمتلازمة تكيس المبايض المتكرر في المدن وخصوصا ذوات الدخل ألمعاشي المتوسط ,وان اغلب تلك النساء يعانين من العقم الأولي(**65%**) أكثر من العقم الثانوي(**35%**) وعددهن في المجموعة الأولى هو 30/18 والمجموعة الثانية 30/21 ,واغلبهن لديها تاريخ عائلي

موجب لنفس المرض أو داء السكري **(55%)**.30/16 في المجموعة الأولى (أ) و30/17 في المجموعة الثانية(ب).إن أكثر مجموعة عمرية سائدة هي التي تتراوح بين **(25-35)** وتمثل **(58%)** من المجموعة الكلية,وقد كشفت الدراسة أيضا إن معظم النساء العقيمات يحملن صنف الدم +O **(38%)**,بينما غالبية النساء الغير عقيمات من مجاميع السيطرة كانوا من مجموعة الدم +A **(30)** كما إن عمر البلوغ(Menarche) في غالبية تلك المريضات كان دون ال**14** سنة**(65%)**.

فيما يتعلق بنسبة الخصر إلى الحوض (WHR) فقد وجدنا زيادة في هذه النسبة مقارنة بمجموعة السيطرة,وان معدل هذه القيمة في مجموعة أ قبل وبعد العلاج هو**(0.9183± 0.0198** , **0.135±0.86)**بالتتابع ,معدل قيمة مجموعة ب هو **(0.895±0.194, 0.858 ± 0.0165)** بالتتابع بينما معدل قيمة مجموعة السيطرة يساوي**(0.09±0.7616)**.

إن معامل كتلة الجسم(BMI) يظهر اختلافا كبيرا بين مريضات تكيس المبايض عن مجموعة السيطرة ,وعليه فان معدل مجموعة(أ) يساوي(**30.64±5.1**) ومعدل مجموعة(**ب**)يساوي **(32±4.97)**مقارنة بمعدل مجموعة السيطرة والذي يساوي**(2.4± 27.2)**.

وان غالبية المريضات يعانين من دورة شهرية غير منتظمة فيبلغ معدل الفترة بين دورة واخرى في مجموعة أ (**78.3** يوم±**42.6**),وفي مجموعة ب (**103.5**يوم ±**49.6**)بينما في مجموعة السيطرة فكان**(2.1± 27.6**يوم).

وفي دراسة الهرمونات فأن هناك ارتفاع ملحوظ في مستوى الهرمون الليوتيني(LH) عن مجموعة السيطرة قبل العلاج وان معدل هذه النسبة في كل من مجموعة أ,ب يساوي(**10.05±5.48**),(**10.25±5.32**)بالتتابع ,مقارنة بمجموعة السيطرة **(3.3± 5.39)**ولكن هناك انخفاض ملحوظ في هذا المستوى بعد العلاج في كل من مجموعة أ,ب كما يلي (**6.76±3.53**),(**6.85**±4.35).مع بقاء نسبة الهرمون الحويصلي بالمستوى الطبيعي (**4.53±1.88**),(**4.06±1.66**)آما مجموعة السيطرة(**2.8± 4.44**),وفيما يخص الهرمون الذكري فهناك زيادة في مستواه الكلي قبل العلاج لدى المريضات بالمقارنة مع مجموعة السيطرة **(1.25±0.75)(1.38±0.725)(0.26±0.67)**بالتتابع.مع نقصان ملحوظ بعد العلاج **(1.05±0.60)(1.05±0.560)**بالتتابع.وفيما يخص هرمون الحليب فنلاحظ زيادة بسيطة في مستواه مقارنة بمجموعة السيطرة ولكن بصورة عامة يبقى ضمن المستوى الطبيعي **(13±8.25)(11.15±5.54)(4.8± 9.52)**.

وان الدراسة كذلك تكشف عن نقصان ملحوظ في مستوى نسبة الدم(PCV) وعدد كريات الدم لبيضاء بعد العلاج في كلا المجموعتين وفيما يخص تمييز الكريات فان هناك زيادة في عدد الكريات اللمفية(lymphocytes) قبل العلاج(**46.3,44)(39.5)**بالتتابع.

كذلك فقد سجلت الدراسة نقصان في إنزيمات الكبد(ALT,AST) بعد العلاج في كلا المجموعتين ,وزيادة في نسبة الكلوبيولين في مصل الدم بعد العلاج .فقد كانت النسبة قبل العلاج(**24.75±8.83**),(**23.67±8.68**)وبعد العلاج(**28.77±8.89**)(**24.67±8.77**)بالتتابع.

وفيما يخص ضغط الدم فلا توجد هناك اختلافات ملحوظة بين المجاميع الثلاثة .

أما درجة المشعرانية(Hirsutism) في الجسم فقد تم قياسها بواسطة (FG Score) فقد وجدنا أن ارتفاع هذه النسبة قد تم تصحيحه بعد العلاج في المجموعة الثانية(التي تتعاطى الدايان وإلانتي اندروجين) أكثر من الأولى بمعدل(**10.8±4.31**)(**13.13±4.95**)وبعد العلاج(**10.2±3.80**)(**9.6±2.58**) بالتتابع.

بسم الله الرحمن الرحيم

وَيَسْأَلُونَكَ عَنِ الرُّوحِ قُلِ الرُّوحُ مِنْ أَمْرِ
رَبِّي وَمَا أُوتِيتُمْ مِنَ الْعِلْمِ إِلَّا قَلِيلًا ﴿٨٥﴾

صدق الله العظيم

سورة الإسراء

الآية (٨٥)

DEDICATION

TO:

Holy IMAM
AL-HUJJA AL-MAHDI

MY FAMILY,

MY PARENTS,

MY SUPERVISORS,

MY HUSBAND

&

MY Daughters

AL-ZAHRAA & MANAR

BAN.

Aknowledgement

Thank God of the world as it should be for His loftiness face and His great authoring and God `s blessing and peace be upon the seal of the prophet, Mohammed .

I would like to express my great thanks and respects to Dr. Naseer J.H. Al-Mukhatar at the department of Physiology and Dr.Bushra J.Al-Rubaie at the Department of Gynecology, College of Medicine in Babylon to suggest the topic of the thesis and for this efficient participation to over-come all difficulties facing me through writing this paper thesis .

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Last but not least, all thanks evaluation and gratefulness to my family &my husband for their help and support in the course of study to carry out the thesis. I thank every person helps me even by sweet word that helps me to submit this thesis.

BAN

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List of Abbreviations:

Symbol	Description
A	Absorbency
Ab	Antibody
Ag	Antigen
ALT	Alanine amino transeferase
AST	Aspartate amino transeferase
BCG	Green of Bromocresol
BMI	Body mass index
C.I	Confidence interval
DHEA	Dihydroepiandrostendione
EDTA	Ethylene di amine tetra acetic acid
FG	Ferriman Gallwey score
FSH	Follicular Stimulating Hormone
GnRH	Gonadotrophin releasing hormone
GOT,GPT	Glutamate Oxaloacetate Transferase,Glutamate Pyruvate Transaminase
HCG	Human Chorionic Gonadotrophin
HCL	Hydrochloric acid
HOMA-IR	Homeostasis Model Assessment for Insulin Resistance
IBC	Interval Between the Cycles
IGF-1	Insulin like Growth Factor -1
IGFBP	Insulin like Growth Factor Binding protein
IgG	Immunoglobulin G
IR	Insulin Receptor
IR	Insulin Resistance

IVF	**Invetrofertilisation**
K2-EDTA	**Dipottassium-EDTA**
LH,LTH	**Lutenizing Hormone, Lutropic Hormone**
mRNA	**Messenger Ribonucleic Acid**
nm	**nanometer**
OCP	**Oral Contraceptive Pill**
O.D	**Optical Density**
PCOS	**Poly Cystic Ovary Syndrome(Poly Cystic Ovarian Syndrome)**
PCV	**Packed cell volume**
PRL	**Prolactin**
RPM	**Round per minute**
S.ALB	**Serum Albumin**
S.D	**Standard deviation**
S.G	**Serum Globulin**
SHBG	**Sex Hormone Binding Globulin**
T2DM	**Type 2 Diabetes Mellitus**
TG	**Triglyceride**
TSP	**Total Serum Protein**
U.S	**Ultrasound**
WBC s	**White Blood Cells**
W.C	**Waist Circumference**
W.H.R	**Waist Hip Ratio**

Summery:

Infertility is one of the important medical problems in our society, Female infertility has many causes, one of these is due to increase in androgens level especially in women with polycystic ovary.

The aim of this study is to describe the beneficial and harmful effect of two types of treatment regimes given to infertile women with PCOS in Babylon province, were recruited between November 2008 to April 2009, to evaluate some of Physiogynecological parameters in relation to PCOS and to assess the effect of metformin alone versus metformin with oral contraceptive pills and antiandrogens on PCOS patients. The parameters of the study were divided into clinical, Haematological and Biochemical paramameters.

Clinical parameters are usually taken from the history and the physical examination including : age of the patients, type of infertility ,economic state ,family history, onset of menarche ,days of bleeding ,intervals between the cycles, blood pressure ,BMI,W.C and WHR.

Biochemical parameters: include LH, FSH, LH/FSH ratio, Testosterone, Prolactin, TSP, ALT, AST, S.G, and S.ALB. and Hematological parameters :like Blood groups, PCV, WBC s count , and Differential WBC s count .These parameters are taken as base line measurement for the three groups(**30** patients takes metformin alone ,**30** patients takes three medications ,**30** subjects as control group). These three groups are reassessed after a period of 6 months by the same parameters.

The results of this study present a highly geographical distribution of infertile women with PCOS among urban areas and a middle economic state population. Most of those patients had primary infertility(**65%**) rather than secondary(**35%**), in group A 18/30 had primary infertility, while in group B 21/30 were primary ,and many of them had positive

family history of the same condition or history of type 2 diabetes(**55%**) .(16) Out of (30) in group A and (17) out of (30) in group B. the most predominant age group was between (**25-35**) represent (**58 %**) of total populations ,the study also reveals that most of infertile women were with blood group O+ (**38%**), while fertile control women were with blood group A+(**30%**), In most of those patients the onset of menarche was below the age of 14 year(**65%**). Regarding WHR patients had greater WHR than control. The mean value of WHR in group A pre and post treatment is (**0.9183± 0.0198, 0.86± 0.135)**, while in group B it is (**0.895±0.194, 0.858± 0.0165**) respectively, where as control mean value is equal to (**0.7617±0.09**).The WC shows significant difference between patient and control pre treatment with a mean of **(37.3±4.3, 37.1±2.6, 27.8±3.4)** while post treatment it was **(35.9±4.6, 35.9±2.2)** respectively. The BMI shows great difference among PCOS patients pre treatment from control groups .So the mean value of group A is equal to(**30.64 ± 5.1**), and group B is (**32 ± 4.97**) compared with control mean (**27.2±2.4**).Most of those patient had irregular interval between the cycles :the mean of group A is equal to(**78.3 day± 42.6**), and the mean of group B is (**103.5day±49.6**), while in control group it is equal to(**27.6day±2.1**).

Hormonal study shows significant increment in LH level pretreatment in both A and B groups: Their mean value are (**10.05± 5.48**) , (**10.25 ± 5.32**)respectively from control group(**5.39±3.3**) but there was a significant decrement in this level after treatment in both groups (**6.76±3.53**) and,(**6.85± 4.35**) with normal FSH level(**4.53± 1.88, 4.06± 1.66 , 4.44±2.8**),The total testosterone level increased in some patient while others remain normal, there may be an increment in their free testosterone level. Also, the study founds a high level of testosterone in patient's groups pretreatment compared with control (**1.25 ± 0.75, 1.38**

± 0.725, 0.67±0.26) especially in the second group with significant decrement after treatment (**1.05 ± 0.60, 1.05± 0.560**)respectively. The prolactin hormone level was increased non significantly from control group but it is within normal range (**13 ± 8.25, 11.15 ± 5.54),** and **(9.52±4.8**) in respect to control group.

This study reveals a significant decrement ($p<0.05$) in the level of PCV, with highly significant decrement ($p<0.001$) in WBC s count post treatment with metformin in both treatment's groups. Regarding differential count there is an increment in the lymphocyte count in both PCOS patients and control group pre treatment(**46.3, 44, 39.5**) respectively.

The study also records a decrement in the level of liver enzyme post treatment in both groups .There was an increment in S.G in both groups after treatment ,whereas the mean value pretreatment were (**24.75± 8.83 , 23.67±8.68**), with mean value post treatment were(**28.77±8.89 , 24.67± 8.77)** respectively. Blood pressure: There is no significant difference between the three groups, and all were present within normal range. And in respect to FG score, we found that the high degree of hirsutism in the second group was more corrected with Diane and Androcur, and their mean level pre treatment were (**10.8 ± 4.31, 13.13± 4.95**) and post treatment were (**10.2 ± 3.80, 9.6± 2.58**) compared with control **(6.3±2.33)**

We certify that this thesis was prepared under our supervision at the University of Babylon as partial fulfillment of the requirements for the degree of M.Sc. in medical physiology …

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Recommendations of the Head of Department of Physiology and Medical Physics :-

According to the Available Recommendations , We Certify Forward this Thesis for Debate by the Examination Committee.

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We, the examiner Committee certify that we have read this thesis entitled **(***PhysioGynecological study of the effects of Metformin ,Diane, and Androcur in treatment of infertile women with Polycystic Ovary Syndrome in Babylon province)* and have examined the student **(***Ban Dhahir thabbah AL-shemmary***)**in it's contents and that in our opinion it is accepted as a thesis for the degree of master of science in medical physiology with **(Excellent estimation)** .

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Conclusions:

The following conclusions based on the findings of the present study and are summarized as follows:

1. The highest incidence of PCOS among adult women occurs in age group (25-35) years and represent 58%, and 55% of patients have positive family history, and 65% of them had a history of primary infertility. Regarding the onset of menarche: higher population of PCOS patients appears to have menarche below the age of 14years, and high geographical distribution of PCOS occurs in urban areas and in those with a middle economic state.
2. From the studies included in this review, and according to the severity of signs and symptoms of PCOS patients, the physician can prescribe either metformin alone or with Diane(OCPs), Androcur. Therefore, there is limited evidence to support that the OCP alone is more effective than metformin in improving menstrual pattern and reducing serum total testosterone levels. So combining a metformin type of insulin sensitizer with an oral estrogen–progestin formulation(Diane) having a mixed effects on androgens, hirsutism and uterine bleeding, other studies recommending the use of metformin as a first line treatment for an ovulation in women with mild symptoms of PCOS and with out hirsutism , Our study shows significant decrement in IBC in both groups (more in group B)that support both previous statements in which the effect of metformin added to that of Diane together give a better response in whom with moderate to sever signs of hyperandrogenism .
3. PCOS were being predominant in blood group O+ of ABO system and there is significant decrement in PCV after treatment in both studied groups, as well as there are an increment in lymphocyte count with significant decrement in total WBC s count after

treatment for both groups A, B .Both treated groups show a decline in the level of liver enzymes (ALT, AST) after the follow up medications.

4. There is a positive significant relationship between W.C, B.M.I. pretreatment with significant decrement after treatment in both groups A, B.
5. Hormonal study shows that: there is larger reduction in LH hormone in both treatment's regimes with increment in LH/FSH ratio .The LH hormone present to have a positive relationship with WC, BMI. Also there is non significant decrement in FSH after treatment. While in prolactin there is increment after (group B) regimes, this may be due to effect of Cyproterone acetate.

Recommendations:

This study presents a number of recommendations to be carried out by the following health institutions:

1. Metformin should be used as an adjuvant to general lifestyle improvements and not as a replacement for increased exercise and improved diet in PCOS patients. Also recommending the use of metformin as a first line treatment for an ovulation in women with polycystic ovary syndrome.

2. Combinations of other OCP s, anti-androgens and insulin sensitizers could also be of benefit. This new approach needs to be further investigated for an optimal treatment of an overweight PCOS patient presenting with high insulin and requesting an oral contraception.

3. Deeper studies were needed for the relation ship between PCOS and liver enzymes,insulin resistance, differential WBC s count .,SHBG.

Chapter One

Introduction

Chapter Two

Literatures Review

Chapter Three

Materials &Methods

Chapter Four

The Results

Chapter Five

Discussion

Conclusions & Recommendations

References

1. Introduction:

1.1. Polycystic Ovary Syndrome (PCOS):

It is a condition that causes irregular menstrual periods and elevated levels of androgens (male hormones) in women. The elevated androgen levels can sometimes cause excessive facial hair growth, acne, and/or male-pattern hair thinning.(Legro, *et al* ,2007) and it is the commonest endocrine disturbance in women of reproductive years. Its aetiology remains unclear, and its clinical features include hyperandrogenism, obesity, menstrual irregularity and anovular infertility, but the clinical presentation can vary (Balen, 1999). PCOS is familial and thought to be the morphological manifestation of a genetically determined disorder, whereas its heterogeneity is believed to result from interaction with environmental factors (Frank, 1995).PCOS had many differential diagnosis include the following:

A. Androgen secreting tumors of the ovary or adrenal gland
B. Hypogonadotropic hypogonadism (nutrition, excessive exercise, chronic disease)
C. Pituitary tumor and other prolactin disorders
D. Cushing's syndrome
E. Nonclassic congenital adrenal hyperplasia
F. Acromegaly
G. Genetic defects in insulin action
H. Primary hypothalamic amenorrhea
I. Primary ovarian failure
J. Thyroid disease
K. Exogenous androgens

The following are the Criteria for Diagnosis of PCOS:

A. Rotterdam Criteria: two of the following, in addition to exclusion of related disorders:

- Oligo- or anovulation
- Clinical and/or biochemical signs of hyperandrogenism either increased total or free testosterone or decreased SHBG. particularly among obese women, or increased LH/FSH ratio.
- Polycystic ovaries on transvaginal ultrasound.

B. For exclusion of other disorders that may cause similar symptoms

- Prolactin to rule out hyperprolactinemia
- 17-hydroxyprogesterone to rule out 21-hydroxylase deficiency (congenital adrenal hyperplasia).

The management of PCOS can be summarized in the following points:

1. **Home treatment** (changing life style)

- Weight control or weight loss (lowers your risk for Diabetes and hypertention.)
- Eat a balanced diet.
- Stay at a healthy body weight.
- Exercise (make a regular physical activity)
- If you smoke, consider quitting (Huang, 2007).

2. **Medical treatment** *:* (Tan ,*et al*. ,2007).

- Lowering of insulin levels (antidiabetics like metformin)
- Restoration of fertility

- Treatment of hirsutism or acne (antiandrogens like Androcur)
- Restoration of regular menstruation, and prevention of endometrial hyperplasia and endometrial cancer . (OCPs like Diane)

3. **Surgical treatment**: ovarian drilling by using laparoscopy (by electrocautery or laser) , or conventional surgery(ovarian wedge resection).

1.2. Aims and objectives of the study:

This study aim to:

1. Presenting the effects of age, residence and economic state , type of infertility, family history and onset of menarche on severity and distribution of PCOS patients in Babylon province.
2. Studying the relation ship of some parameters like WHR(waist hip ratio),BMI,WC(waist circumference)and physiological parameters (blood group, PCV, WBC s count, differential WBC s count) and biochemical parameters (liver enzymes ,TSP S.G ,S.ALB, Hormones like LH, FSH ,LH/FSH ratio, Testosterone , Prolactin) to PCOS.
3. Comparing the effect of metformin alone and metformin with contraceptive pills like Diane, and antiandrogen (Androcur) on progression of symptoms between two populations of PCOS patients (compare the efficacy and safety of metformin versus OCP in the treatment of women with PCOS).
4. Estimating the benefits and side effects of medications and follow up the patients before and after treatment in a regular period of one month for a total period of 6 month.

2: Literatures review:

2:1 Anatomical and physiological review of female reproductive system

The human female reproductive system (figure 2-1) is a series of organs primarily located inside the body and around the pelvic region of a female that contribute towards the reproductive process. It contains three main parts: the vagina, which acts as the receptacle for the male's sperm, the uterus, which holds the developing fetus, and the ovaries, which produce the female's ova and hormones .The breasts are also an important reproductive organ during the parenting stage of reproduction. At certain intervals, typically approximately every 28 days, the ovaries release an ovum, which passes through the fallopian tube into the uterus. The lining of the uterus, called the endometrium, and unfertilized ova are shed each cycle through a process known as menstruation. (Kimball, *et al*., 2006).and the main function of this system (figure 2-1) includes:

1. Produces (ova)
2. Secretes sex hormones
3. Receives the male spermatazoa during sexual intercourse.
4. Protects and nourishes the fertilized egg until it is fully developed.
5. Delivers fetus through birth canal
6. Provides nourishment to the baby through milk secreted by mammary glands in the breast.

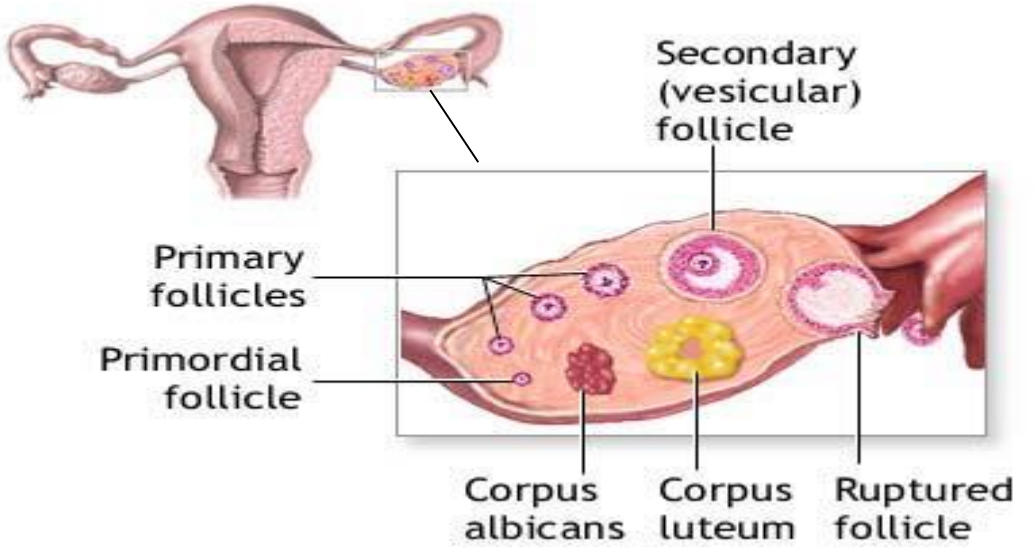


Figure (2-1) shows the female reproductive system anatomy with ovarian follicle (Peter**, 2008**).

2:2.Hormonal regulations during menstrual cycle in fertile female.

2:2:1The Female Reproductive Cycle (Menstrual cycle):

The normal reproductive years of the female are characterized by monthly rhythmical changes in the rates of secretion of the female hormones and corresponding physical changes in the ovaries and other sexual organs. This rhythmical pattern is called the female monthly sexual cycle (or, less accurately, the menstrual cycle) figure (2-2). The interval between the cycle averages 28 days. It may be as short as 20 days or as long as 45 days in some women, although abnormal cycle length is frequently associated with decreased fertility. There are two significant results of the female sexual cycle. First, only a single ovum is normally released from the ovaries each month, so that normally only a single fetus will begin to grow at a time. Second, the uterine endometrium is prepared in advance for implantation of the fertilized ovum at the required time of the month (Guyton and Hall, 2006).

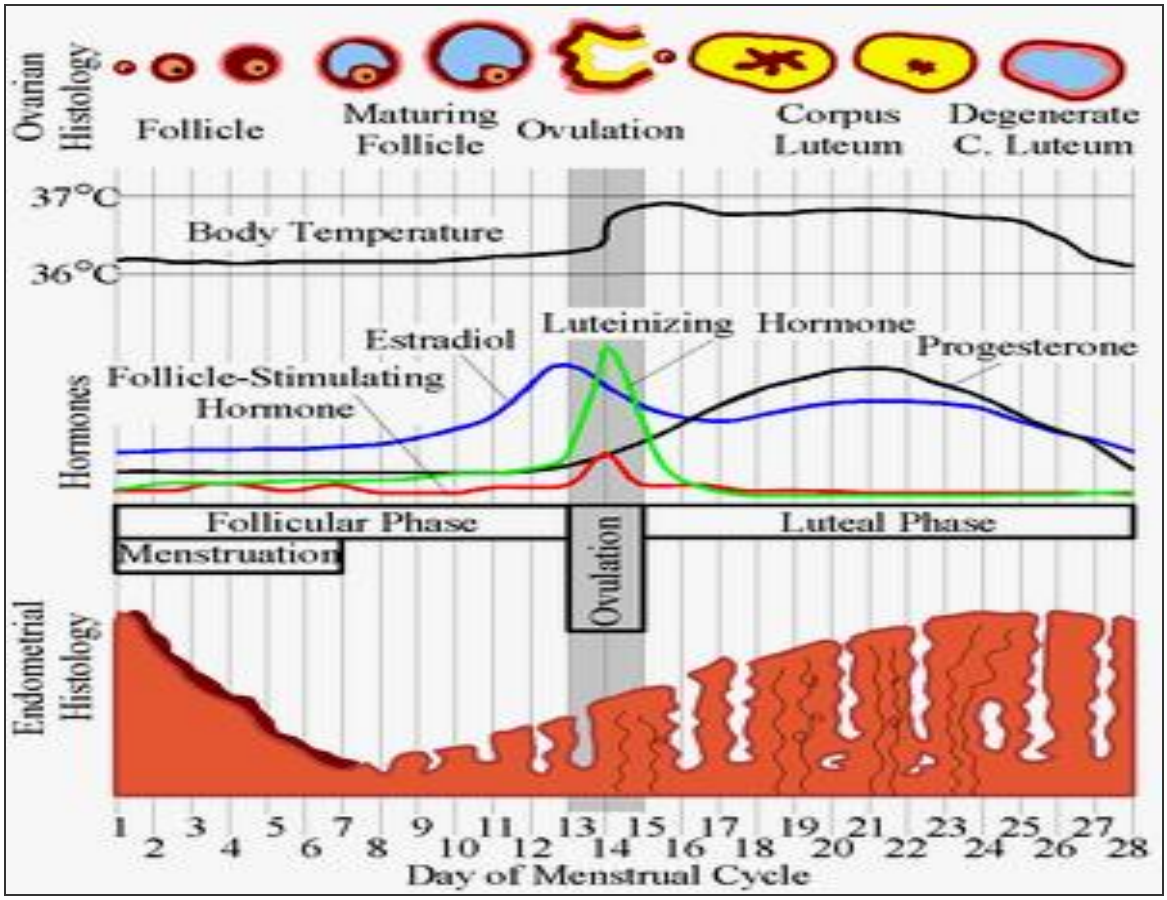


Figure (2-2) normal female menstrual cycle (Valerie and Tina, 2009).

The usual duration of the menstrual flow is 3–5 days, but flows as short as 1 day and as long as 8 days can occur in normal women. The amount of blood lost may range normally from slight spotting to 80 ml; the average amount lost is 30 ml. Loss of more than 80 ml is abnormal (Ganong, 2005).

Although women can become pregnant at any time during their menstrual cycle, peak fertility occurs during just a few days of the cycle: usually two days before and two days after the ovulation date (Creinin, *et al*., 2004). This fertile window varies from a woman to another, just as the ovulation date often varies from cycle to cycle for the same woman. The ovule is usually capable of being fertilized for up to 48 hours after it is released from the ovary. Sperm survive inside

the uterus between 48 to 72 hours on average, with the maximum being 120 hours (5 days). These periods and intervals are important factors for couples using the rhythm method of contraception (Creinin, *et al*., 2004).

2:2:1:1. The Hormonal Patterns During The Menstrual Cycle and Feed Back Mechanism:

The menstrual cycle is divided into two sequential phases, the "follicular phase" begins with the onset of menstrual bleeding .The luteal phase has a rather constant length of (13 – 14) days and ends with the onset of menstrual bleeding (Leavelle , 1994).

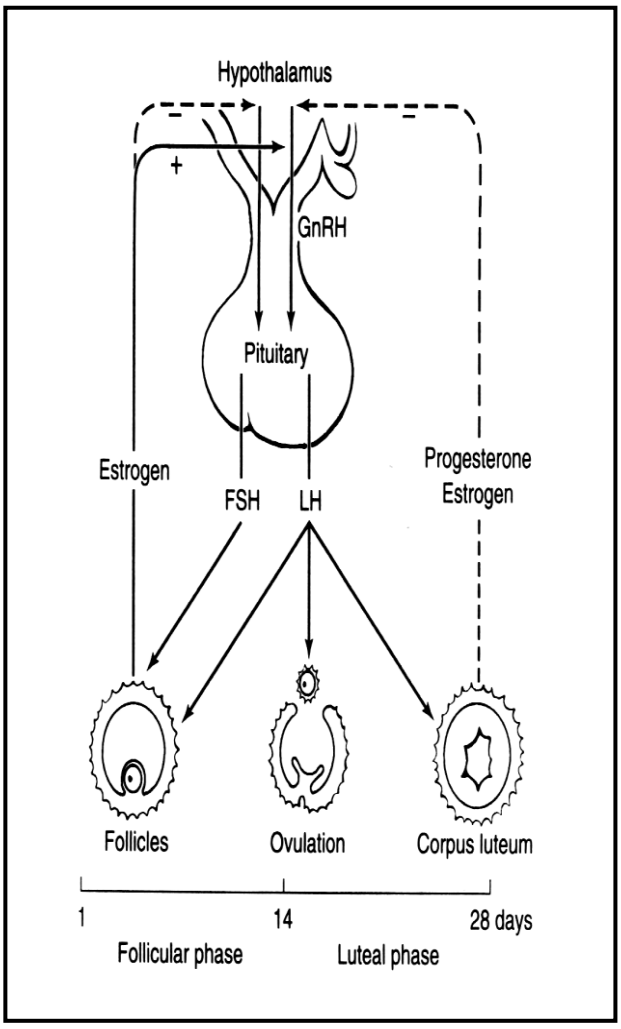


Figure (2-3): A feedback mechanism regulates the secretion of hormones produced during the menstrual cycle (Junqueira ,*et al*. ,1998**)**.

2:2:2 Menstrual Abnormalities:

One of the causes of infertility is the **anovulatory cycles;** they fail to ovulate but have menstrual periods at fairly regular intervals. Anovulatory cycles are the rule for the first 1–2 years after menarche and again before the menopause. **Amenorrhea** is the absence of menstrual periods. If menstrual bleeding has never occurred, the condition is called **primary amenorrhea.** Some women with primary amenorrhea have small breasts and other signs of failure to mature sexually. Cessation of cycles in a woman with previously normal periods is called **secondary amenorrhea.** The commonest cause of secondary amenorrhea is pregnancy; other causes of amenorrhea include emotional stimuli and changes in the environment, hypothalamic diseases, pituitary disorders, primary ovarian disorders, and various systemic diseases.

The terms **hypomenorrhea** and **menorrhagia** refer to scanty and abnormally profuse flow, respectively, during regular periods. **Metrorrhagia** is bleeding from the uterus between periods, and **oligomenorrhea** is reduced frequency of periods. **Dysmenorrhea** is painful menstruation (Ganong, 2005).

2: 3 Hormones:

A hormone is a substance that is produced in a special tissue, where it is released into the blood stream, and travels to distant responsive cells in which the hormone exerts its characteristic effects. Hormones, therefore, are substances that provide a means of communication and should now be viewed broadly as chemical regulatory and signaling agents.

Traditionally the endocrine system has been distinguished from the nervous system by the fact that the nervous system is connected to its target tissues through neurons that carry and transmits chemical signals while the endocrine system acts through the hormones one of these major pathway is the hypothalamic pituitary –target gland system (Sperroff, 1999).

2. 3.1 The Testosterone Hormone:

Testosterone is one of androgens, the major circulating androgens in women are testosterone, dihydrotestosterone, Dihydroepiandrostendione (DHEA), and Dihydroepiandrostendione-sulphate (DHEA-S) (Pesce and Kaplan,1987 and Rang, *et al*. ,2001). Both of adrenal gland and ovaries normally synthesize and secrete testosterone and the other androgens (Butcher, 1999; Goldfien and Monroe, 2001and Alhadidi, 2003).

Approximately 50% of testosterone arises from the peripheral conversion of androstendione and 25% is secreted by ovary, the remaining 25% is secreted by adrenal gland (Speroff, *et al*., 1999).While testosterone circulating in the blood is bound to a protein carrier known as sex hormone binding globulin (SHBG) produced in the liver(Lobo and Carmina,2000 and Kahn, *et al*. ,2003)., It is glycoprotein containing a single binding site for testosterone about 80% bound to SHBG, another 19% is loosely bound to albumin, leaving only 1% unbounded "Free". The testosterone binding capacity is decreased by androgens (Speroff, *et al*., 1999).

2. 3.1.1 Mechanism of Action for Androgens Hormone:

The mechanism includes:

1- Diffusion across the cell membrane.

2- Androgens hormone binding to receptor protein.

3- Interaction of a hormone receptor complex with nuclear DNA.

4- Synthesis of mRNA.

5- Transport of the mRNA to ribosomes.

Protein synthesis in the cytoplasm that results in a specific cellular activity (Speroff, *et al*., 1999 and Beaker, 2001).

2. 3.1.2 Hirsutism:

Hirsutism is defined clinically as excessive sexual hair that appears in a male pattern. Hirsutism is commonly graded according to the Ferriman-Gallwey system, which quantitates the extent of hair growth in the most androgen-sensitive areas .In adult women, a score of <8 is normal, 8 to 15 indicates mild hirsutism, and >15 indicates moderate to severe hirsutism (Martin, *et al*., 2008).

Hirsutism must be distinguished from hypertrichosis, the generalized excess growth of hair that sometimes occurs on a hereditary basis, or in patients taking glucocorticoids, phenytoins, diazoxide, or cyclosporine. Hypertrichosis is distributed in a nonsexual pattern (e.g., generalized distribution or more prominent distribution on the forehead or shoulders) and is not caused by excess androgen, although it may be aggravated by excess androgen (Martin, *et al*., 2008).

Hirsutism is the presence of terminal hair in females in a male-like pattern. Furthermore, hirsutism may be a sign of underlying androgen excess (hyperandrogenism) in the majority of sufferers. Hence, it is important to evaluate every patient that complains of unwanted facial or body hair and not to dismiss the problem as simply cosmetic (Knochenhauer ,*et al*.,1998).

In hirsute women only 25% of the circulating testosterone arises from peripheral conversion and most is due to direct glandular secretion. The ovary is the most important source of increased testosterone and androsteindione in hirsute women (Speroff, *et al*., 1999).The most common cause of hirsutism is persistent un ovulation and excessive androgen production by the ovaries and adrenal gland however ,the adrenal causes are almost uncommon (Vijaya, *et al*. ,1996 and Alhadidi,2003).

. 2.3.1.2.1 Pathogenesis of Hirsutism :

Many hormones have androgenic potential in the human body but testosterone is the key circulating androgen. It is produced by the ovaries and adrenals either as testosterone or prohormones. These prohormones (mainly androstenedione or dihydroepiandrosterone sulfate) are metabolized into testosterone in peripheral tissues, such as fat (Quinkler ,*et al*.,2004). Large quantities of circulating androgens are bound to specific plasma proteins, including sex hormone-binding globulin (SHBG), cortisol-binding globulin, and albumin. The free fraction of testosterone acts as the main bioactive component of plasma testosterone (Rosner, 1990) . The SHBG levels can decrease in the body in many conditions such as obesity, hyperinsulinemia, acromegaly, or hypothyroidism, or after administration of androgens, synthetic progestins, glucocorticoids, and growth hormones. Under such circumstances, the level of free testosterone may be elevated even when the total testosterone level is normal in hirsute women (Rosenfield, 2005).

2.3.1.2.2 Insulin-sensitizing drugs (ISD) and hirsutism:

These are anti diabetic drugs, not indicated for the treatment of hirsutism alone. ISD are prescribed for symptomatic management of

poly cystic ovarian syndrome PCOS, particularly in women with additional metabolic and cardiovascular risk factors. Treatment of insulin resistance using metformin, troglitazone, or rosiglitazone can improve many of the hormonal disturbances and restore menses in a considerable proportion of patients with PCOS(De Leo ,*et al*., 2006; Krstevska, *et al*. ,2006 and Meyer, *et al*.,2007).However, its effectiveness in the treatment of PCOS-associated hirsutism is less clear. It has been argued that blood insulin lowering effect of ISD may result in a decrease of free androgen concentration, thereby improving hirsutism as a result (Dawber, 2005).

2.3.2. Gonadotrophins (Lutenizing Hormone, Follicular - stimulating Hormone) (LH, FSH):

2.3.2:1 Biosynthesis and function:

LH and FSH are glycoproteins gonadotrophins composed of alpha and beta subunits and secreted by the same cell from the anterior pituitary gland .The specific beta subunit confers on these hormones their unique biologic activity ,as it does with Thyroid stimulating hormone(TSH), Human Chorionic Gonadotrophin (HCG). They bind to receptors in the ovary and testis and regulate gonadal function by promoting sex steroid production and gametogenesis .

In women LH stimulates estrogen and progesterone production from the ovary. A surge of LH in the mid menstrual cycle is responsible for ovulation ,and continued LH secretion subsequently stimulates the corpus luteum to produce progesterone by enhancing the conversion of cholesterol to pregnenolone .Development of ovarian follicle is largely under FSH control ,and the secretion of estrogen from this follicle is dependent on both FSH and LH. (Greenspan and Gardner, 2002) .

2.3.3. Prolactin :

Prolactin (PRL) or Luteotropic hormone (LTH) is a peptide hormone primarily associated with lactation. In breastfeeding, the act of an infant suckling, the nipple stimulates the production of prolactin, to fill the breast with milk via a process called lactogenesis, in preparation for the next feed. Oxytocin, another hormone, is also released, which triggers milk let-down (Greenspan and Gardner,2002).

2.3.3.1Production and regulation:

Prolactin hormone is synthesized and secreted by lactotrope cells in the adenohypophysis (anterior pituitary gland). As well as secreted from the breast, the decidua, parts of the central nervous system and the immune system (Mancini,2008). Pituitary prolactin secretion is regulated by neuroendocrine neurons in the hypothalamus. Although PRL does not appear to play a physiologic role in regulation of gondal function , hyperprolactinemia in humans leads to hypogonadism . In women initially there is a shortening of luteal phase ,subsequently an ovulation , oligomenorrhea or amenorrhea ,and infertility occurs the exact mechanism of PRL inhibition of gondal function are unclear ,but the principal are appear to be alteration of hypothalamic pituitary control of gonadotrophin secretion .basal LH,FSH level are usually normal .however their pulsatile secretion is decreased and the midcycle LH surge is suppressed in women (Greenspan and Gardner,2002) .

2.4 Female Infertility:

2.4.1 Human Fertility

Both women and men have hormonal cycles which determine both when a woman can achieve pregnancy and when a man is most virile. The female cycle is approximately twenty-eight days long, but the male cycle is variable. Furthermore, age also plays a role, especially for women (Creinin, *et al*., 2004). The average age of menarche in the United States is about 12.5 years. Women's fertility peaks around the age of 19-24, and often declines after 30 (Anderson ,*et al*.,2003).

2.4.2. **Infertility**:

Infertility primarily refers to the biological inability of a person to contribute to conception. There are many biological causes of infertility, some which may be bypassed with medical intervention.
Women who are fertile experience a natural period of fertility before and during ovulation, and they are naturally infertile during the rest of the menstrual cycle. Fertility awareness methods are used to discern when these changes occur by tracking changes in cervical mucus or basal body temperature (Makar and Toth, 2002).

Reproductive endocrinologists, the doctors specializing in infertility, consider a couple to be infertile if the couple has not conceived after 12 months of contraceptive-free intercourse if the female is under the age of 34, the couple has not conceived after 6 months of contraceptive-free intercourse if the female is over the age of 35. (declining ovum quality of females over the age of 35 account for the age-based discrepancy as when to seek medical intervention the female is incapable of carrying a pregnancy to term) .

2.4.2.1 Primary Infertility:

Couples with primary infertility have never been able to conceive.

2.4.2.2 Secondary Infertility: is the difficulty in conceiving after already having conceived (and either carried the pregnancy to term, or had a miscarriage). Technically, secondary infertility is not present if there has been a change of partners (Khan, *et al*., 2005).

2.4.2.3 The main causes of infertility:

Causes of infertility can be found in about 90% of infertile couples, and 10% are without underlying cause :it means unexplained. The underlying causes of female infertility (Michel, 2002.) includes:

A. Causes of failure to ovulate:

Ovulatory disorders are one of the most common reasons why women are unable to conceive and account for 30% of women's infertility. Approximately 70% of these cases can be successfully treated by the use of drugs. The causes of failed ovulation can be categorized as follows (Peterson, 2002.):

A.1 Hormonal problems:

These are the most common cause of an ovulation. There are two main sources causing this problem **(**Alhadidi, 2003.):

A.1.1 Failure to produce mature ova : In approximately 50% of the cases of an ovulation , the ovaries do not produce normal follicles in which the ova can mature (Peterson,2002). Ovulation is rare if the ova are immature and the chance of fertilization becomes almost non existent, Polycystic ovary Syndrome is the most common disorder responsible for this problem (Tronic,2001)., and hyperprolactinemia that prolactin stimulate breast milk production then affecting ovulation (Dan,2002).

A.1.2 Hypothalamic – Pituitary disorder:

The hypothalamus controls of all the secretion of pituitary involved FSH, LH which stimulate the ovaries to initiate ovum maturation, if hypothalamus fails to trigger and control this process immature ova will be resulted, and if the pituitary responsibility lies in producing and secreting FSH, LH the ovaries will be unable to ovulate (Guyton and Hall,2001 and Peterson,2002).

A.2 Scarred Ovaries: Physical damage to the ovaries may result in failed ovulation, or multiple surgeries for repeated ovarian cysts may cause the capsule of the ovary to become damaged or scarred such that the follicles cannot mature and ovulation does not occur (Peterson, 2002).

A.3 **Premature Menopause:** Some women cease menstruation and begin Menopause before normal age(Peterson,2002) .It is a significant cause of infertility ,and women having this condition have only 5-10% chance to conceive without fertility treatments (Michel ,2002).

A.4 **Follicle Problems:** Called "un ruptured follicle syndrome" occurs in women who produce a normal follicle with an ovum inside of it every month; yet, this follicle fails to rupture (the ovum); Therefore it remains inside the ovary and ovulation does not occur (Beaker,2001 and Peterson,2002).

B. Uterine factors:

B.1 Uterine malformations (Raga, *et al*., 1997).

B.2 Uterine fibroids (leiomyoma)

B.3 Asherman's Syndrome(Magos ,2002).

C. Cervical factors:

C.1 Cervical stenosis (Tan and Bennett, 2007).

C.2 Antisperm antibodies (Francavilla, 2007).

C.3Non-receptive cervical mucus (Farhi, 1995).

D -Vaginal factors:-

D.1 Vaginismus

D.2Vaginal obstruction

E. Poorly Functioning Fallopian tube: Tubal disease affects approximately 25% of infertile women, the main causes of tubal damage include (Peterson,2002):

E.1 Infection: Caused by both bacteria and viruses ,and usually transmitted sexually (Peterson,2002).

E.2 **Abdominal disease:** This disease causes inflammation of abdominal cavity which can affect the fallopian tube and lead to scarring and blockage (Peterson,2002).

E.3 Previous Surgeries: This is an important cause of tubal disease damage pelvic or abdominal surgery which can result in adhesions (Michel, 2002) that compromise tubal motility and prevent ovum pick up (Beaker, 2001).

E.4 Ectopic Pregnancy:

This pregnancy occurs in the tube itself that may cause tubal damage and increase the risk for infertility (Michel, 2002. and Peterson, 2002).

E.5 Congenital defects: In rare cases women may be born with tubal abnormalities (Peterson, 2002)**.**

E.6 Endometriosis:

This condition characterized by excessive growth of lining of the uterus called endomatrium, occurs not only in the uterus but also elswhere in the abdomen such as in the fallopian tubes, ovaries and the pelvic peritoneum (Beaker,2001; Michel ,2002 and Peterson,2002).It occurs in women between 30 - 40 years of age although it is found in younger women . Approximately 10% of infertile women are affected by endometriosis, 30 – 40% of patient with endometriosis are infertile.

F. Additional Factors:

F.1 Behavioural factors:

It is well – known that certain personal habits and lifestyle factors impact health, many of these same factors may limit women ability to conceive (Peterson, 2002).

F.1.1- Diet and Exercise:

Optimal reproductive functioning requires both proper diet and appropriate level of exercise. Women who are significantly overweight or underweight may have difficulty in becoming pregnant. 5% of cases of infertility are explained as micronutrient deficiencies (e.g. Zinc, magnesium and potassium) (Peterson, 2002).

F.1.2- Smoking:

It reduces the chance of conceiving with each cycle (Peterson, 2002).

F.2 Environmental factors:

The ability to conceive may be affected by exposure to various toxins or chemicals in the work place or the surrounding environment (Michel, 2002 and Peterson, 2002).

F.2.1-Lead:

Exposure to lead sources had a negative impact on fertility in human (Peterson, 2002).

F.2.2-Medical treatments and materials:

Repeated exposure to radiation, ranging from simple x-rays to chemotherapy has been seen to contribute to a wide array of ovarian problems (Peterson, 2002).

G. Genetic factors

Various intersexed conditions, such as androgen insensitivity syndrome.

H- General factors

H.1 Significant liver or kidney disease

H.2 Thrombophilia (Middeldorp, 2005 and Qublan, 2006).

H.3 Age; Fertility starts declining after age 27 and drops at a somewhat greater rate after age 35.

2:5 Polycystic ovary syndrome:

It is one of the most common causes of ovulatory infertility, affects 5–10% of women (Ehrmann, 2005). Polycystic ovarian Syndrome (PCOS) is also known as Stein Leventhal Syndrome after Stein and Leventhal who first described the condition in 1935. They described an association between the presence of multiple cysts in the ovaries, menstrual disturbances, hirsutism (excess hair growth) and obesity (Nishan, 2008). It occurs amongst all races and nationalities.It is the most common hormonal disorders among women of reproductive age, and is a leading cause of infertility (Goldenberg and Glueck ,2008 and Boomsma, *et al*., 2008).

PCOS is a heterogeneous condition (Clinically and Biochemically) in affected women presenting in clinical practice seeking treatment for reproductive disorders such as menstrual cycle disturbances (oligomenorrhoea, amenorrhoea), infertility, hirsutism or acne (Michelmore and Balen, 1999).Patient who are diagnosed as consistently having adequate level of oestrogen but who are acyclic are often described as having PCOS .This is a common presentation that almost certainly represents several disorders and not a single specific abnormality ,Further more ,the typical ovarian appearance ,characterized by multiple small cysts in the ovarian cortex producing a "string of pearls" is certainly not the basis of this problem .Rather, the endocrinopathy associated with hyperandrogenism and often hyperinsulinism with insulin resistance is the hormonal bases that produces the multicystic –appearing ovaries in many such women

(Hacher and moore,1998). In PCOS patients, an increase in insulin resistance and in central body fat accumulation has been observed independent of obesity (Escobar, et *al*., 2003; Kirchengast and Huber, 2004; Ibanez and de Zegher, 2004; Mohlig, *et al*., 2004 and Ehrmann, 2005).

Its clinical features include hyperandrogenism, obesity, menstrual irregularity and anovular infertility, but the clinical presentation can vary. PCOS is the commonest cause of anovulatory infertility and hirsutism world-wide (Franks, 1995). PCOS is associated with insulin resistance and hyperinsulinaemia, with a risk of glucose intolerance (Dunaif,1995).

The characteristic biochemical abnormalities are elevation of serum androgen concentrations and luteinizing hormone (LH) concentrations, but with normal levels of follicle-stimulating hormone (FSH) and elevated LH / FSH ratio (Zawadzki and Dunaif, 1992). Importantly, PCOS is also associated with metabolic abnormalities, central to which are insulin resistance and hyperinsulinaemia, which carry an increased risk of developing type 2 diabetes in later life (Franks, 1995 and Ehrmann, 2005).

2:5:1Epidemiology:

Approximately 20% of asymptomatic women who have ultrasound are found to have polycystic ovaries - but without the clinical features of the syndrome (irregular or absent menses and signs of excess androgen); intervention is rarely required. European estimates of prevalence are between 2-20%. Most clinical data suggests a prevalence of 6–7% of the United Kingdom population (Azziz, *et al*., 2004).The highest reported prevalence of Poly Cystic

Ovaries in a community survey was 52% in south Asian immigrants in Britain, of whom 49% had menstrual irregularity .There is a paucity of data on the prevalence of Poly Cystic Ovaries and PCOS in south Asian women (Rodin, *et al*., 1998) .

2:5:2 A etiology:

We don't know why some women get polycystic ovary syndrome. But it seems to run in families. So, the Genes you inherit from your parents may play a part.

- About 4 in 10 women with PCOS have a sister who has it.
- About 2 in 10 women with PCOS have a mother who has it. (Richardson, 2003).

The cause of PCOS is not fully understood, but genetics may be a factor. If you have PCOS, your sisters and daughters have a 50% chance of developing PCOS (Barbieri, 2007).

The exact cause of PCOS is unknown (Griffing, 2002). , certainly a miscommunication occurs among the hypothalamus, pituitary gland ovaries and fatty tissue (Waheed, 2003.and Polson, *et al*., 1998). Current theories emphasize genetic and intraovarian origin (Webber**,** *et al.,* 2003.)coupled with environmental factors such as diet and altered lifestyle patterns (Norman ,2004).

2:5:3 Pathophysiology:

PCOS is one of the most common hormonal disorders in women of reproductive age affecting up to 10% of population (Oberfield, 2000).

- Current theories indicate that above normal anterior-pituitary LH secretion leads to over stimulation of the ovarian theca cells,

causing excessive androgen production (predominantly testosterone and androstenedione).

- Low FSH levels mean that ovarian granulosa cells fail to adequately convert these androgens into oestrogens, leading to ovulatory impairment and the development of unruptured cysts.
- Associated hyperinsulinaemia causes dyslipidaemia and increased plasminogen activation, thought to increase the risk of intravascular thrombosis. This condition is often familial, but no culprit genes have been identified (Homburg, 2005).

2:5:4 Symptoms:

PCOS symptoms tend to start gradually. Often, hormone changes that lead to PCOS start in the early teens, after the first menstrual period. Symptoms may be especially noticeable after a weight gain. With PCOS, you may have only a few symptoms or many symptoms. And the early symptoms of PCOS include:

- Oligomenorrhea, amenorrhea irregular, few, or absent menstrual periods. 30% (Ehrmann, 2005).
- Hirsutism — excessive and increased body hair, typically in a male pattern on the face, chest, back, stomach, thumbs, or toes. About 70% of women in the United States with PCOS complain of these hair problems caused by high androgen levels (Huang, 2007.), also Hair loss appearing as thinning hair on the top of the head.
- Acne and oily skin, caused by high androgen levels.
- Depression or mood swings. Hormonal changes are a known cause of emotional symptoms (Barnard, et *al*., 2007).

While PCOS symptoms that may develop gradually include:

- Weight gain or upper body obesity (more around the abdomen than the hips). This is linked to high androgen levels, one in two women with PCOS are obese 40% (Huang, 2007).
- Male-pattern baldness or thinning hair (alopecia). This is linked to high androgen levels.
- Repeat miscarriages. This may be linked to high insulin levels.
- Inability to become pregnant (infertility). This is because the ovaries are not releasing an ovum (not ovulating)(Boomsma,*et al*.,2008).
- Symptoms of hyperinsulinemia and insulin resistance. Which can include upper body weight gain and skin changes, such as skin tags or dark, velvety skin patches under the arm, on the neck, or in the groin and genital area (Ehrmann, 2005).
- Breathing problems while sleeping (Ehrmann, 2005).
- Pain in the lower abdomen and pelvis (chronic pelvic pain).
- High blood pressure may be more common in women with PCOS, especially if they are very overweight.

2:5:5 Risk:

By the age of 40, up to 40% of PCOS patients will have type 2 diabetes or impaired glucose tolerance (Legro, 2001).Polycystic ovary syndrome is therefore an important health concern and may represent a major health issue affecting young women today.

They are at risk for the following:

1. Endometrial hyperplasia and endometrial cancer (cancer of the uterine lining) are possible, due to over accumulation of uterine lining, and also lack of progesterone resulting in prolonged

stimulation of endometrial cells by estrogen(chronic anovulation). It is however unclear if this risk is directly due to the syndrome or from the associated obesity, hyperinsulinemia, and hyperandrogenism (Navaratnarajah, *et al*., 2008).

2. Insulin resistance/Type II diabetes:

As a consequence of insulin resistance, women with PCOS exhibit a greater risk for dyslipidemia (Legro, *et al*.,2001.), coagulation disorders and endothelial dysfunction (Paradisi ,*et al*., 2001.), increased incidence of hypertension(Bjorntorp,1996)., and type 2 diabetes mellitus (Solomon, *et al*. ,2001and Legro ,2002). in later life, which are strong and established risk factors for cardiovascular disease (Legro ,2003) .

2:1 Insulin resistance (IR) (Glucose Intolerance):

Insulin resistance is a state in which a given concentration of insulin produces a less-than-expected biological effect. Insulin resistance with PCOS defined as unexplained hyperandrogenism and chronic an ovulation, about 40% of PCOS women will have IR (Gambineri, *et al*.,2002). Studies in American, Asian, and Italian subjects have shown that women with PCOS have an increased risk for the selective development of impaired glucose tolerance and type 2 diabetes, with a tendency to early development of glucose intolerance states, when compared to the general population (lergo, 1999). The high insulin levels associated with IR stimulate the ovary to make excessive amounts of androgens. Additionally, high insulin levels decrease levels of SHBG, increasing the androgens potency. High insulin levels may also work at the level of the brain, causing increased LH secretion (which in turns stimulates more ovarian androgen

production) and stimulating appetite. Increased LH secretion, high androgen levels, and obesity disrupt ovulation. These complex and interrelated effects lead to "unexplained hyperandrogenic chronic an ovulation" that is, PCOS (Wong, *et al*.,1995).

3. High blood pressure

4. Dyslipidemia (disorders of lipid metabolism — cholesterol and triglycerides)

5. Cardiovascular disease (.Nishan ,2008) **.**

6. Strokes

7. Weight gain

8. Miscarriage (Goldenberg and Glueck 2008 and Boomsma,*et al*., 2008).

9. Acanthosis nigricans (patches of darkened skin on the back of the neck, under the arms, and in the groin area)

10. Infertility

2:2.Relationship between Hyperandrogenism and Hyper-insulinaemia :

Hyperinsulinemia may cause hyperandrogenism by binding to insulin like growth factor -1(IGF-I) receptors in the ovary(Gill,*et al*.,1996; Beaker,2001and Waheed,2003). which are similar in structure to insulin receptors. When insulin receptors are blocked it is to be expected that insulin would bind to type I- IGF receptors (Hunter and garvey, 1998 and Speroff, *et al*., 1999). The activation of the receptor by insulin can lead to further increase androgen production by theca cells (Beaker, 2001). Increasing in insulin will decrease the hepatic synthesis of SHBG (Hunter and garvey, 1998; Eagleson, *et al*., 2000; Beaker, 2001and Peterson,

2002). by inhibiting insulin like growth factor binding – protein-1 in the liver permitting greater local activity of IGF-I in the ovary (Imani, *et al*.,2000 and Beaker,2001).The androgen is converted to oestrogen in the periphery (Jack,2002). The oestrogen stimulates GnRH synthesis and secreting in the hypothalamus causing preferential LH release by the pituitary gland, figure (2-4). Then LH leads to hyperplasia of theca cells, increased androgens production and also decreased the SHBG (Braunwald, *et al*., 2002 and Waheed, 2003). The low level of SHBG facilitates tissue uptake of free androgens leading to increase peripheral formation of oestrogens that stimulate chronic unovulation and stimulate fat cell proliferation leading to obesity (Gill, *et al*., 1996; Speroff, *et al*., 1999and Jack, 2002).

The frequency of obesity in women with PCOS has been reported to be from 35-60 %(Speroff, *et al*.,1999.), obesity play a major role beside hyperinsulinemia (Pasquali ,*et al*.,2000.) predominantly via insulin's anabolic effects on fat metabolism, figure (2-4), it increases lipogenesis in adipocytes, increases glucose uptake into adipocytes then synthesized into triglyceride (TG) and inhibit the catabolism of fat (Cosford,1999).

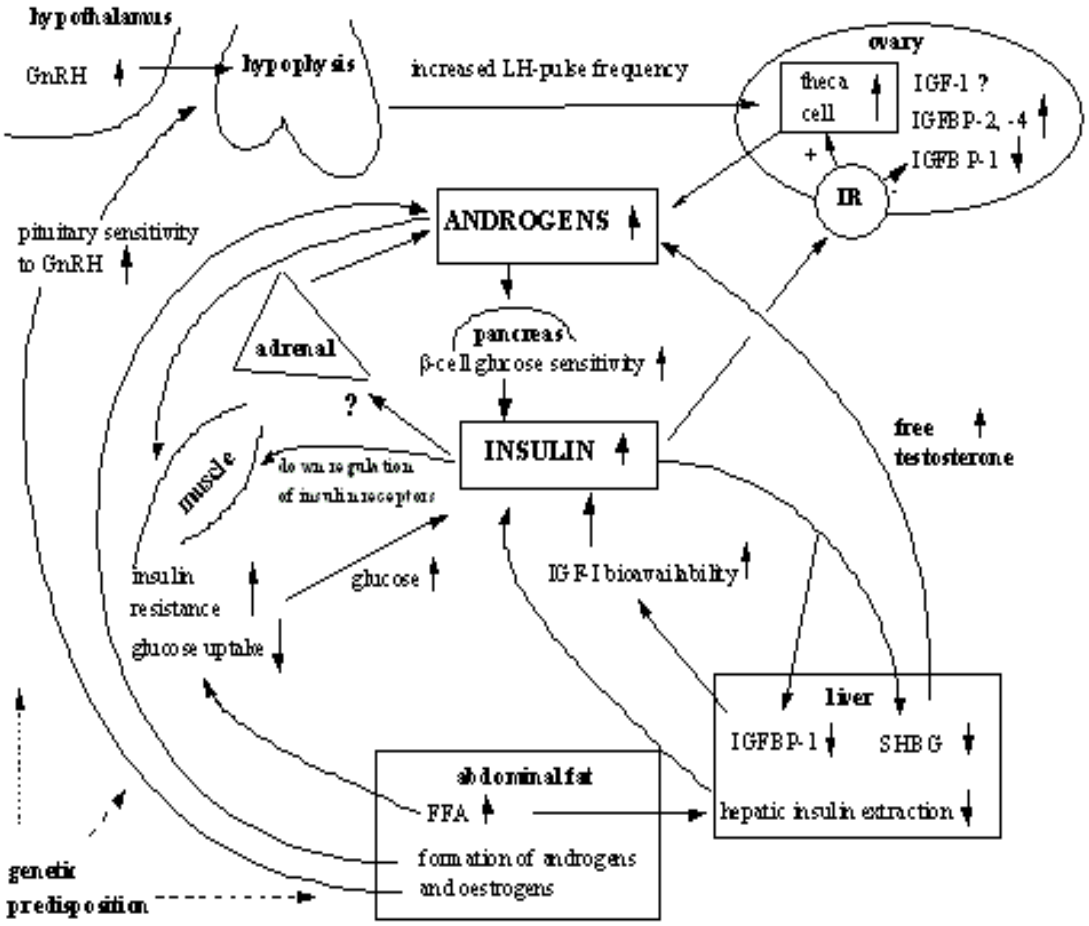


Figure (2-4) Possible mechanisms causing hyperandrogenism and hyperinsulinemia in women with PCOS (Konerman, 2003). In the follicles increased levels of insulin-like growth factor-binding protein-2 and -4 (IGFBP-2, -4) and the absence of IGFBP proteases may sequester the insulin-like growth factor-I (IGF-I) and thereby reduce its synergistic action with FSH leading to arrest of follicle development. Adrenal androgen production is increased. Hyperandrogenism increases the proportion of insulin resistant muscle fibers in the muscle tissue and serum free fatty acid (FFA) levels. The increased FFAs in the serum compete with glucose for uptake and oxidation in the muscle cell, inducing further insulin resistance. In addition FFAs have been shown to decrease hepatic insulin extraction.

Androgens may indirectly modify β -cell sensitivity to glucose. Hyperinsulinemia, either primary or secondary to insulin resistance, induces hyperandrogenism directly by increasing ovarian androgen secretion, probably via its own receptor, and indirectly by decreasing the secretion of sex hormone-binding globulin (SHBG) and IGFBP-1 in the liver. Insulin down regulates the number of its own receptors on the membranes of the target cells. Obesity may contribute to androgen excess due to the capacity of the adipose tissue to form testosterone and oestrone from inactive precursors. Elevated level of estrogen can augment pituitary sensitivity to gonadotrophin releasing hormone (GnRH). IR = insulin receptor

2:5:6 Diagnoses;

Standard diagnostic assessments done by:

A. **History-taking**, specifically for menstrual pattern, obesity, hirsutism, and the absence of breast discharge. A clinical prediction rule found that these four questions can diagnose PCOS with a sensitivity of 77.1% (95% CI (confidence interval) 62.7%–88.0%) and a specificity of 93.8% (95% CI(confidence interval) 82.8%–98.7%) (Pedersen, *et al*., 2007).

B. Diagnostic criteria (Rotterdam criteria): Two of the three following criteria are diagnostic of the condition: (Chang, 1996; Balen, 1999; Legro, 1999; Rotterdam, 2004 and cho, 2007).

- Polycystic ovaries (either 12 or more peripheral follicles or increased ovarian volume (greater than 10 cm^3).
- Menstrual irregularities. **(**Balen and Rutherford ,2007).
- Clinical and/or biochemical signs of hyperandrogenism.

C. Investigations :

- This may show LH elevated, LH: FSH ratio increased, with FSH normal. If you have PCOS your pituitary gland secretes higher levels of LH throughout the month instead of just before ovulation and ovulation may fail. Therefore LH/FSH ratios are increased to > 1 in women with PCOS (Richardson, 2003).

- Exclude other potential causes e.g. thyroid dysfunction, congenital adrenal hyperplasia (check basal serum 17-hydroxyprogesterone levels and seek expert advice), hyperprolactinaemia, androgen-secreting tumours and Cushing's syndrome (Chang, 1996; Balen, 1999 and Legro, 1999).
- Testosterone levels may be elevated (total or free).
- Laparoscopy or ultrasound shows characteristic ovaries: The diagnosis of PCO, based on transvaginal ultrasound, figure (2-5), requires the presence of 15 cysts of 2 to 10 mm diameter arranged in a single plane (Kyei-Mensah ,*et al*., 1996).

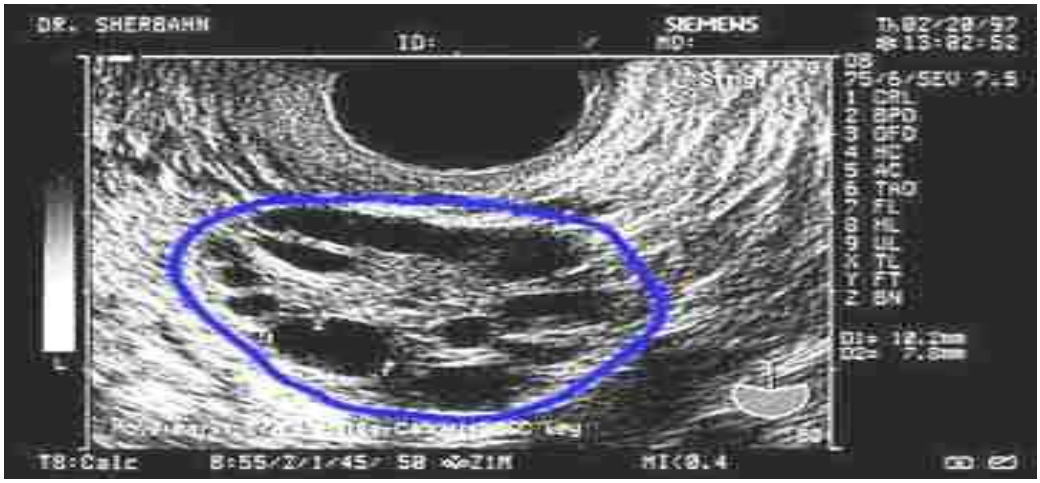


Figure (2-5) Ultrasound picture of a typical polycystic ovary .Black circle around ovary shows numerous antral follicles (2-9mm diameter cysts) are visible (Gurnee, *et al*., 2009).

- Fasting glucose and oral glucose tolerance test are useful in assessing insulin resistance/diabetes.
- Fasting lipid levels should be checked.
- If late-onset congenital adrenal hyperplasia is suspected then it is worth checking.

2:5:8. Management:

2:5:8: 1. Home Treatment

This can help you manage the symptoms of polycystic ovary syndrome (PCOS) and live a healthy life.

- Weight control or weight loss lowers your risks for diabetes, high blood pressure (hypertension), and high cholesterol (Speroff and Fritz ,2005).Weight loss of as little as 5% to 7% over 6 months can reduce androgen levels enough to restore ovulation and fertility in more than 75% of women with PCOS. (Huang, 2007).Being more active and eating healthy foods are key parts of weight control.

- Exercise. Make physical activity a regular and essential part of your life. Walking is one of the best activities
- Eat a balanced diet. Includes: lots of fruits, vegetables, whole grains, and low-fat dairy products supplies your body's nutritional needs, satisfies hunger, and decreases your cravings.
- Stay at a healthy body weight.
- If you smoke, consider quitting. Women who smoke have higher levels of androgens than women who don't smoke so it increases the risk for heart disease (Barbieri, 2007).

2:5:8:2 Medical treatments*:*

Medical treatment of PCOS is tailored to the patient's goals. Broadly, these may be considered under four categories:

- Lowering of insulin levels
- Restoration of fertility
- Treatment of hirsutism or acne
- Restoration of regular menstruation, and prevention of endometrial hyperplasia and endometrial cancer.

Regular exercise and maintaining a healthy weight will help reduce the hormonal imbalance, restore ovulation and fertility, and improve acne and hirsutism (Marsh and Brand-Miller, 2005).

2:5:8:2:1.Insulin Lowering Medications:

Many women find insulin-lowering (where insulin sensitivity is improved, and insulin resistance is reduced) medications such as metformin hydrochloride (Glucophage), pioglitazone hydrochloride (Actos), and rosiglitazone maleate (Avandia) helpful, and ovulation may resume when they use these agents(Solomon ,1999). When combined with exercise and a low glycemic index diet, up to 85% of patients will get improvement in menstrual cycle regularity and ovulation within about six months. Glucophage, Actos and Avandia are drugs that reduce insulin levels and therefore, lower blood levels of the male hormone, testosterone (Nestler ,*et al*.,1998 and Unluhizarci ,*et al*., 1999).A cohort study has shown that metformin can also improve insulin resistance in thin PCOS patients without clinically apparent insulin resistance as measured by the Homeostasis Model Assessment for Insulin Resistance (HOMA-IR).Besides

positive effects on insulin resistance, metformin treatment was also shown to improve hirsutism, acne, and menstrual irregularities in thin PCOS patients (Tan ,*et al*. ,2007).

2:5:8:2:1.A. Metformin:

Metformin is an oral anti-diabetic drug from the biguanide class. It is the oldest and still the most used insulin sensitizer worldwide in the treatment of states of glucose intolerance, particularly type 2 diabetes mellitus(T2DM). So, it is the first-line drug for the treatment of type 2 diabetes, particularly in overweight and obese people and those with normal kidney function and evidence suggests it may be the best choice for people with heart failure. It is also used in the treatment of polycystic ovary syndrome(Eurich,*et al*. ,2007) .There is an evidence that clinical effects of metformin may take prolonged time to show it's full manifestations on the patient ,whereas almost all studies were of short duration (4-6)months at most (Lord , *et al*.,2003).Many benefits had been shown with metformin such as :

1. Decrease in body mass index in the region of 4%
2. Decrease in androgen (male hormone) activity for around 20% i.e(the degree of hirsutism was attenuated.)
3. Increase in ovulatory cycles – normal menstrual cycles achieved within 3 months of starting treatment in some groups of patients (Ehrmann, 2005).
4. Increased rates of pregnancy reported in several trials and reduce risk of miscarriage (Nishan, 2008).

5. Prevention or delay of onset of diabetes and reduce risk of gestational diabetes (Knowler, 2002).

6. Lowering of insulin and glucose level (Nestler, 1996).

It is considered an insulin sensitizer since it lowers glucose levels without increasing insulin secretion. In fact, it lowers hepatic glucose production by reducing gluconeogenesis and by decreasing glycogenolysis, it increases peripheral glucose uptake by skeletal muscle and adipose tissue and reduces intestinal glucose absorption (Hundal & Inzucchi, 2003). In women with PCOS, metformin administered at doses of up to 1500 mg/day decreases insulin, testosterone and LH levels and it also appears to favor some weight loss (De Leo, *et al*., 2003) .It seems possible that insulin-sensitizing drugs may be useful in the restoration of normal endocrinological and clinical parameters of PCOS by lowering insulin secretion (Harborne ,*et al.*, 2003). Also metformin may cause many side effects like: lactic acidosis, vit.B12 malabsorbtion(McCarty,2000.) ,anaemia, gastrointestinal (G.I) disturbance and liver or kidney problems (Boss, 2001) .

2:5:8:2:1.B. Metformin and Antiandrogens *:*

Treatment with antiandrogens is also effective in PCOS. Notably, antiandrogens markedly reduce androgens (Diamanti-Kandarakis, *et al*.,1995 ; De Leo, *et al*.,1998 ; Eagleson ,*et al*.,2000 and Ibánez ,*et al* .,2000) and improve gonadotropin secretion (Diamanti-Kandarakis ,*et al*.,1998). and hirsutism (Diamanti-Kandarakis, *et al*.,1995; De Leo, *et al*,1998; Eagleson ,*et al*.,2000 and Ibánez ,*et al* .,2000). Several studies have shown that antiandrogens may also improve the lipid

profile (Paoletti ,*et al*.,1999and Eagleson ,*et al*.,2000), whereas it is still unclear whether this treatment can reduce insulin resistance (De Leo, *et al*.,1998; Eagleson ,*et al*.,2000 and Gambineri ,*et al*.,2004). There is an evidence that hyperandrogenism, insulin resistance and associated hyperinsulinemia and obesity play as a key and coordinating roles in the pathogenesis of PCOS, contributing in different ways to the clinical expression of the syndrome. , Therefore; their specific actions, insulin sensitizers and antiandrogens are thought to display potential complementary effects in the treatment of PCOS. In the majority of available studies, however, insulin sensitizers have been investigated as the sole treatment or in combination with other drugs, such as oral contraceptives and antiandrogens, but not with lifestyle interventions(dietary therapy and exercise) (Pasquali& Gambineri,2002). As we show in this chapter there is evidence that hyperandrogenism, insulin resistance and associated hyperinsulinemia and obesity play key and coordinating roles in the pathogenesis of PCOS, contributing in different ways to the clinical expression of the syndrome. Due to their specific actions, insulin sensitizers and antiandrogens are therefore thought to display potential complementary effects in the treatment of PCOS. The two drugs(Metformin+ Androcur) showed an additional effect in reducing testosterone concentrations . Metformin treatment maintained the same positive effects shown after the 6-month period, chiefly in improving menstrual cycles. Interestingly, however, the combined (metformin+antiandrogen)therapy further and significantly improved insulin resistance, which suggests that long-term sustained decrease of androgen excess may favor improvement of insulin sensitivity (Diamanti- Kandarakis, *et al*.,1998).

The improvement of insulin resistance and the decrease of insulin concentration and action can be achieved in different ways: (i) by reducing body weight with lifestyle modifications, if overweight or if obesity is present; (ii) by using insulin-sensitizing agents; (iii) by using antiandrogens. In fact It has been demonstrated that long-term treatment with antiandrogens may improve insulin sensitivity in both of normal weight and obese PCOS women presenting an insulin-resistant state (Poretsky, *et al*., 1999).

2:5:8:2:2.Treatment of Infertility:

Not all women with PCOS have difficulty in becoming pregnant. For those who do, an ovulation is a common cause. For overweight women with PCOS, who are anovulatory, diet adjustments and weight loss are associated with resumption of spontaneous ovulation. For those who after weight loss still are anovulatory or for anovulatory lean women, clomiphene citrate and FSH are the principal treatments used to help infertility. Previously, even metformin was recommended treatment for an ovulation (Thessaloniki ,2008).The most drastic increase in ovulation rate occurs with a combination of diet modification, weight loss, and treatment with metformin and clomiphene citrate (Andy ,*et al* .,2005). For patients who do not respond to clomiphene, diet and lifestyle modification, there are options available including assisted reproductive technology procedures such as controlled ovarian hyperstimulation with FSH injections and in vitro fertilization (IVF).

2:5:8:2:3.Treatment of Hirsutism and Acne

A standard contraceptive pill may be effective in reducing hirsutism. A common choice of contraceptive pill is one that contains

cyproterone acetate; in the United Kingdom/United State the available brand is Diane. Metformin can reduce hirsutism, perhaps by reducing insulin resistance, and is often used if there are other features such as insulin resistance, diabetes or obesity that should also benefit from metformin. Individuals may vary in their response to different therapies, and it is usually worth trying other drug treatments if one does not work, but drug treatments do not work well for all individuals. For removal of facial hairs, electrolysis or laser treatments can be used for sever conditions (Krstevska, *et al*. ,2006).

2:5:8:2:3.A. Cyproterone acetate (Androcur):

This is a progestogen with antiandrogen properties. It decreases testosterone and androstenedione levels through a decrease in circulating LH levels. It also antagonizes the effect of androgens at the peripheral level. It blocks androgen receptors and reduces androgen synthesis by inhibiting androgen-synthesizing enzymes (Dawber, 2005.) so suppresses gonadotropin secretion by maintaining the negative feedback on the pituitary ,so selectively inhibits pituitary function and suppresses ovulation.

Cyproterone acetate is often combined with a synthetic estrogen ethinylestradiol in a single pill (cyproterone acetate 2 mg, ethinylestradiol 0.035 mg). It can take up to a year of treatment with cyproterone acetate before the desired hair loss is achieved. The recommended dosage for treating hirsutism is usually within the range of 50 to100 mg daily (Azziz, 2003).Hepatotoxicity is a serious adverse reaction potentially induced by both steroidal and nonsteroidal antiandrogens (Kacar, *et al*., 2002). This powerful anti-androgen is usually taken on days 1 to 10 of the menstrual cycle (conventionally, day 1 is the first day of menstruation). Spironolactone and cyproterone

may be combined with Diane or other oral contraceptive agent, partly because they cause menstrual irregularities and partly to prevent pregnancy. The combined treatment is not necessary in post-menopausal women. These medications suppress release of the pituitary hormone, gonadotrophin, which in turn reduces androgen production by the ovaries. They also have a direct peripheral effect on the sebaceous gland. Higher doses of cyproterone acetate are indicated for more severe cases of androgenetic skin conditions. It is effective for 70% of those with hirsutism. The medication is usually combined with cyproterone acetate/ethinyloestradiol or other oral contraceptive agents:

- To regulate menstrual cycle irregularities caused by the high dose cyproterone
- To prevent pregnancy; there are concerns that cyproterone could harm a male fetus by "feminising" it.

2:5:8:2:4. Treatment of menstrual irregularity, prevention of endometrial hyperplasia

If fertility is not the primary aim, then menstruation can usually be regulated with a contraceptive pill. The purpose of regulating menstruation is essentially for the woman's convenience, and perhaps her sense of well-being. There is no medical requirement for regular periods, so long as they occur sufficiently often. Most brands of contraceptive pill result in a withdrawal bleeding every 28 days if taken in 3-weeks periods. Diane (a contraceptive pill containing cyproterone acetate) is also beneficial for hirsutism, and is therefore often prescribed in PCOS .If a regular menstrual cycle is not desired, then therapy for an irregular cycle is not necessarily required - most

experts consider that if a menstrual bleeding occurs at least every three months, then the endometrium (womb lining) is being shed sufficiently often to prevent an increased risk of endometrial abnormalities or cancer. If menstruation occurs less often or not at all, some form of progestogen replacement is recommended.

However, recent limited and contradictory observational evidence has raised the concern that OCP s may reduce insulin sensitivity and glucose tolerance in PCOS women but there is no evidence that OCP s modify the risk of T2DM or cardio vascular disease (CVD) either negatively or positively (Diamanti-Kandarakis ,*et al*. , 2003 and Vrbikova and Cibula, 2005).

2:5:8:2:4 .A. (Diane-35) Ethinyloestradiol -cyproterone- acetate:

The oral contraceptive pills (OCP s) is the traditional therapy for the chronic (long term) treatment of PCOS, exerting a number of beneficial effects including regularization of menses, amelioration of hirsutism and acne by reducing ovarian androgen production(Diamanti-Kandarakis, *et al*., 2003 and Vrbikova and Cibula, 2005), and protection from the development of endometrial cancer , it inhibits ovulation and causes changes in cervical secretion. Provides contraceptive protection and stabilizes cycle (O'Brien, *et al*.,1991). And because it contains the same antiandrogenic substance (cyproterone acetate), also enhances the therapeutic effect of Androcur. OCP s can be used alone or in conjunction with antiandrogens, gonadotropin-releasing hormone agonists, or insulin-sensitizing agents.

• In women with PCOS not trying to conceive, menstrual irregularity is usually best treated with an estrogen-progestin contraceptive. In

some women, it is possible that sustained metformin treatment may induce regular, ovulatory menstruation (Barbieri, 2003).

• In women with PCOS not trying to conceive, hirsutism treatment initially usually consists of an estrogen-progestin contraceptive, an anti-androgen such as Cyproterone acetate, and mechanical cosmetic treatment (Wong, *et al* .,1995).

2:5:8:3 Surgery:

Surgical treatment is occasionally used for women with infertility caused by polycystic ovary syndrome (PCOS) who do not start ovulating after taking medicine. During surgery, ovarian function is improved by reducing the number of small cysts. Though surgery is not commonly performed, the polycystic ovaries can be treated with a laparoscopic procedure called "ovarian drilling" (puncture of 4-10 small follicles with electrocautery or laser), which often results in either resumption of spontaneous ovulations or ovulations after adjuvant treatment with clomiphene or FSH. Other choices of surgical treatments (conventional) were done by ovarian wedge resection (Stegmann, 2003).

3.1 Materials

3:1:1. Subjects:

The study protocol was conducted in Babylon province .The samples were taken from two sources (from the infertility center of maternity and pediatrics teaching hospital and from those who referred from out patient clinics to the hospital) .

Women with PCOS who previously diagnosed by gynecologist (n=60), with control group (n=30), aged 18-45 (28±6) years, whose chief complaints were menstrual disturbances and infertility and /or clinical or biochemical signs of hyperandrogenism (e.g: hirsutism and acne) were recruited from November 2008 to April 2009, and/or ultrasound (polycystic ovaries) evidence. History was taken whether any of the participants had diabetes mellitus or took any other medications which may alter insulin sensitivity. Also whether the subjects of the included studies met the new proposed internationally agreed definition of PCOS (Fauser, *et al*., 2004).

The diagnosis of PCOS was based on at least two of the three following abnormalities .According to our including criteria all patient were expected to have disturbed ovulatory function with chronic oligomenorrhea (cycle length more than 35 day ;less than 9 cycle per year) or amenorrhea (cycle length more than 12 week) and typical appearance of polycystic ovaries by ultrasound (U.S) according to the criteria of Rotterdam consensus meeting 2003,which all patients fulfilled. Also we confirm absence of heart, liver, or kidney disease (predisposing lactic acidosis), and un suspected pregnancy in all participants before inclusion in the study. Some patients prematurely were excluded from the study in case of diagnosis of pregnancy or because of loss to follow up.

3:1:1:1. Study Design:

The subjects study consisted of a total number of (90) married women in the reproductive age. Their age ranged between 18-45(28±6) years. The women included in this study were classified into three groups as follows :

Treatment's groups were allocated according to the severity of symptoms to one of two groups :

A . (n=30) received metformin only .

B.(n=30)received Metformin (Glucophage)+ ethinyloestradiol - cyproterone acetate(Diane)+cyproterone acetate (Androcur).

Metformin was administered at a dose of 3×500 mg daily ,except for the first week of treatment when 500mg were given only twice a day to reduce the incidence and severity of gastrointestinal side effects. (De Leo ,*et al*. ,2003.) , while cyproterone acetate was administered Per oral rout (50 mg) for first 10 days of cycle to provide contraceptive protection., Diane was administered During first 21 days of cycle (O'Brien ,*et al*.,1991).

C . Thirty women were normal, married and fertile as a control group. Complete history and clinical examinations were carried out according to special form designed for this purpose.

3:1:2. Equipments and apparatuses the equipments and apparatuses used in this study shown in table **(3-1):**

Table (3-1) Equipments and Apparatuses

Equipment	Company	Origin
Micropipette(1000)µL	Oxford	Japan
Micropipette(100) µL automatic	Slamed	Germany
Micropipette(50) µL	Eppendorf	Germany
Centrifuge	Hettich	Germany
Light microscope	Nikon	Japan
PCV diagnostic reader	Syrbio	Germany
Water bath thermoelectron corporation	Haake	Germany
oven	Memmert	Germany
Incubator	Memmert	Germany
freezer	Concord	Lebanon
refrigerator	Concord	Lebanon
Microscope slides	Sail brand	China
Microscope cover glass	Ataco	China
Automated differential counter	Erma	Japan
Elisa	Beckman coulter	Austria
Filter paper	Macherey-Nagel	USA
Disposable plastic tips	Plastilab ®	Lebanon
Tape measure	RIAZ	China
Plain tubes	Afco-dispo	Jordan
Weight/tall measure	Seca -unicef	Australia
Syringes(29G×1/2)	Micro-fine plus	USA
Syringes(23G×1)	Proton	Malaysia
EDTA tubes	Afco	Jordan
Glass tubes spectrophotometer	Apel	Japan
Capillary tubes	Modulo HM A/S	Denmark
1 ml plastic plugged tubes	Eppendorf	Germany
Spectrophotometry	JeNway 6310	England

3:1:3. Chemicals

The chemical materials that were used in our study are shown in table (3-2) :

Table (3-2) :Chemicals

Chemicals	Company	Origin
Dipottassium ethylenediamine tetra-acetic acid(k2-EDTA)	Afma-Dispo	Jordan
Glacial acetic acid	Afma-Dispo	Jordan
70% alcohol	Fluka chemika	Switzerland
Leishman stain	Crescent	Saudi Arabia
ALT Kit	Biomagrib	Morocco
AST Kit	Biomagrib	Morocco
TSP Kit	Biomagrib	Morocco
Serum albumin Kit	Biomagrib	Morocco
Testosterone kit	Biocheck	USA
Prolactin kit	Monobind	USA
LH Kit	Monobind	USA
FSH Kit	Monobind	USA

3.2. Methods: As we said previously that all patients underwent clinical , hematological ,and biochemical evaluations at base line and at the end of treatment period of 6 months . Clinical assessment included menstrual cycle frequency , body mass index (BMI), hirsutism score(FG) ,waist circumference ,WHR and blood pressure.

Hematological like PCV, WBC s, Differential WBC s count, and blood group. Hormonal studies were performed on day 2-5(early follicular phase) of cycle a non heparinized venous blood sample was obtained between 8 O'clock and 9 O'clock to measure the circulating concentration of Prolactin , LH,FSH, LH/FSH ratio ,total testosterone ,complete blood count ,differential WBCs count, liver function tests (ALT,AST), TSP, S.G, and S.ALB.

3:2:1. **Menstrual cycle**: Improvement of cycle disorders was defined as a change among clinically classified cycle groups (amenorrhea/ oligomenorrhea/ eumenorrhea)or a reduction in cycle length of at least 4weeks or pregnancy.

3:2:2.BMI was calculated using the equation :weight (kilograms) /height (meters)^2.

3:2:3. Hirsutism was clinically evaluated using the Ferriman-Gallwey(F-G)score (assessment of 11 body areas):a score greater than 8 was defined as hirsutism (Morin-Papunen, *et al*., 2000; Morin-Papunen, *et al*.,2003). Hair growth is rated from 0 (no growth of terminal hair) to 4 (complete and heavy cover). A score of 8 or higher is regarded as indicative of androgen excess. With other ethnic groups, the amount of hair expected for that race should be considered. The 11 measured locations are the upper lip, chin, chest, upper back, lower back, upper abdomen, lower abdomen, the upper arms, forearm ,legs and the thighs(Ferriman and Gallwey,1961).This rating scale has been modified by other researchers to include a total of 19 locations, with the 10 extra locations being: sideburns, neck, buttocks, inguinal area, perianal area, foot, toes and fingers. Each area has its own specified definition of the 4 point scale (Goodman, *et al*.,2001).

3:2:4.**Waist Circumference (W.C):**Was calculated by tape measure, it located just above the upper hip bone and was calculated by inches.

3:2:5. WHR measurement: Was done also by tape measure. It was calculated by measuring the waist circumference and dividing by the hip circumference at its widest part (waist/hip). The ratio is applied both to women and men. A WHR of 0.7 for women and 0.9 for men have been shown to correlate strongly with general health and fertility (Dixson, *et al* .,2007) .

3:2:6. **Blood Pressure** : was measured by mercury sphygmomanometer.

3:2:7 .Blood Collections:

Venous blood samples were obtained from antecubital vein at early follicular phase (2-5) day of the cycle . By means of disposable plastic syringes (23G×1), the skin is cleaned with 70%alcohol. About 8 ml of blood were withdrawn , 2ml of this blood are put into (k2-EDTA)tube and the blood mixed well to prevent clotting of the blood, the other 6 ml are delivered into disposable sterile plain tubes . The blood in the plain tubes is allowed to clot, and then the sera are separated by centrifugation for 2 minutes. at 3000 round per minutes (RPM)and the sera obtained are put in another disposable sterile plain tubes and stored in deep freeze for biochemical and hormonal test.

3:2:7:1.Haematological Tests:

3:2:7:1:1. Determination of ABO and Rh (D) blood groups ABO blood groups:

There are four main blood groups ,which are A,B,AB and O according to the presence or absence of two antigens ;A and B as shown in table (3-3):

Table (3-3) classification of ABO blood groups

blood groups	Antigens in red blood cells	Antibodies in serum
A	A	Anti-B
B	B	Anti-A
AB	A+B	none
O	Neither A or B	Anti-A + Anti-B

Principle :

Determination of ABO blood groups of red cells depends on testing them with known anti-A and anti-B sera .The presence or absence of agglutination indicates the group of each cell sample.

Rh (D) blood groups

Besides the A and B agglutinogens , human red cells ,carries many other antigen .The only one which needs specific consideration are those of Rh system **(**Dacie and Lewis,2005).

Principle :

Determination of Rh blood groups of red cells depends on testing them with known anti-D sera .The presence or absence of agglutination indicates the presence or absence of D- antigen in each cell sample.

Procedure :

The slide method :which includes the following steps the microscope slide is divided into three areas A,B,D. one drop of anti-A serum, anti-B serum and anti-D serum is placed in area A,B and D division respectively. The finger is prick with sterile lancet and the first drop of blood is removed.

One drop of blood is added into each division (A, B and D) next to the drop of test serum and mixed them with clean match stick. after 2 minutes. The area is observed for the evidence of agglutination of the red cells (read microscopically).

Rh is found using the following table(3.4):

Table (3.4) Rh types and their Anti-D

Rh types	Anti-D
Rh+	+
Rh-	-

(+) indicate agglutination.

(-) indicate no agglutination.

3:2:7:1:2. Determination of Packed Cell Volume(PCV)

Microhematocrit method was used to determine PCV, heparinized capillary tubes used ,and blood was filled to approximately three quarters of their lengths then the unmarked end is closed with modeling clay and put in the microhematocrit centrifuge. After centrifugation for 15 minutes, 3000 RPM, the red blood cells were separated from plasma and remain a band of Buffy coat at the interface between them consisting of leukocytes and blood platelets (Lewis, *et al*.,2006).

3:2:7:1:3.Determination of Total and Differential White Blood Cells (WBCS)

A-**Determination of Total WBC s Count:**

About 0.02ml of the blood had been taken from the anticoagulant tube and mixed with 0.4ml of glacial acetic acid , then one drop is taken and placed on a Neubauer's chamber at the space between the cover slip and the chamber, it is waited for three minutes to let the cell for setting down, then the WBCS are counted in the four corners secondary sequares under the microscope (x40) (Dacie and Lewis,2005).

WBC s count Normal Findings (Adult): 5,000-10,000×10^9/L.

B **.Determination of Differential WBCS Count:**

From previous anticoagulant tubes ,one drop of blood is taken and smeared to make blood film and then left to dry .these smeares are stained with Leishman's stain for five minutes then flooded with distal water for ten minutes and washed with distal water also and left to dry again and examined under the microscope (with oil immersion)(Lewis ,*et al.* 2006).

Normal Findings (Adult): neutrophile 55-70% and absolute 2500-8000, lymphocyte 20-40% and absolute 1000-4000 , monocyte 2-8% and absolute 100-700 , eosinophile 1-4% and absolute 50-500 ,basophile 0.5-1.0% and absolute 25-100.

3:2:7:2 . Biochemical tests :

3:2:7:2:1. Hormonal Measurement

A.Testosterone Standard Curve:

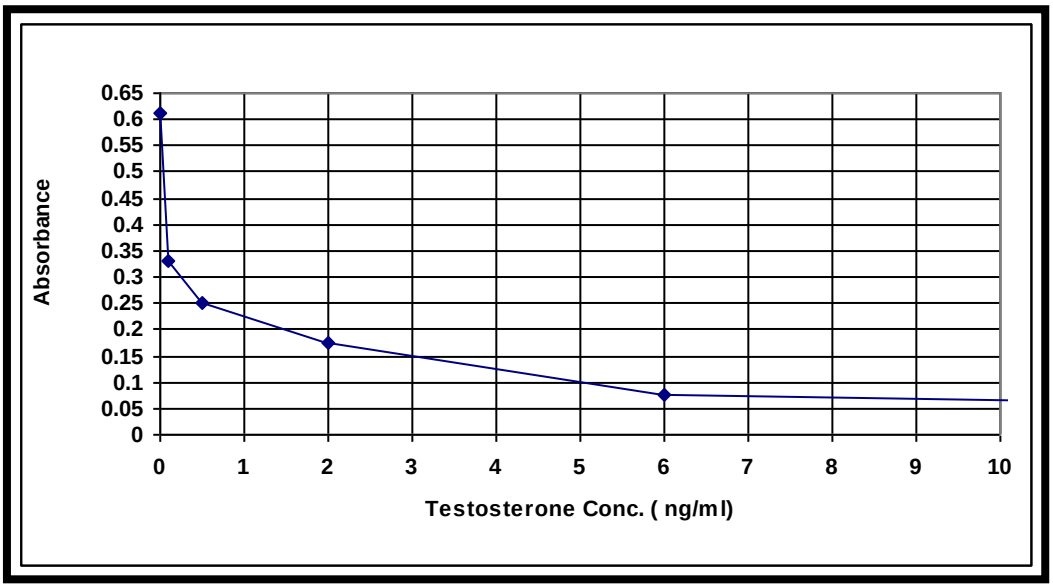


Figure (3-1) shows the testosterone standard curve.

Testosterone enzyme immunoassay(EIA) test kit, catalog number: BC-1115,Biocheck,inc. ,Determination of total serum testosterone (chen, *et al* .,1991.and Teitz,1995).

Principle of the test :

The testosterone EIA is based on the principle of competitive binding between testosterone in the test specimen and testosterone-HRP conjugate for a constant amount of rabbit anti-testosterone .In the incubation , goat anti rabbit IgG-coated wells are incubated with 10µL of testosterone standards ,control, patient samples , 100µL testosterone HPR conjugate reagent and 50 µL rabbit anti-testosterone reagent at 37c° for 90 minutes.

During the incubation , a fixed amount of HRP(Hourse Reddish Peroxidase) labeled testosterone competes with the endogenous testosterone in the standard ,sample ,or quality control serum for affixed number of binding sites of the specific testosterone antibody .Thus the amount of testosterone peroxidase conjugate immunologically bound to the well progressively decreases as the concentration of testosterone in the specimen increases.

Unbound testosterone peroxidase conjugate is then removed and the wells washed .Next, a solution of TMB(3,3′,5,5′ Tetra Methyle Benzedene) reagent is then added and incubated at room temperature for 20 minutes, resulting in the development of blue color .The color development is stopped with the addition of 1 normality HCL(Hydrochloric acid), and the absorbance is measured spectrophotometrically at 450 nm(nanometer) .the intensity of the color formed is proportional to the amount of enzyme present and is inversely related to the amount of unlabeled testosterone in the sample .A standard curve is obtained by plotting the concentration of the standard versus the absorbance .The testosterone concentration of the specimens and controls run concurrently with the standards can be calculated from the standard curve .

Assay procedure:

1. Secure the desired number of coated wells in the holder.

2. Dispense 10 µL of standards, specimens and controls into appropriate wells.
3. Dispense 100 µL of testosterone-HRP conjugate reagent to each well.
4. Dispense 50 µL of rabbit anti-testosterone reagent to each well.
5. Thoroughly mix for 30 seconds .it is very important to mix them completely.
6. Incubate at 37c° for 90 minutes.
7. Rinse and flick the microwells 5times with distilled or deionized water .(please do not use tap water).
8. Dispense 100 µL of TMB reagent into each well .gently mix for 10 seconds.
9. Incubate at room temperature (18-25c°)for 20 minutes.
10. Stop the reaction by adding 100 µL of stop solution to each well.
11. Gently mix 30 second .it is important to make sure that all the blue color changes to yellow color completely.
12. Read absorbance at 450 nm with the microtiter well reader within 15 minutes.

Expected normal value:

in female at follicular phase:0.2-0.8ng/ml.

B. Prolactin Standard Curve

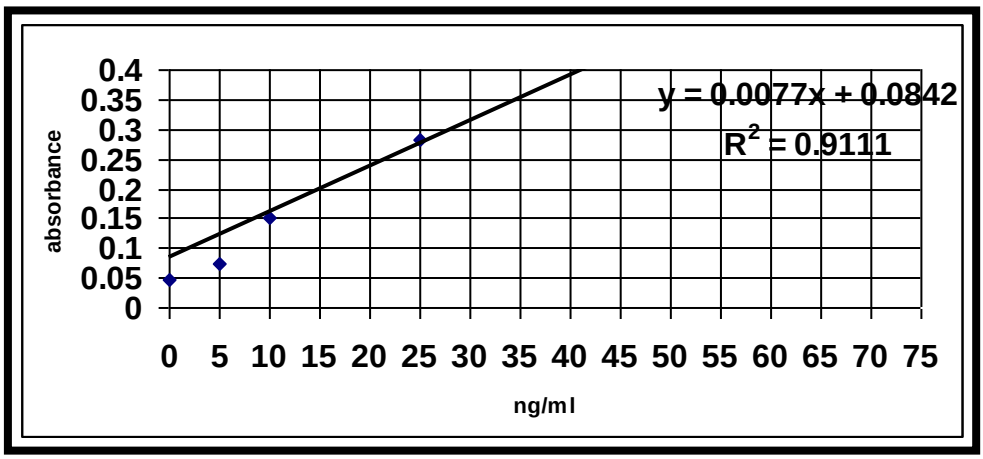


Figure (3-2) shows the prolactin standard curve

Prolactin hormone (PRL):Monobind Inc. ,ELISA microwells . (Maddox, *et al*., 1991and Teitz, 1992).

Principle:

Immunoenzymometric assay:

The essential reagents required for an immunoenzymometric assay include high affinity and specificity antibodies (enzyme labeled and immobilized),with different and distinct epitope recognition ,in excess, and native antigen .In this procedure the immobilization takes place during the assay at the surface of amicroplate well through the interaction of streptavidin coated on the well and exogenously added biotinylated monoclonal anti-PRL antibody.

Upon mixing monoclonal biotinylated antibody, the enzyme labeled antibody and aserum containing the native antigen, reaction results between the native antigen and the antibodies without competition or steric hindrance ,to form a soluble sandwich complex .the interaction is illustrated by the following equation:

$$\textbf{Enz Ab(p)+Ag PRL+Btn Ab (m)} \underset{\textbf{K-a}}{\overset{\textbf{Ka}}{\longleftrightarrow}} \textbf{Enz Ab(p)-Ag PRL-Btn Ab (m)}$$

Enz **Ab(p)**= Enzyme labeled antibody (excess quality)

Btn Ab (**m**)=biotinylated monoclonal antibody (excess quality)

Ag **PRL** =native antigen(variable quality)

Enz **Ab(p)**-Ag **PRL-Btn** Ab (**m**)=antigen -antibody sandwich complex

Ka=rate constant of association

K-a=rate constant of dissociation

Simultaneously the complex is deposited to the well through the high affinity reaction of streptavidin and biotinylated antibody .This interaction is illustrated below:

Enz Ab(p)-Ag PRL-Btn Ab (m)+streptavidin c.w. $\longrightarrow$ Immobilized complex

streptavidin c.w.=streptavidin immobilized on well

immobilized complex =sandwich complex bound to the well

after equilibrium is attained ,the antibody –bound fraction is separated from unbound antigen by decantation or aspiration.

The enzyme activity in the antibody bound fraction is directly proportional to the native antigen concentration .By utilizing several different serum references of known antigen value, a dose response curve can be generated from which the antigen concentration of an unknown can be ascertained.

Reagent preparation:

1. Wash Buffer

Dilute content of wash solution to 1000ml with distilled or deionized water in asoluble storage container. Store at room temperature 20-27c ° for up to 60 days.

2. Working Substrate Solution

Pour the contents of the amber vial labeled solution (A) into the clear vial labeled solution (B) place the yellow cap on the clear vial for easy identification .mix and label accordingly .store at 2-8c°.

Test procedure:

Before proceeding with the assay .bring all reagents, serum references and controls to room temperature (20-27 c°).

1. Format the microplate wells for each serum references, control, and patient specimen to be assayed in duplicate. Replace any unused microwell strips back into the aluminum bag, seal and store at 2-8 c°.
2. Pipette 0.025ml (25 µL) of the appropriate serum references, control or specimen into the assigned well.
3. Add 0.100(100 µL) of prolactin enzyme reagent solution to all wells.
4. Swirl the microplate gently for 20-30 seconds to mix and cover.
5. Incubate 60 minutes at room temperature.

6. Discard the content of the microplate by decantation or aspiration . if decanting ,blot the plate dry with absorbent paper.
7. Add 3000 µL of wash buffer , decant (tap and blot)or aspirate. Repeat two (2)additional times for atotal of three (3)washes . An automatic or manual plate washer can be used .Follow the manufacturers instruction for proper usage, if a squeeze bottle is employed , full each well by depressing the container (avoiding air bubbles)to dispense the wash . Decant the wash and repeat two(2) additional times
8. Add 0.100ml (100 µL) of working substrate solution to all wells. Always add reagent in the same order to minimize reaction time differences between wells

Do not shake plate after substrate addition

9. Incubate at room temperature for fifteen (15) minutes.
10. Add 0.05ml(50 µL) of stop solution to each well and gently mix for 15-20 seconds . always add reagents in the same order to minimize reaction time differences between wells.
11. Read the absorbance in each well at 450 nm (using reference wave length of 620-630 to minimize well imperfections) in a microplate reader. The results should be read within thirty(30) minutes of adding the stop solution .

Expected ranges of value: in women adult age group: 1.2-19.5ng/ml.

C. LH standard curve:

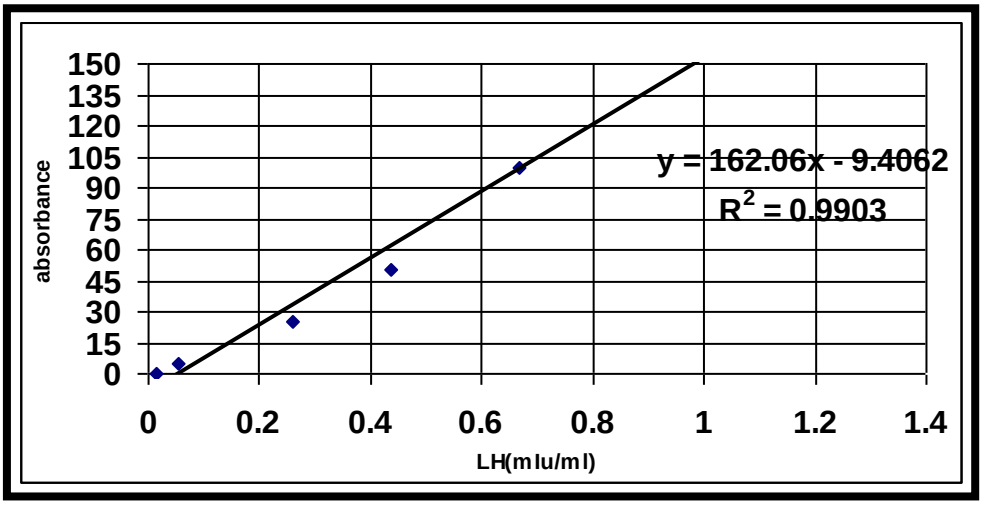


Figure (3-3) shows the LH standard curve

Luteinizing hormone (LH):Monobind Inc . Product code: 625-300 (Kosasa ,1981) .

Principle and procedure :had similar steps to that of prolactin.

Expected ranges of value: in women adult age group in follicular phase 0.5-10.5mIU/ml.

D. FSH Standard Curve :

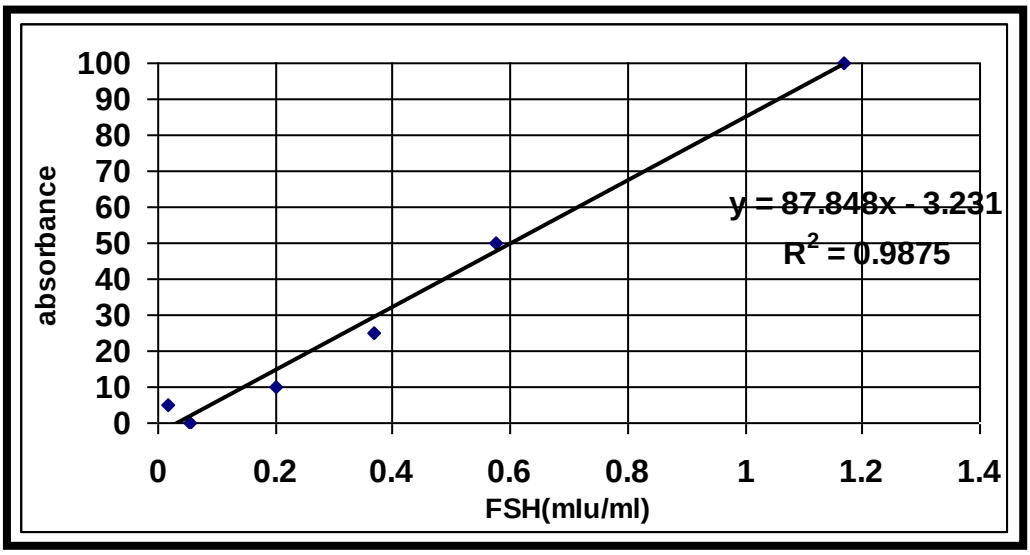


Figure (3-4) shows the FSH standard curve

Follicular stimulating hormone(FSH),Monobind Inc. product code 425-300 (Odell ,*et al*. ,1981andVitt, *et al*.,1998).

Principle and procedure: had similar steps to that of prolactin.

Expected Ranges of Value: in women adult age group in follicular phase 3-12mIU/ml.

3:2:7:2:2.Measurement of Amino Transferases (GOT(AST)-GPT (ALT)) Colorimetric method (Cabaud , 1956)**.**

Principle:

Colorimetric determination of GOT or GPT activity according to the following reactions:

$$\text{L.Aspartate + alpha ketoglutatate.} \xrightarrow{\text{GOT}} \text{Oxaloacetic + L.glutamate}$$

$$\text{Alanine+ketoglutatate} \xrightarrow{\text{GPT}} \text{pyruvate+glutamate}$$

GPT:Alanine+a-ketoglutatateGPT pyruvate+glutamate

The pyruvateor oxaloacetateformed is measured in its derivated form ,2.4dinitrophenylhydrazone.

Table (3.5) Reagents that had been used in measurement of ALT,AST

Reagent1 GOT substrate	Phosphate buffer ph 7,5 Aspartate Alpha ketoglutarate	85mmol/l 200mmol/l 2mmol/l
Reagent 2 GPT substrate	Phosphate buffer ph 7,5 Alanine Alpha ketoglutarate	95mmol/l 200mmol/l 2mmol/l
Reagent 3 Colour reagent	2.4dinitrophenylhydrazine	1mmol/l
Reagent 4 standard	Standard pyruvate	

Samples :serum heamolysis will interfere.

Procedure:

Wave length: …… 550 nm (490-520)

Zero adjustment:……. distilled water

1. Standard curve

Pipette into test tubes (ml):

Table (3.6) procedure for measuring amino transferases

Tube no	1	2	3	4	5
Distilled water	0.2	0.2	0.2	0.2	0.2
Reagent 1	1	0.9	0.8	0.7	0.6
Reagent 2	-	0.1	0.2	0.3	0.4
Reagent 3	1	1	1	1	1

Mix let stand for 20 minutes at room temperature.

NaOH 0.4N 10 10 10 10 10

Mix .wait 5 minutes, measure.

GOT units/ml	0	22	55	95	150
GPT units/ml	0	25	50	83	126

Plot the standard curve:

Number of units/ml in x axis .OD (millimeter paper) or percentages of transmission (semi-log paper) in Y axis.

2. Measurement:

Set up the following tubes for each serum as shown in table (3.7):

Table (3-7) measurement of Amino Transferases

	GOT	GPT
Reagent 1 Reagent 2	1ml -	- 1ml
Incubate for 5 minutes at 37cº.		
serum	0.2ml	0.2ml
Mix and incubate at 37cº for	exactly 1 hour	exactly 30 minutes.
Reagent 3	1ml	1ml
mix. let stand for 20 minutes at room temperature.		
NaOH 0.4N	10ml	10 ml

Mix waits 5 minutes, measure under conditions identical to those used for the standard curve.

Reference value: GOT less than 40 units/ml, GPT, less than 45 units/ml.

3:2:7:2: 3.Measurement of total serum protein (TSP):

Principle :(Valey, *et al*.,1991).

serum protein formed in alkaline media a colored complex whose intensity is proportional proteins in the sample (Biuret reaction).

Table (3.8) Reagents of total serum protein :

Reagent1 Alkaline reagent	potassium –sodium tartrate, Sodium hydroxide , Potassium iodide.	12 mmol \l 0.6 mmol \l 30 mmol \l
Reagent2 Colouring Reagent	cooprrsulphate (nocive)	0.6 mol \l
Reagent3 Standard	bovine albumin	5g\dl

Procedure :

Three set of tubes are prepared as shown in table (3-9):

Table (3-9) procedure for measuring TSP.

	Blank reagent	Standard	sample
sample	-	-	20 µL
R3:standard 1	-	20 µL	-
Working reagent (R1 &R2)	1 ml	1 ml	1 ml

Tubes are mixed and incubated minutes at 20-25 Cº. ,and the absorbency of standard, blank and sample is read by spectrophotometer at absorbency (A) 546 nm(the colour of reaction is stable for 30 minutes)

Calculation:

$$\text{Total protein} = \frac{\text{A sample – A blank}}{\text{A standard - A blank}} \times n$$

n = 5g\l , n=50 g\dl

3:2:7:2 :4. Measurement of Serum Albumin (S.ALB.):

Principle :(Doumas, *et al*.,1971).

Albumin ,in a buffered solution ,reacts with the a green of bromocresol (BCG) to form a red-colour complex.

Table (3-10) Reagents of serum albumin:

Reagent1	bromocresol green Succinate Buffer, Brij	0.14 g\l 75 µ mol\l 7mol\l
Reagent2	bovine albumin	50 g\l 724 µ mol\l

Procedure: Three set of tubes are prepared as shown in table (3.11):

Table (3.11) procedure for measuring S.ALB.

	Blank reagent	Standard	sample
sample	-	-	10 µL
R3:standard 1	-	10 µL	-
Working reagent (R1 &R2)	2 ml	2 ml	2 ml

Tubes are mixed. ,and the absorbency of standard, blank and sample is read by spectrophotometer at absorbency (A) 628 nm after 5 minutes (the colour of reaction is stable for 30 minutes)

Calculation:

$$\text{Serum albumin level} = \frac{\text{A sample – A blank}}{\text{A standard - A blank}} \times n \quad ; \quad n = 5 \text{ g\dl ,n=50 g\l}$$

3:2:7:2: 5.Measurement of Serum Globulin

serum globulin is measured by subtraction of serum albumin from total protein as follows: serum globulin = Total protein -serum albumin.

3.3 Statistical Analysis :

Statistical analysis is performed using SPSS program .Mean and standard deviation are used to evaluate changes over time between the same group pre and post treatment through paired samples T test (paired sample statistics) and between the two patients groups and control through General linear model-univariate. P values of less than or equal to 0.05 are considered to indicate statistical significance ,while p values larger than 0.05 indicate statistical non significance .Also p values of less than or equal to 0.001 are considered to indicate statistical highly significance.Correlation test is also used to show the relationship between some biochemical and physiological parameters through Bivariate test (Daniel ,1999).

4. The Results:

As we studied previously in chapter three , that we classify our subjects into three equal groups: group A(those who treated with metformin), group B(those who treated with metformin, Diane and Androcur) and group C(those who are considered as control).

4:1 Results from History:

4:1:1 .Distribution According to Age Groups:

According to the age of the patients, as shown in table (4-1)the most common age group was (25-35)years which represents the highest percentage of total PCOS patients ,35 out of 60(58%) followed by other groups (15-25)that represents ,19 out of 60(32%) then the lowest percent in the third group ,6 out of 60(10%).

Table (4:1) shows the distribution of all PCOS patients (60) according to age groups

Table (4-1)Distribution of PCOS patients according to age group

Name of patient's groups	No. of patient in each Age group with percentage.		
	(15-25)years	**(25-35)years**	**(35-45)years**
Group A	11(37%)	17(57%)	2(7%)
Group B	8(27%)	18(60%)	4(13%)
Total no. (percentage)	19(32%)	35(58%)	6(10%)

4:1:2.Family History:

33 persons out of 60 (55%) presenting to have positive family history of PCOS or diabetes.53% of them were in group A, and 57% were in group B.

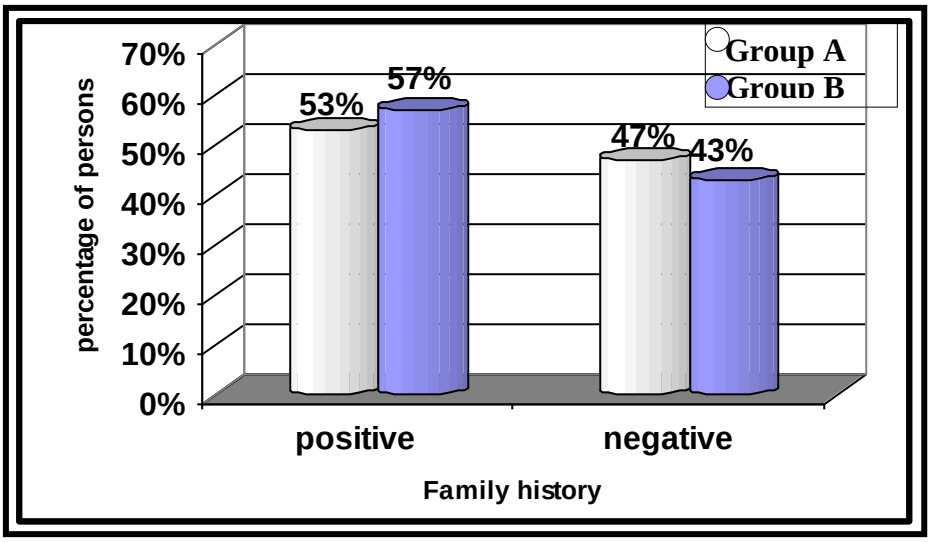


Figure (4:1): Distribution of PCOS patients according to the family history.

4:1:3.Type of Infertility:

39 patients out of 60(65%) had history of primary infertility, 18 one was in group A, and 21 was in group B, while the other 21 had history of secondary infertility.

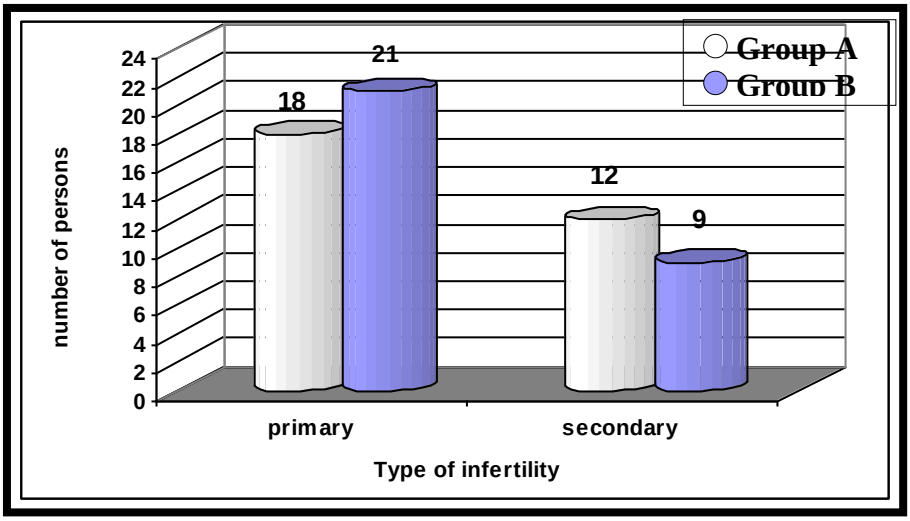
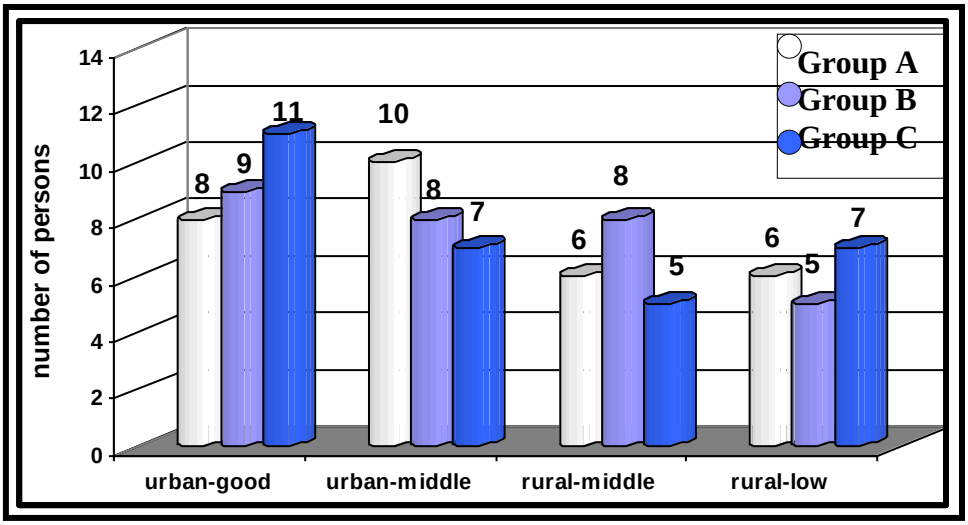


Figure (4:2): Distribution of PCOS patients according to the type of infertility.

4:1:4. Economic state and Residence:

32 patients out of 60 PCOS patients were living in middle economic state (18 one live in urban and 14 live in rural), 17 in good economic state(urban areas), while the other 11 live in low economic state.(rural areas).



Figure(4:3): Distribution of PCOS patients according to their economic state and residence .

4:1:5. Onset of Menarche:

In our study : age of menarche were classified into 2 age groups from 10-18, as follow: (10-14)year,(14-18)year ,that reports 39 out of 60(65%) ,and 21 out of 60(35%) respectively, The study shows that most of patient's menarche were below the age of 14 year ,while the control were mostly above the age of 14 .

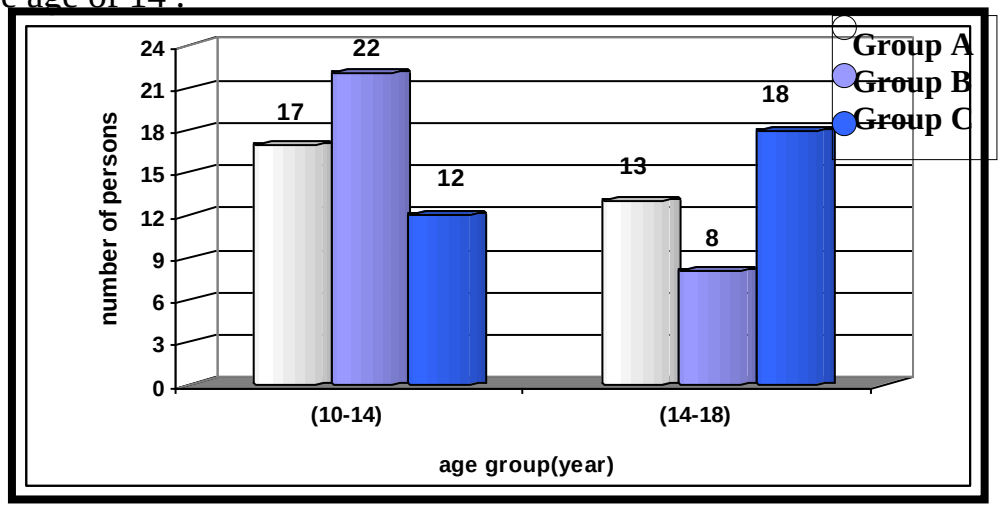


Figure (4:4) The difference in menarche between patients and control.

4:1:6 .Days of Menstrual cycle (M.C) Bleeding:

The study shows in most of patients that although they have irregular cycle but when they have a cycle the bleeding prolong or even not stop only by treatment.

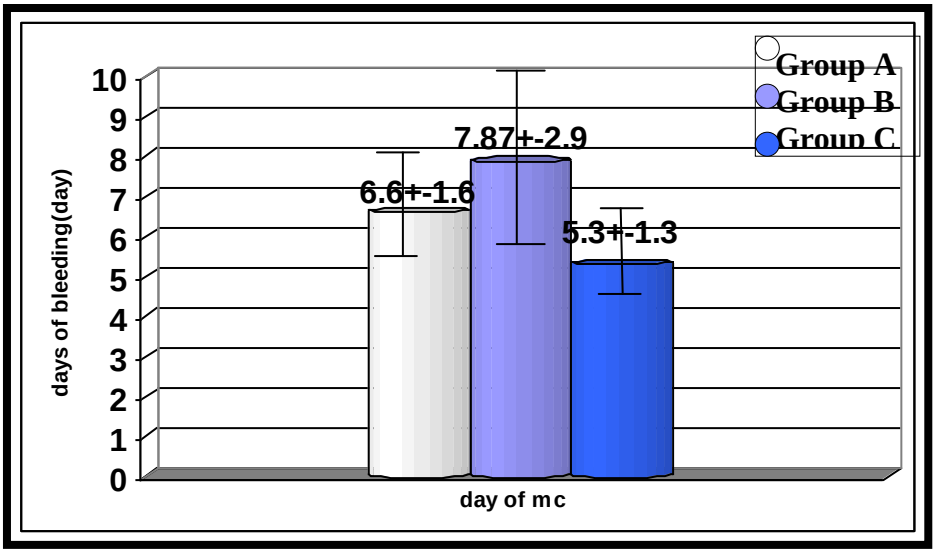


Figure (4:5) show the difference in days of bleeding between patients and control.

4:1:7 .Interval between the cycles (IBC):

Figure below reveals a highly significant decrement in IBC p< 0.001 between PCOS patients pre and post treatment in treatment groups from control group as shown in figure below:

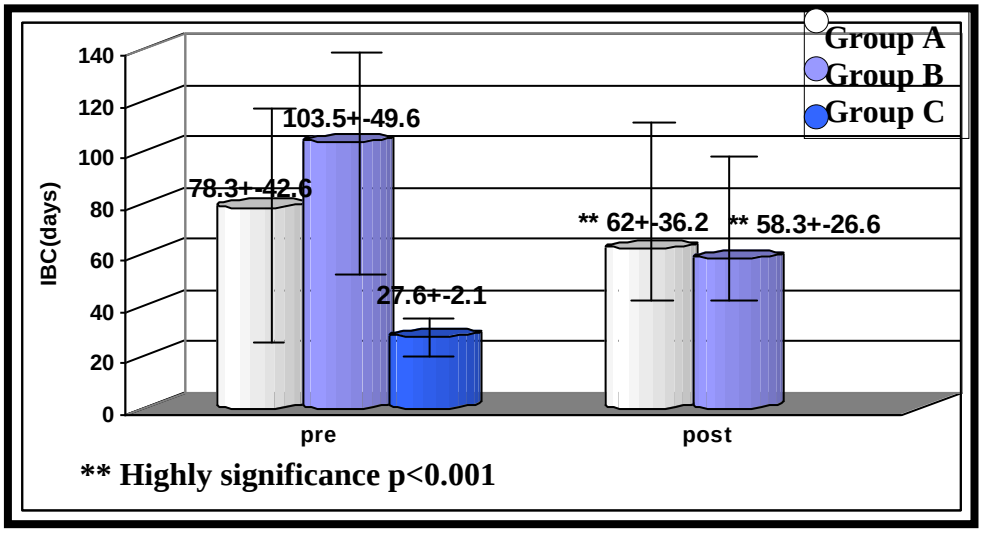


Figure (4:6) represent the difference in the interval between the cycles between the three studied groups.

4:2 Results From Physical Examination:

4:2:1 .Waist hip ratio (WHR):

WHR shows a significant decrement after treatment in groups A, B. P< 0.05, compared with control group.

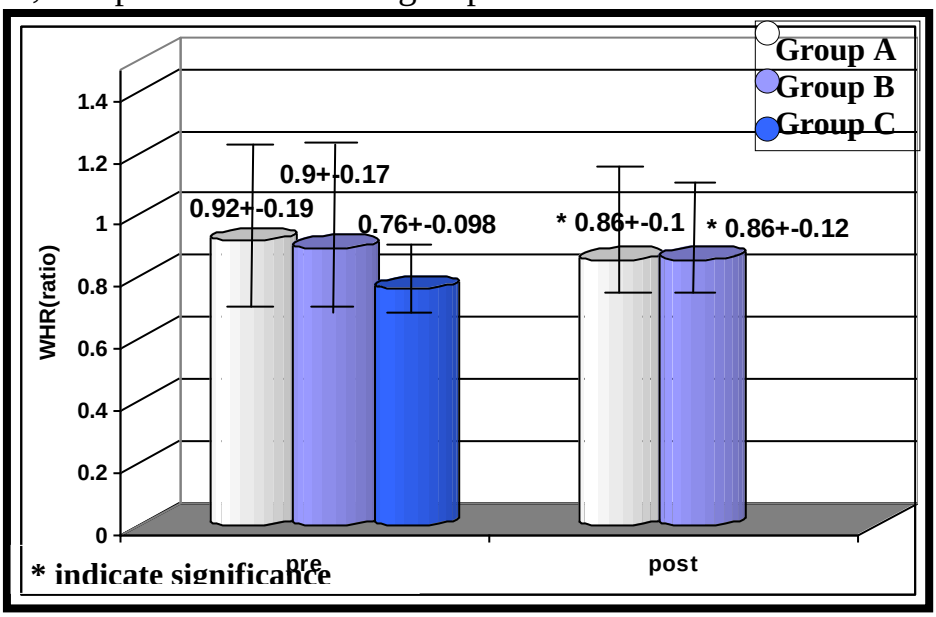


Figure (4:7) waist hip ratio in both patients and control groups.

4:2:2 .Blood pressure:

There are no significant differences in blood pressure between the three studied groups, and all groups were presented within the normal range as in the following table that show the Mean Arterial Pressure (MAP) which equal to:

MAP=Diastolic blood pressure+1/3(systolic blood pressure- diastolic blood pressure) (pulse pressure) (James, 1999).

Table (4-2) blood pressure values among the three studied groups

groups	MAP±S.D	Range of Blood pressure
group C	89.56±5.5	(120/70-130/80)mmHg
group A	90.11± 8.2	(110/60-135/80) mmHg
Group B	90.22 ±8.7	(110/70-135/85) mmHg

4:2:3 .BMI (Body Mass Index):

This figure shows great differences between patients and control group pre treatment, and also found highly significant differences p< 0.001 between pre and post treatment's groups, in which the mean value of BMI in group A was(30.6±5.1),and in group B was(32.05±4.4), while in group C was equal to (27.2±2.4).

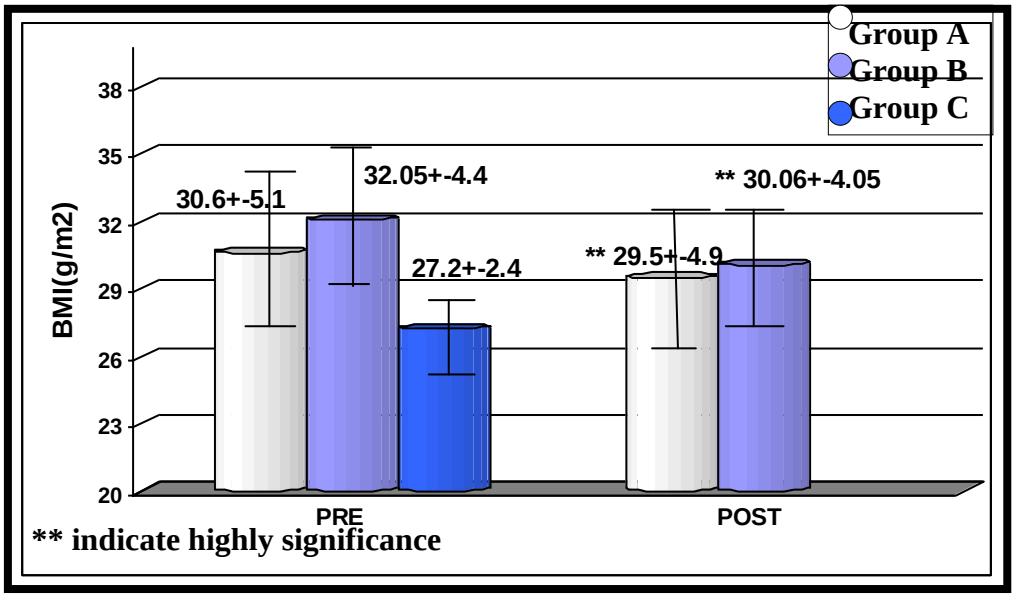


Figure (4-8) shows the values of BMI in the three studied groups .

4:2:3:1. Relationship between BMI and Hormones in group A, B, and C.

There is positive significant relationship between BMI , LH, FSH.in group A, p< 0.05, and there is positive non significant relationship between BMI and LH,FSH hormones in group B, with negative significance to LH P<0.05, and positive non significance to FSH in group C. as shown in figure (4-9):

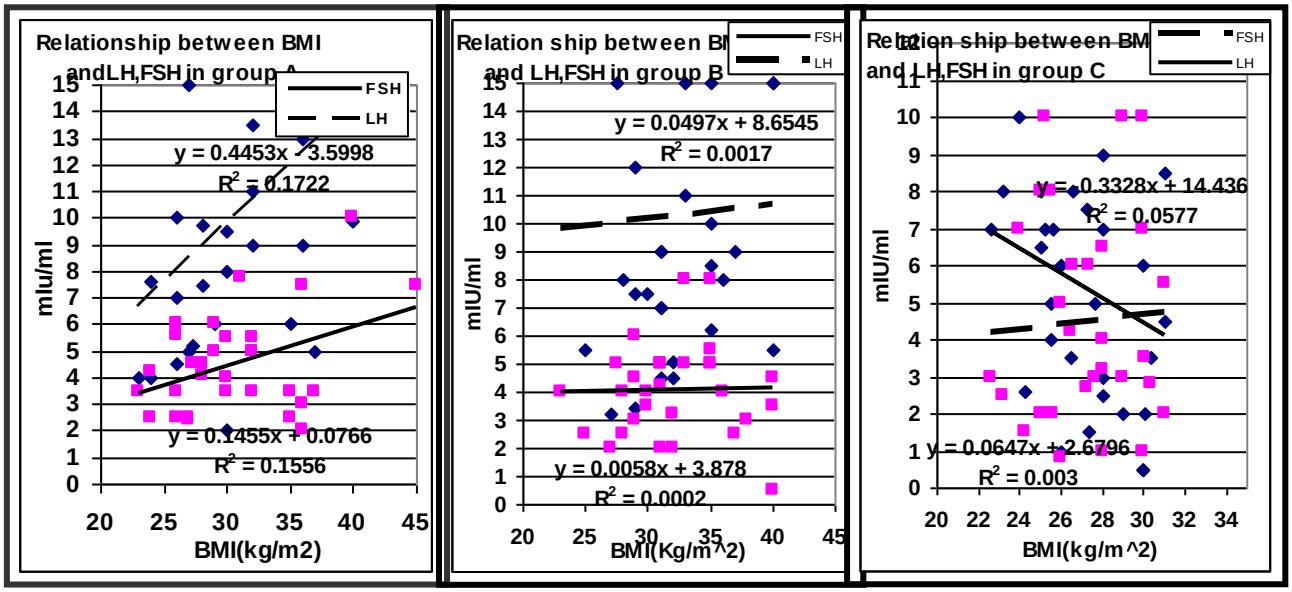


Figure (4-9) Relationship between BMI, and hormone in the three studied groups.

4:2:4. Ferriman, and Gallwey (F.G) score :

Regarding degree of hirsutism there is great difference between patients and control as shown in table (4-3) and figure (4-10):

Table (4-3) Relationship between FG score and hormones in the three groups

Group name	FG score±S.D		Testosterone Total(ng/ml) pre± S.D	LH pre± S.D(mIU/ml)	FSH pre ± S.D (mIU/ml)
	pre	post			
Group C	6±2.3		0.67±0.26	5.29±3.3	4.44±2.8
Group A	10.8±4.3	10.2±3.8	1.25±0.75 *	10.05±5.5*	4.53±1.9
Group B	13.13±4.9	9.6 ±2.6	1.38±0.73 **	10.25±5.3*	4.06±1.7
* indicate significance p< 0.05, ** indicate highly significance p<0.001					

According to the FG score the high degree of hirsutism in the group B are more corrected with Diane and Androcur, and there is significant difference between patients and control as shown in figure (4-10).

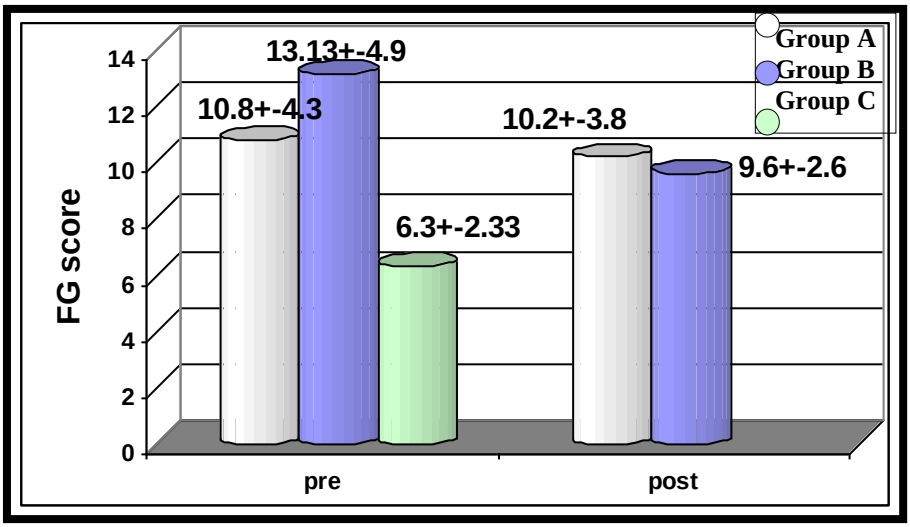


Figure (4-10) shows the mean and standard deviation of FG score pre and post treatment in patients and control.

4:2:4:1.Relationship between FG score and testosterone hormone in group A, B, and C.

Figure (4-11) shows that there is positive significant relationship between FG score and testosterone level in group A , p< 0.05.And there is highly positive significant relationship between FG score and testosterone level in group B p< 0.001, with negative non significant relationship in control group.

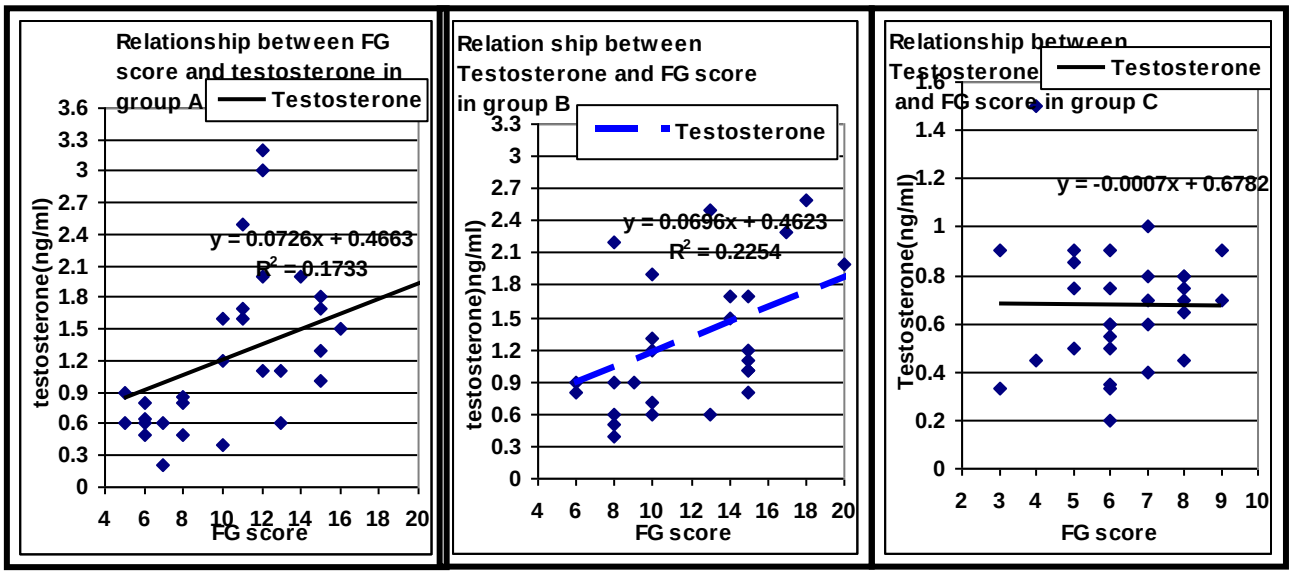


Figure (4-11) Relationship between FG score and testosterone in group A, B, and C.

4:2:4:2 .Relationship between FG score and LH, FSH hormone in group A, B, and C.

There is negative significant relationship between FG and LH, P<0.05 with positive non significant relationship with FSH in group A, and there is positive significant relationship between FG score, LH. P< 0.05, with negative non significant relation ship with FSH in group B, and there is negative significant relationship between FG score and FSH with negative non significance to LH in control group.

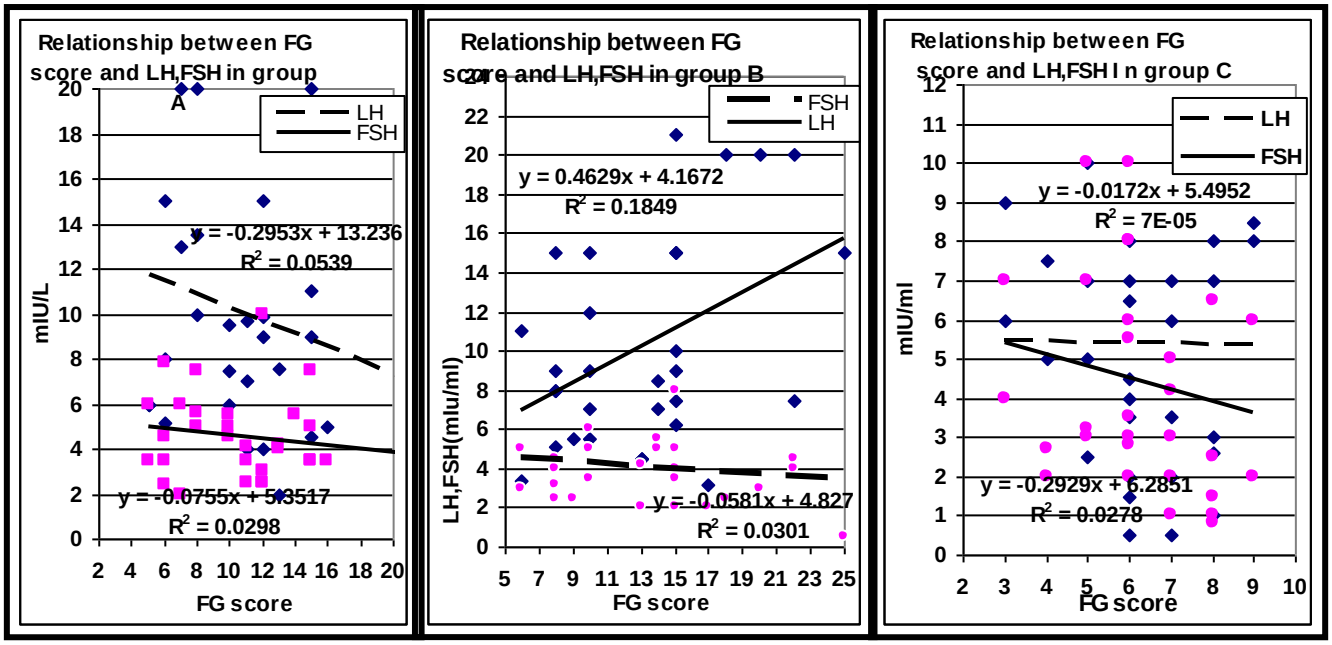


Figure (4-12) Relationship between FG score and LH, FSH in group A, B, C.

4:2:5 .Waist circumferences (WC):

There is significant changes in (WC) after treatment for both studied groups, p<0.05.as shown in figure (4-13) in which the mean value of WC. In group A pre treatment was equal to (37.3±4.3) , while post treatment was(35.9±4.6) and in group B pre treatment was equal to (37.1±2.6), while post treatment was (35.93±2.2) and regarding control it equal to (27.8±3.4).

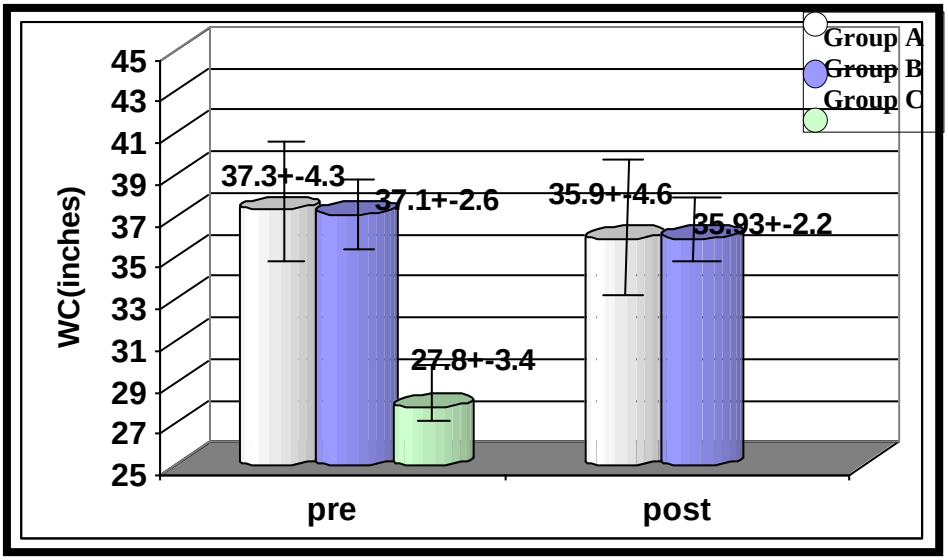


Figure (4-13) the difference in WC value pre and post treatment among the studied groups.

3:2:5:1 Relationship between BMI and WC in group A, B, and C.

There is highly positive significant relationship between BMI, WC. In group A p< 0.001, and there is highly positive significant relationship between BMI, WC. In group B, p< 0.001, with positive non significant relationship in control group. as shown in figure below.

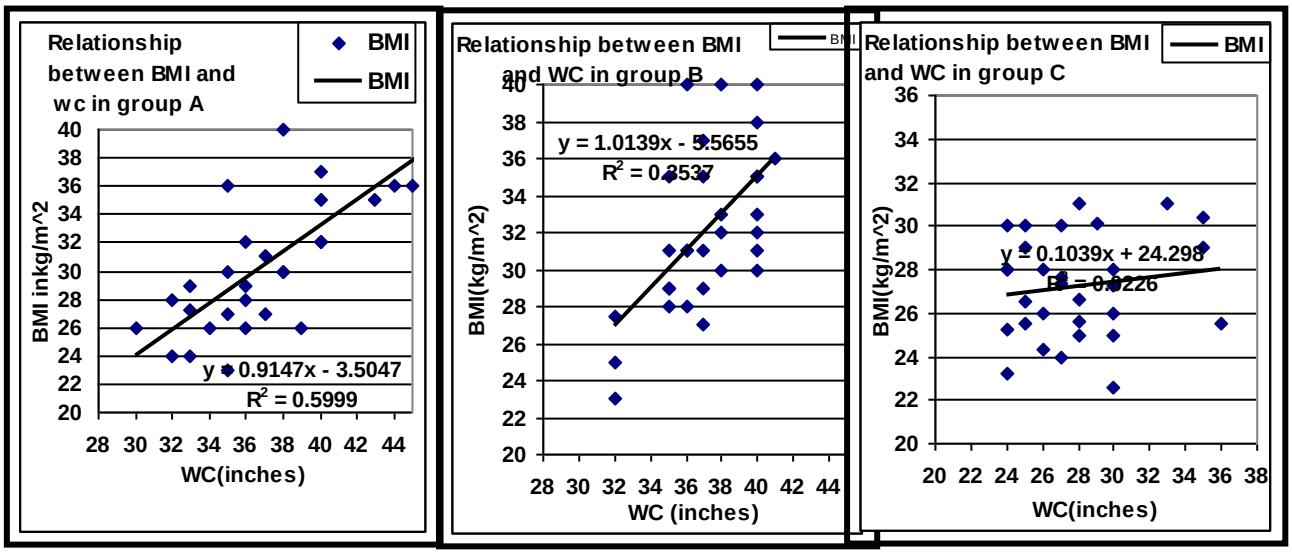


Figure (4-14) Relation ship between BMI and WC in the three studied groups.

4:3 Results from Investigation

4:3:1. Biochemical Investigations: 4:3:1:1.Hormonal Investigations: 4:3:1:1:1 LH

The result of LH level had been used as an indicator for PCOS patients compared with control group, but there was significant decrease in this level after treatment in both groups. p< 0.05.as follow:

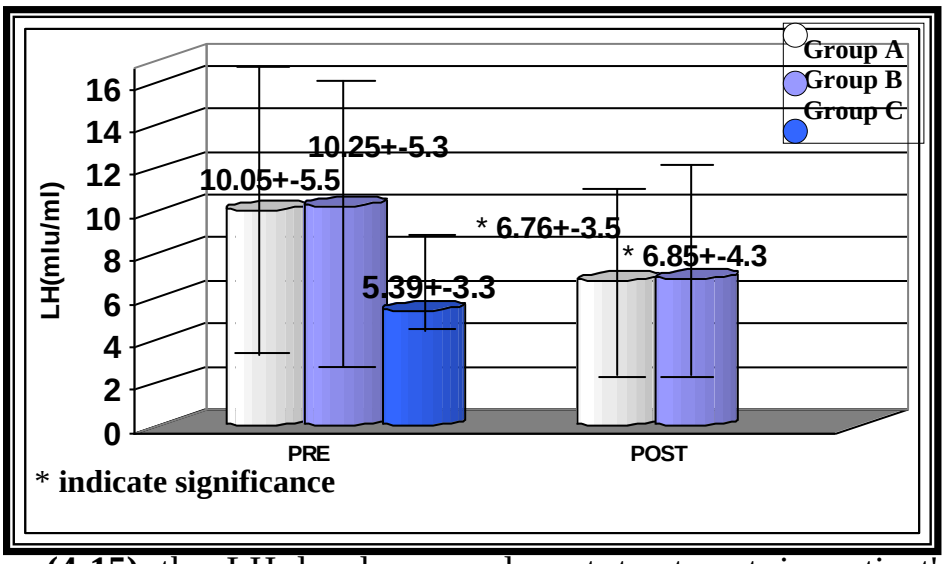


Figure (4-15) the LH level pre and post treatment in patient's groups compared with control.

4:3:1:1:1.A LH/FSH ratio in the three groups:

The mean value of LH/FSH ratio reveals highly significant increment after treatment in group A.P< 0.001. With only slight increment in group B.

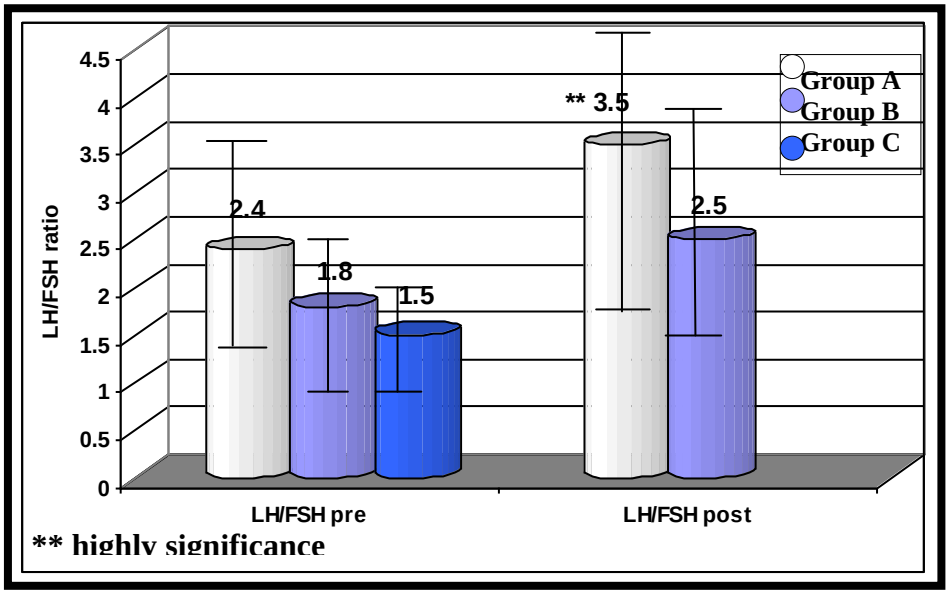


Figure (4-16) LH/FSH ratio in patients compared with control group.

4:3:1:1:1:B Relationship between age and hormones(LH,FSH) in control group:

There is positive significant relationship between age, LH, FSH. In group C, $p< 0.05$.

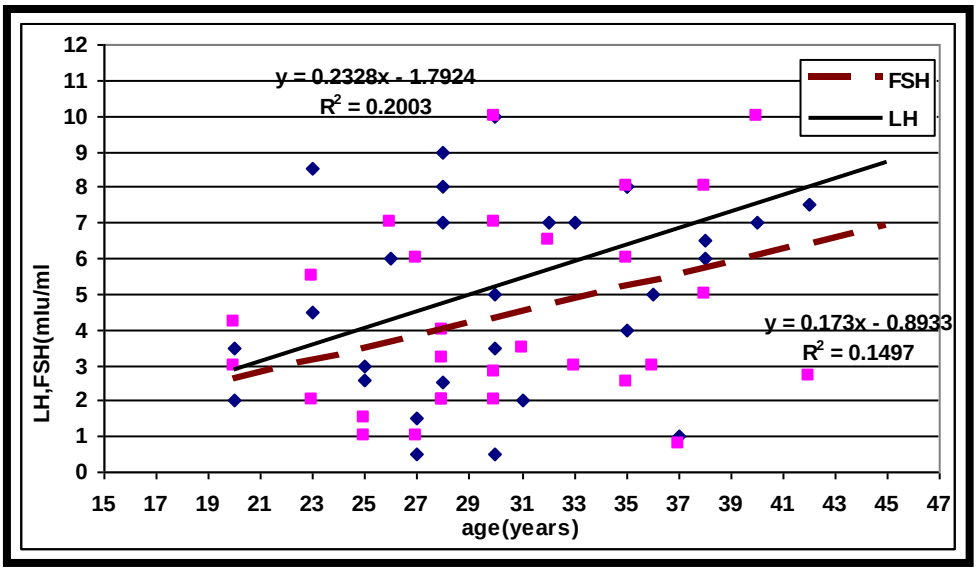


Figure (4-17) Relatioship between age and LH, FSH in control group.

4:3:1:1:2.FSH:

There was decreased or normal level of FSH in patient groups pre treatment with highly significant decrement after treatment in group A $p<0.001$, and significant decrement in group B $p< 0.05$.

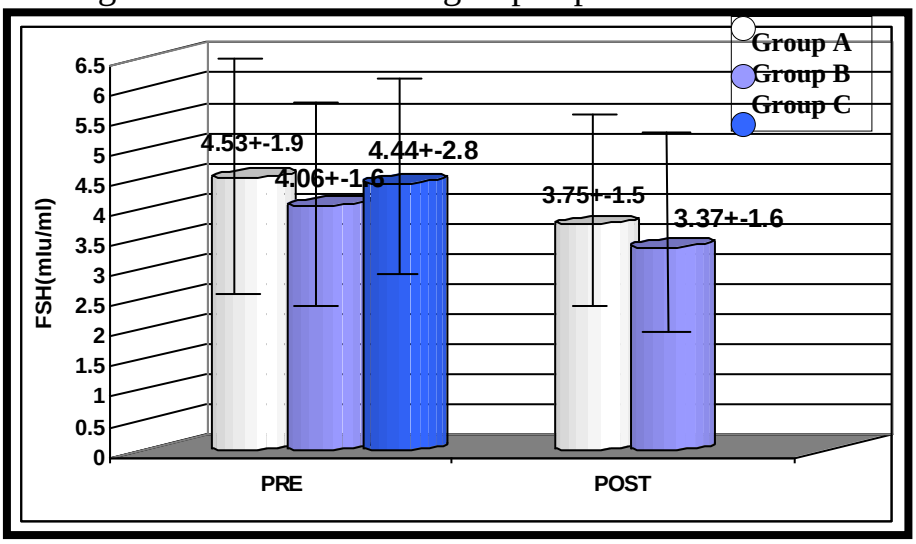


Figure (4-18) the FSH level pre and post treatment in patient's groups compared with control.

4:3:1:1:3.Testosterone:

The resulted study illustrate that the high level of testosterone in patients groups (A, B) show highly significant decrement after treatment p< 0.001.The mean value of total testosterone pre treatment in group A and B was equal to (1.25±0.75, 1.38±0.72) while post treatment it equal to (1.05±0.6,1.05±0.56) respectively, compared with control group (0.67±0.26).as shown in figure (4-19):

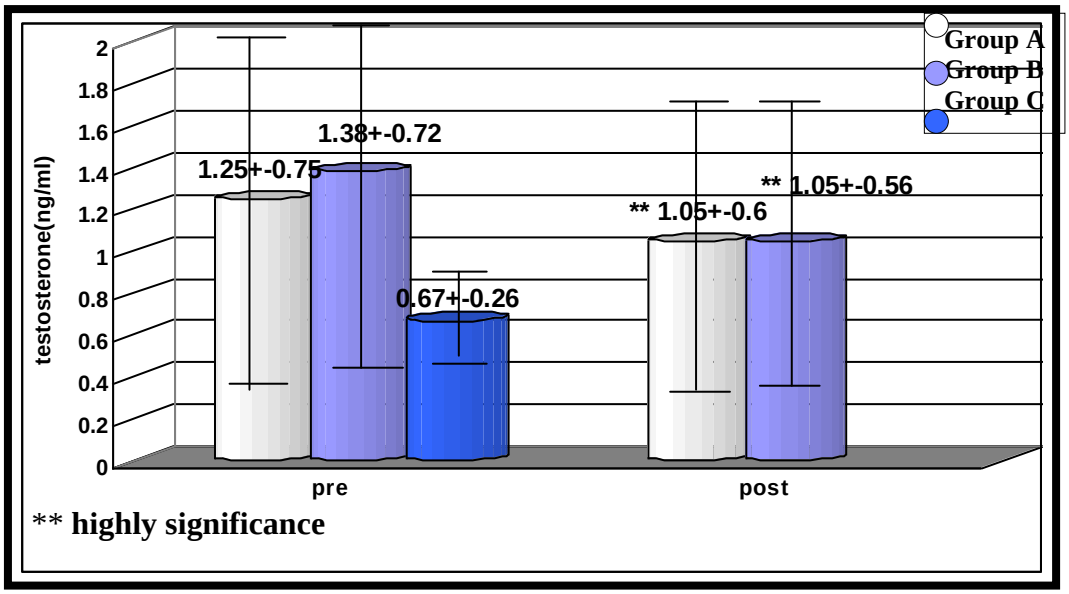
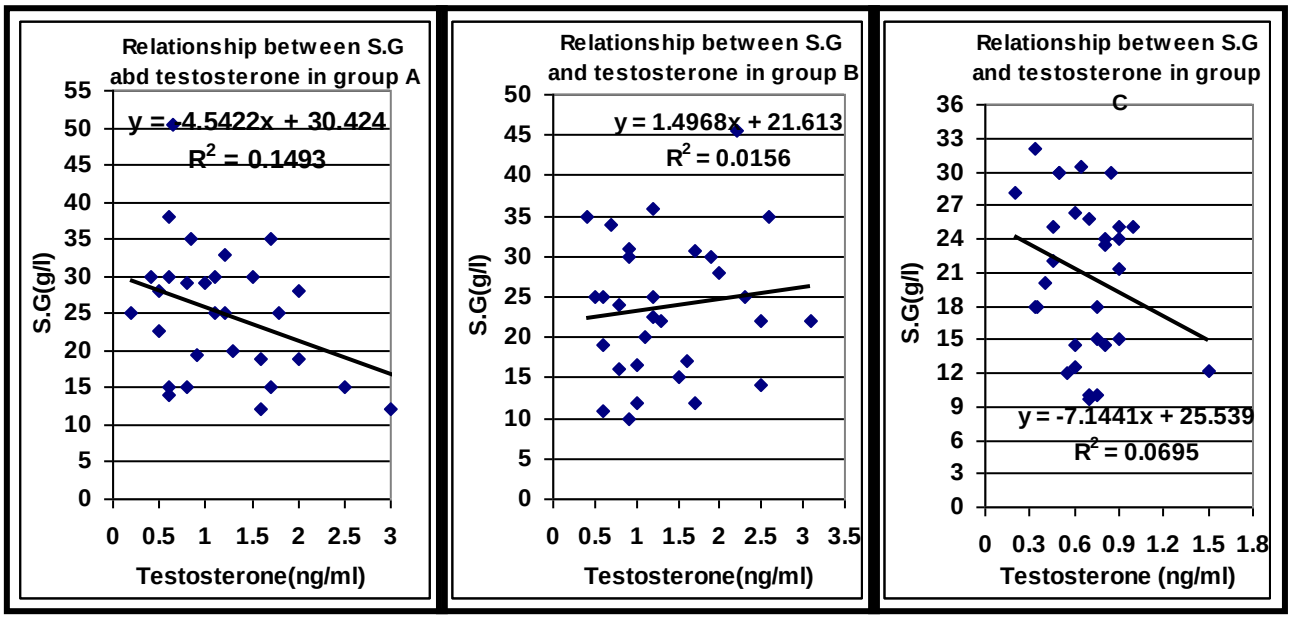


Figure (4-19) the testosterone hormone level pre and post treatment in patient's groups compared with control.

4:3:1:1:3:A. Relationship between serum globulin and testosterone in group A.:

There is negative significant relationship between testosterone level and S.G in group A p< 0.05.while there is positive non significant relation ship in group B. with negative non significance in control group as in figure (4-20):



Figure(4-20) Relationship between testosterone and S.G. in the three groups.

4:3:1:1:4 .Prolactin:

There was normal level of prolactin pre treatment with significant increment in group B patients p< 0.05 after treatment. The mean value of prolactin pre treatment in both group A, B was equal to 13±8.3, 11.2±5.5, while post treatment was equal to 12.26±5.7, 14.14±8.8 respectively ,compared with control group 9.52±4.8. as shown in figure below:

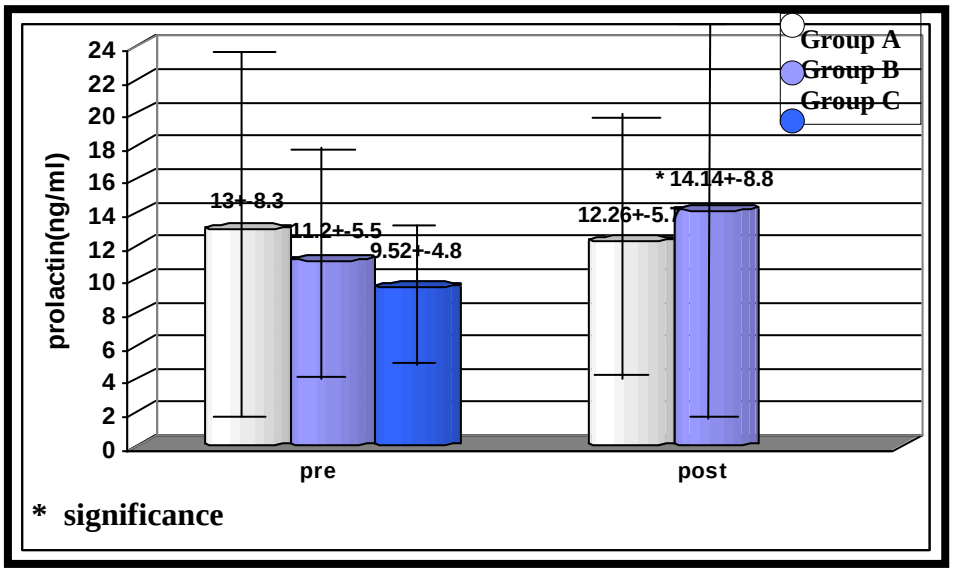


Figure (4-21)the prolactin level pre and post treatment in patient's groups compared with control.

4:3:1:1:4.A. Relation ship between prolactin and testosterone in group A,B and C.:

There is positive non significant relation ship between testosterone hormone and prolactin pre treatment in group A, and B, with negative non significant relation ship in group C, as shown in figure (4-22):

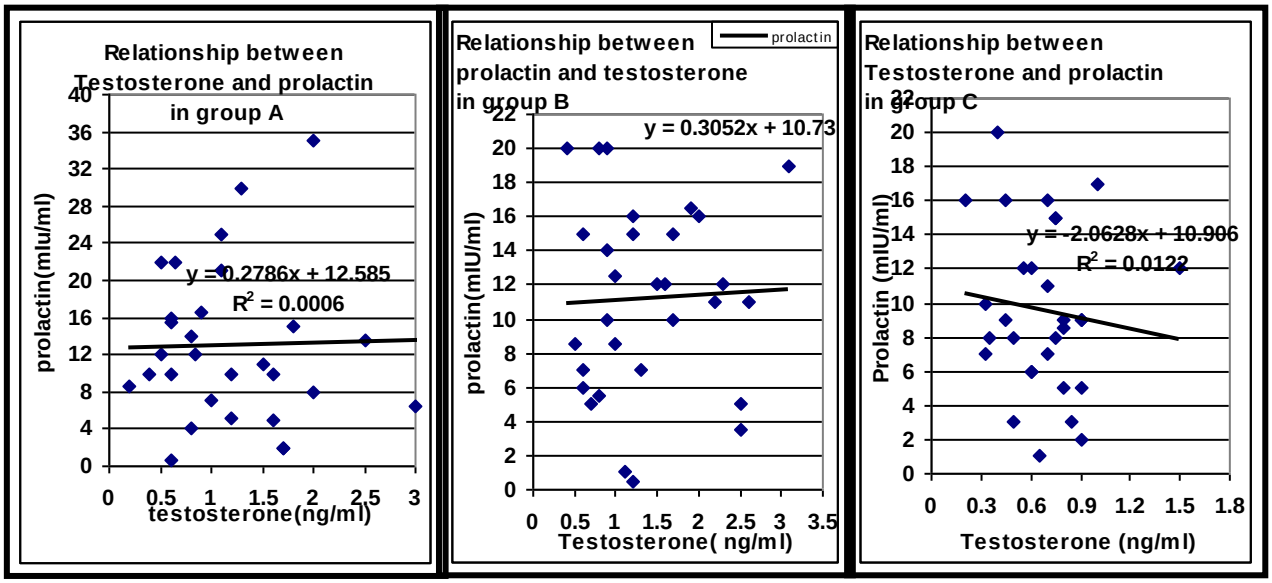


Figure (4-22) shows the relationship between prolactin hormone and testosterone in the three studied groups.

4:3:1:2.Liver enzymes (ALT, AST):

There were significant increment in the level of enzymes pre treatment especially in the second group with significant decrement after treatment p< 0.05.as shown in table (4- 4):

Table (4-4) Describe the difference in the level of ALT,AST a mong patients and control and among patients themselves pre and post treatment .

Group name	ALT(Unit/L)± S.D		AST(Unit/L)± S.D	
	Pre	Post	Pre	Post
Group C	8.4±3.5	-	7.8±3.2	-
Group A	10.2±3.3	7.3 *±3.3	11.2±3.7	7.13 *±7.3
Group B	13±7.4	9.5 *±8.2	12.7±8.2	9.9 *±6.7
* indicate significance (p< 0.05)				

4:3:1:2:1 Relationship between ALT and AST in group C .

There was highly positive significant relationship between ALT, AST .in group C. as shown in figure (4-23), and p <0.001.

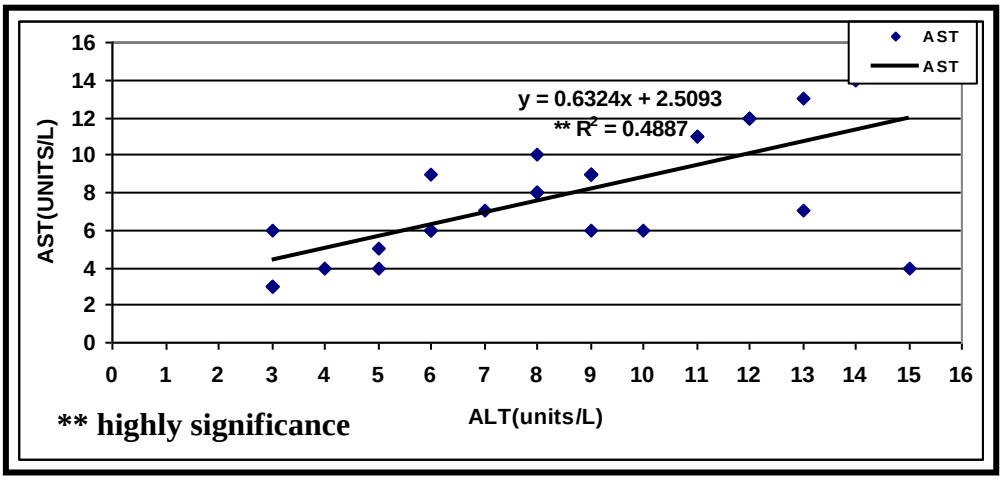


Figure (4-23) Relation ship between liver enzymes (ALT,AST) in control group.

4:3:1:3.Total Serum Protein, Serum Albumin, and Serum Globulin (TSP, S.ALB, And S.G.):

TSP totally not significantly changed while there is significant decrement in S.ALB .in group A. p< 0.05.with increment in S.G after treatment as shown in the following table:

Table (4-5) shows the difference in the level of TSP, S.G., S.ALB. a mong patients and control and among patients them selves pre and post treatment .

Group name	TSP(g/l)±S.D		S.G(g/l)±S.D		S.ALB.(g/l)±S.D	
	pre	post	pre	post	pre	post
Group C	71±7.2	-	20.7±6.98	-	50.7±8.2	-
Group A	70.9±7.2	69.1±6.3	24.8±8.8	28.8±8.9	45.8±8.7	40.4±10.3
Group B	71.8±7.7	70.7±6.5	23.67±8.68	24.67±8.8	46.51±10	46±11.8

4:3:1:3:1 Relationship among TSP, S.G, and S.ALB. in group C.

There is highly significant positive relationship between TSP, S.ALB in control group. p< 0.001.with positive non significant relationship to S.G.

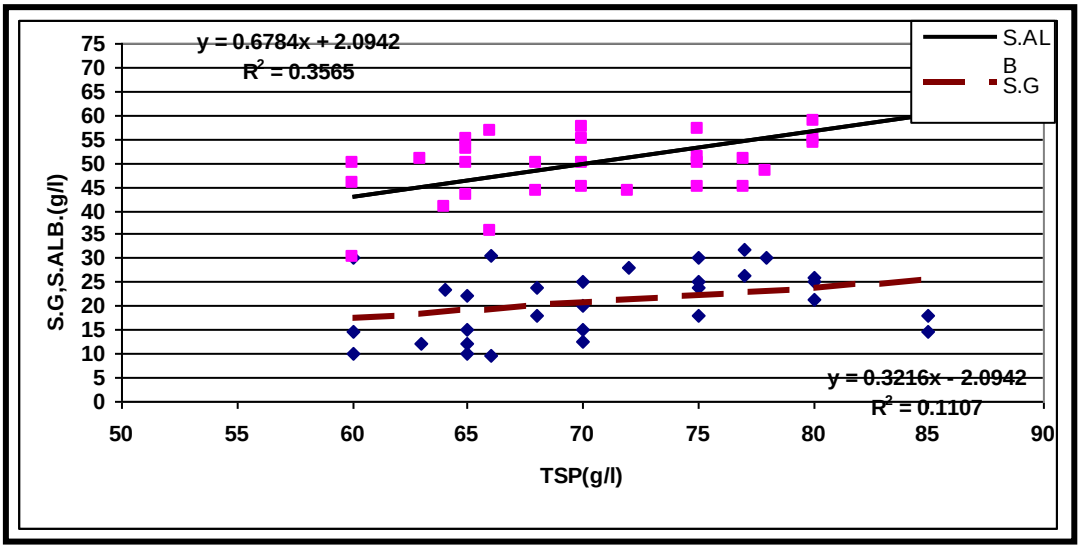


Figure (4-24) Relationship among TSP, S.G, and S.ALB.

4:3:1:3:2 Relationship between S.G, and S.ALB. in group C.

There is highly negative significant relationship between S.G, S. ALB in control group .p< 0.001.

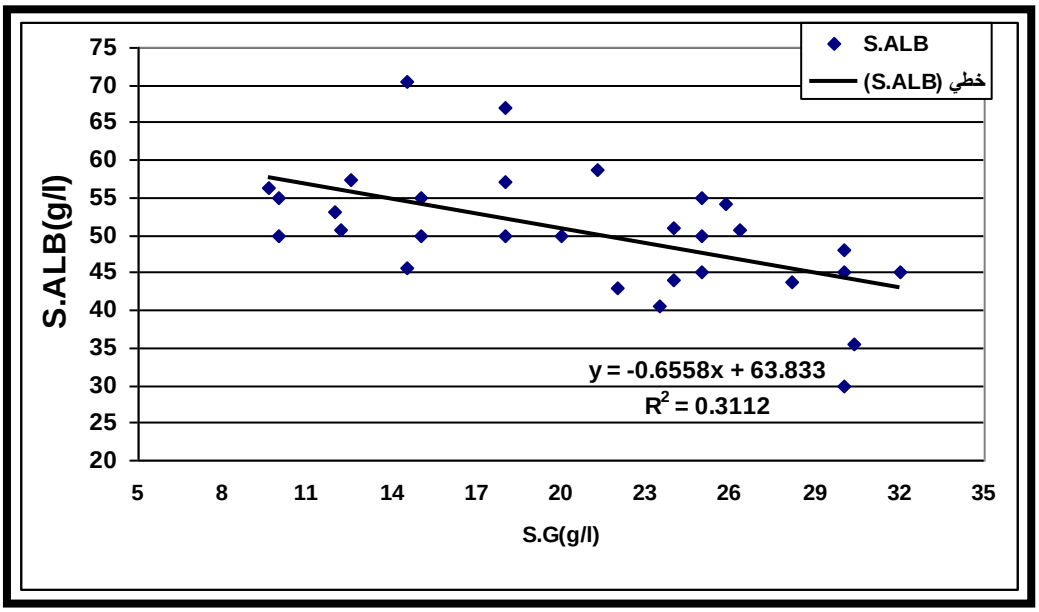


Figure (4-25) shows the relationship between S.G, and S.ALB.

4:3:2 Hematological Investigations:

4:3:2:1 Blood Groups:

In this study we found that the most predominant blood group in patient with PCOS was blood group O+(38%) ,A+(27%), B+(20%), AB+(10%) Respectively.

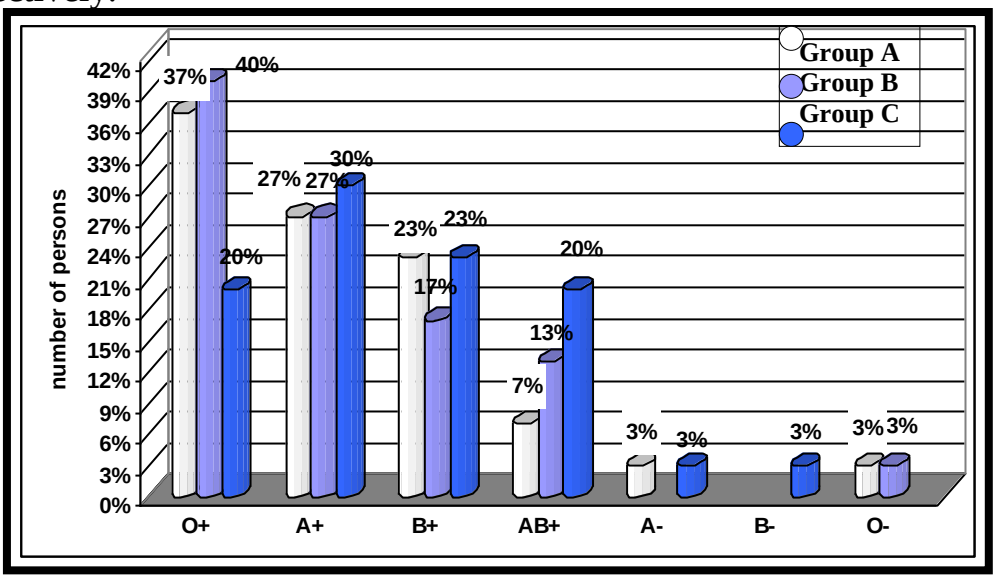


Figure (4-26) Distribution of PCOS patients according to their blood groups.

4:3:2:2 .PCV (packed cell volume):

In patient groups there was normal level of PCV, but there was significant decrement in PCV level after treatment P< 0.001.

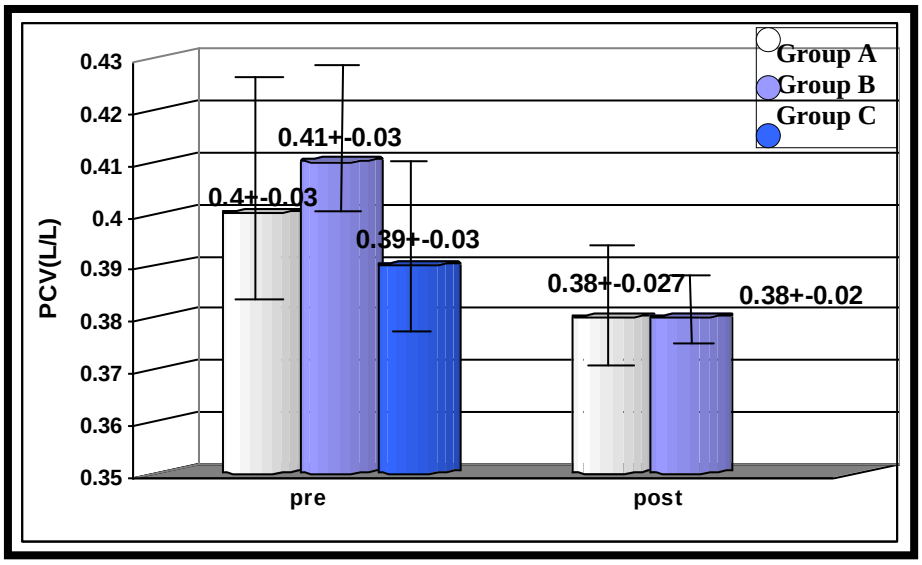


Figure (4-27) PCV value among studied groups pre and post treatment.

4:3:2:3 .WBC s (white blood cell) counts:

There were normal level of WBC pre treatment with highly significant decrement after treatment in group A, p< 0.001,and significant decrement after treatment in group B ,p<0.05.

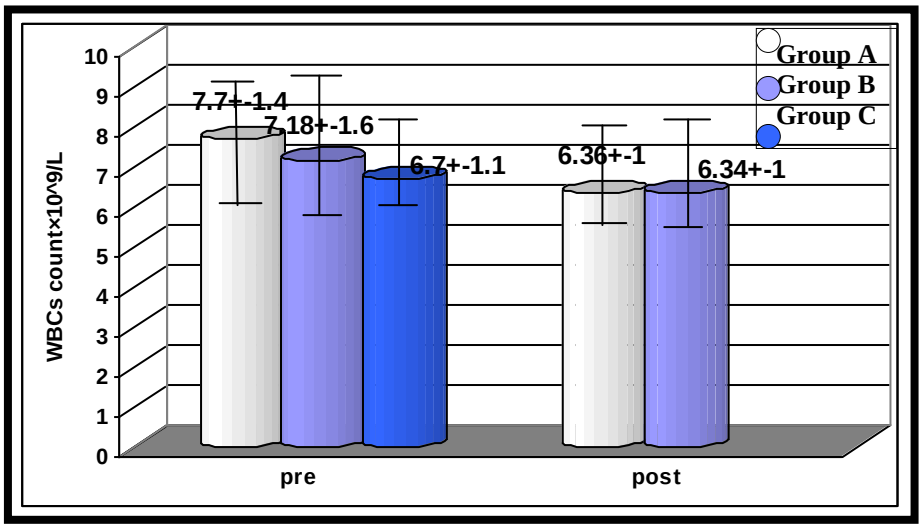


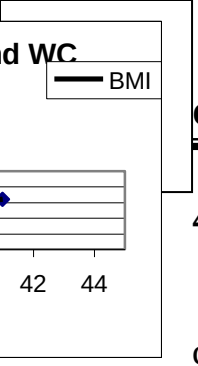
Figure (4-28)WBC s count among studied groups pre and post treatments.

4:3:2:4 .Differential WBC s counts:

In pretreatments groups There was a slight increment in lymphocyte count in patients compared to control group suggest low grade chronic inflammation , with slight decrement in neutrophile count compared with control but remain within the normal range as shown in table (4-6):

Table (4-6) differential WBC s count among PCOS patients compared with control group.

Group name	Differential WBC s count ×10^9			
	neutrophile	mphocytely	monocyte	other
Group C	55.5	39.5	3.06	1.9
Group A	47.8	46.3	3.5	2.3
Group B	50.3	44	3.4	2.2



4:3:3. Ultrasound (U.S) findings:

Table (3-3) show the presence of U.S finding among PCOS women compared with control group .also the result of this study reveals a difference in U.S. findings among PCOS patients regarding the size of the ovary (59% of them had large ovary and the other shows normal ovarian size) and presence or absence of tinny follicles inside the ovary with ovarian thick capsule.(44% shows presence of tinny follicles)while the rest had other ovarian changes like absence of mature follicle, dense stroma ,or shows normal ultrasound findings.

Table (4-7) Ultrasound findings among PCOS patients and control groups.

Group name	Percentage of ultrasound evidence			Normal evidence	Total changes
	Large ovarian size	**Thick capsule and tinny follicle**	**Other changes**		
Group C	0%	0%	0%	100%	0%
Group A	27%	23%	23%	27%	73%
Group B	32%	21%	27%	20%	80%
Total percentage	59%	44%	50%	-	-

5. Discussion:

The present study try to cover the effects of two types of treatment regimes :(metformin alone and metformin +OCP(Diane) + antiandrogens (Androcur)) that had been applicable and descriped nowadays in Iraq and world wide in the treatment of PCOS women according to their symptoms and it's severity. In addition to continuous follow up investigations of their Clinical improvement, Hematological assessment, Biochemical changes after 6 month of treatment.

And the following changes were obtained from the results of the 6 month follow up study:

5.1. Distribution of Age:

Table (4-1) shows the distribution of PCOS patients according to their age. The most common age group was ranged between 25-35(28±2) year, which represent 58% of total number .Followed by (15-25) year with mean of (21±2), 35-45(36±2) year, in a percentage of 32%,10% respectively . And this result agrees with several studies like; (Carmina, 1999) who mentions that PCOS is the most common endocrinopathy in women, occurring in about 5% of reproductive-aged women. Some cases are also seen in young teenagers. But it is rare above the age of 40 years. The onset of symptoms of PCOS is linked to menarche, and puberty is accompanied by a physiological insulin resistance (Ibanez, *et al*., 1997). Women with PCOS usually present in their mid-20s .Menstrual disturbance is the earliest manifestation, and hirsutism and infertility become apparent later. The evolution of symptoms with age appears to be associated with increase of adiposity (Conway, 1996).

5.2. Family history:

Figure (4:1) shows that 55% of PCOS patients had positive family history and this is in agreement with most studies about family history such as (Kidson, 2001.), who states that 40% of women in families with PCOS or type 2 Diabetes have PCOS, indicating a possible inheritable tendency or underlying cause. Also Family history is important in establishing links between onset of puberty, menstrual irregularities, diabetes, and familial patterns of hair growth. However PCOS appears to follow a familial distribution; 40 percent of the sisters and 20 percent of the mothers of affected women also have the syndrome to varying degrees (Legro ,*et al*. ,1998). A report of a large number of sisters of affected women showed that 22% of sisters had well characterized PCOS, and another 24% had hyperandro-genaemia with regular cycles (Legro, 1999). Studies show that PCOS has a genetic component, most likely with an autosomal dominant mode of transmission (Carey, *et al*., 1993).

5.3. Type of infertility:

Primary infertility is more common than secondary infertility as shown in figure (4:2).The percentage of primary infertility was 65%(39/60)18/60 in group A, and 21/60 in group B, whereas that of secondary infertility was 35%.This result was not differ from most studies about infertility which found out that most of PCOS women who had a symptom may ignore it like menstrual irregularities and only they consult their physician when they married and want to conceive as shown in our study.

Many women with PCOS do not ovulate regularly, and these women may take longer time to become pregnant. An infertility evaluation is often recommended (as in primary infertility) after 12 months of trying to become pregnant (Robert and Ehrmann, 2008).

The use of insulin sensitizers has many benefits, since it has been clearly demonstrated that a decrease in insulin concentration, as a result of improved insulin resistance, may have important effects on hyperandrogenism, metabolic alterations and, particularly, on fertility. (Mioni, *et al*., 2003).

5.4. Economic state and Residence:

According to their residence and economic state (lifestyle), (figure 4-3) the distribution of PCOS patients in this study reveals that a 53% of PCOS patients were living in a middle economic state, whereas 28% were living in a good economic state in urban areas while the last 18% were living in low economic state and rural areas, and 58% of those were living in urban areas regardless to their economic state.

Economic state and life style like feeding pattern are most dangerous environmental factor that strongly correlated to PCOS. Extensive research has been performed during the past decades to investigate the molecular basis of polycystic ovarian syndrome (PCOS), hyperandrogenism, and hirsutism. Familial clustering of these disorders strongly suggests a genetic basis, which could be inherited together with insulin resistance and metabolic disorder (Benitez, *et al*., 2001; Legro, *et al*. ,2002; Yldz, *et al*.,2003 and Chang, *et al*.,2005). Despite the data suggesting genetic predisposition, the possibility cannot be ruled out that such clustering may be the result of some environmental factors that are present in the affected families. Some studies have suggested that obesity has a triggering role in the development of these disorders (Gambineri ,*et al*.,2002). There is little doubt that dietary factors interact with the putative genetic influences to affect the phenotype of PCOS. Many studies suggests that environmental factors may play a dominant role in determining individual susceptibility to metabolic disorders, which

is probably more important than genetic background, as supported by recent long-term epidemiological studies demonstrating that the appearance of type 2 diabetes can be prevented by adequate lifestyle intervention, focusing on dietary habits and increased physical activity (Kanaya & Narayan ,2003 and Norris ,*et al.* ,2004).

Insulin sensitizers can be added to lifestyle intervention, when obesity is present, although there is preliminary evidence that some behavioral modification in dietary habits may have a positive effect even in normal-weight insulin-resistant PCOS women (Mioni , *et al.* ,2003). The South Asian subcontinent has a high prevalence of type 2 diabetes, with central obesity strongly associated with diabetes in women (Trevisan, *et al.*, 1998). The prevalence of diabetes in urban and rural women in Pakistan is reported to be 10.6% and 4.8% respectively (Shera, *et al.*, 1999).

5.5. **Distribution of PCOS patients according to Onset of Menarche, Days of Menstrual Bleeding, The Interval Between Menstrual Cycles:**

Often in PCOS patients, the menarche occurs at the age of 12-13 years. Some PCOS patients may start menstruating earlier. Some PCOS patients have quite regular 28 days cycles, but the diagnosis should be suspected in individuals with cycle length over 35 days. Figure (4:6) shows that the OCP was superior to metformin in improving menstrual pattern .This finding was not surprising and consistent with that of systematic review which showed that metformin results in the restoration of regular menses in only 60% of PCOS women with oligomenorrhea or amenorrhea (Costello and Eden, 2003).By six months over 90% of women treated with insulin-lowering agents, diet and exercise will resume regular menses (Velazquez, *et al.*, 1994). On the other hand, cyproterone acetate/ethinyloestradiol (Diane) has advantages: It regulates the menstrual cycle in the majority of women. Also spontaneous ovulation can occur rapidly, and normal menstrual rhythm can be achieved within 3

months of metformin treatment (Nestler ,*et al*.,1998 and Pasquali ,*et al.* ,2000) .

In accordance to our results, several studies show that the ovulation rate increased with no changes in weight, suggesting that the effect is independent of weight loss(Morin-Papunen ,*et al*.,1998.and Pirwany ,*et al*. ,1999).other studies report that metformin can cause improvement in spontaneous menstruation in 20%-50% of women, some women became pregnant Nestler ,*et al*.,(1998).

5 .6 .Waist hip ratio (WHR), Waist circumference (WC):

There was a significant change in WC. After treatment in both groups, figure (4-13) together with significant decrement in WHR in both groups as shown in figure (4-7). WHR has been found to be a more efficient predictor of mortality in older people than waist circumference or body mass index (BMI) .If obesity is redefined using WHR instead of BMI, the ideal ratio for women is considered to be about 0.7.

Other studies have found that waist circumference, not WHR, to be a good indicator of cardiovascular risk factors, body fat distribution, and hypertension in type 2 diabetes (Singh, 2002). In a 6-month study comparing the effect of metformin with that of Diane in obese PCOS women, it was found that whereas metformin significantly decreased waist hip ratio, fasting glucose and insulin levels without any effect on insulin sensitivity, treatment with Diane slightly worsened glucose tolerance, without, however, any change in insulin sensitivity, while it significantly decreased serum androgen levels .Obesity tends to be less of a problem in women with PCOS in the adolescent population. However, Women with PCOS have "android obesity", with fat deposition confined to the upper two-thirds of the body, and a waist-hip ratio

exceeding 0.85. Increased waist-hip ratio occurs independent of body mass index (Amowitz and Sobel ,1999).

5.7 .Blood pressure:

Table (4-2) shows that both treatment groups were within normal range of blood pressure and there is no significant difference in mean blood pressure and standard deviation among the three studied groups. This result may agrees other studies who state that increased blood pressure are one of the late onset complications and is associated with insulin resistance and T2DM.

5.8. Body Mass Index (BMI):

As shown in figure (4-8) there is a great difference between patients and control pre treatment with highly significant difference after treatment in both groups $p< 0.001$.The mean value of BMI in group A pre treatment was 30.6±5.1, and post treatment was 29.5±4.9, while in group B, pre treatment was 32.05±4.4 and post treatment was 30.06±4.05, compared with control group that equal to (27.2±2.4).Also in figure (4-9) and (4-14) that describe the relation ship between BMI and LH,FSH, and WC. In the three studied groups, in which there is positive significant relationship between BMI, LH, FSH. in group A, $p< 0.05$, and there is positive non significant relationship between BMI and LH,FSH hormones in group B, with negative significance to LH $P<0.05$, and positive non significance to FSH in group C. Also there is highly positive significant relationship between BMI, WC. In group A $p< 0.001$, and there is highly positive significant relationship between BMI, WC. In group B, $p< 0.001$, with positive non significant relationship in control group. This result was consistent with some studies about BMI. Like (Balen, 1995.) who states that Metformin appears to work in three ways. First, it decreases the absorption of dietary carbohydrates through the intestines. Second, it

reduces the production of glucose by the liver. The liver uses the raw material in your food to create a reserve supply of blood sugar. When your body experiences stress, the liver releases the reserve glucose to supply your brain and muscles with an immediate source of energy to cope with the stress. Glucophage suppresses the production of this reserve fuel.

Third, and perhaps most importantly, Metformin increases the sensitivity of muscle cells to insulin (Damico, 2002). Obesity has also been linked to increased androgen production and hirsutism in most PCOS patients, however, obesity probably represents a secondary additional pathogenetic condition, capably of amplifying primary factors leading to exaggerated ovarian androgen secretion, such as an increased luteinizing hormone (LH) stimulation (Pasquali & Casimirri, 1993; Soule, 1996.and Dunaif, 1997). However, studies have also shown weight loss through exercise and changes in diet and lifestyle alone to be as effective in regulating menstrual cycles and showing improvement in hyperandrogenism (Kolodziejezyk, 2000.and Knowler, *et al*., 2002).

So that Metformin promotes weight loss and favorable changes in the lipid profile. In fact: weight loss can significantly improve menstrual cycles and ovulation, thereby favoring pregnancy in otherwise infertile obese PCOS women. Since infertility represents a major complaint of adult PCOS women, weight loss should therefore be encouraged in all obese anovulatory PCOS women wishing to become pregnant, regardless of whether these women are willing to treat their obesity in the long term. This should ultimately increase patient compliance with short-term weight-reducing treatment (Mioni, *et al*., 2003) .

5. 9.FG score and Degree of Hirsutism :

Cyproterone acetate/ethinyloestradiol(Diane) has advantages in that more than 40% of women with facial hair find it clears within 9 months, and many get worthwhile reduction in hair growth elsewhere.

Figure(4-10) shows that the high degree of hirsutism are more corrected with OCP+ antiandrogens. And there is positive significant relationship between FG score and the level of testosterone in group A, with highly positive significant relation in group B. the mean value of FG score in group A and B pretreatment was 10.8±4.3 ,13.13±4.9 respectively while post treatment was 10.2 ±3.8,9.6±2.6 compared with control group 6.3±2.33.Also in figure (4-11) and (4-12) we see the relation ship between FG score and Testosterone, LH, FSH, respectively. We see that there is positive significant relationship between FG score and testosterone level in group A ,$p< 0.05$.And there is highly positive significant relationship between FG score and testosterone level in group B $p< 0.001$, with negative non significant relationship in control group. And there is negative significant relationship between FG and LH, $P<0.05$ with positive non significant relationship with FSH in group A, and there is positive significant relationship between FG score, LH. $P< 0.05$, with negative non significant relation ship with FSH in group B, and there is negative significant relationship between FG score and FSH with negative non significance to LH in control group.

Cyproterone acetate: is an antiandrogen, i.e. it suppresses the actions of testosterone (and its metabolite dihydrotestosterone) on tissues. It acts by blocking androgen receptors which prevents androgens from binding to them and suppresses luteinizing hormone (which in turn reduces testosterone levels .In addition, cyproterone acetate has weak progestational activity (e.g., it acts like progesterone) and can be used to

treat hot flashes. As part of some combined oral contraceptive pills (Dianette in UK and Diane-35 in other countries) it decreases acne and hirsutism (male-pattern hair growth) (Honer, *et al*., 2003). Hirsutism may be a sign of underlying androgen excess (hyperandrogenism) in the majority of sufferers (Knochenhauer , *et al*. ,1998). Number of studies indicates Glucophage reduces insulin, testosterone and glucose levels which reduces acne, hirsutism, abdominal obesity, amenorrhea and other symptoms (Nestler, *et al*., 1996). In addition to Morin-Papunen, *et al*., (2000.) and Pasquali, *et al*., (2000.) they found out that metformin decreases hirsutism.

5.10 .LH and FSH hormones:

There is a high LH level pre treatment in both patients groups with significant decrement after treatment, as shown in figure (4-15).The mean value of it pre treatment was 10.05±5.5, 10.25±5.3.respectively, while post treatment was 6.76±3.5, 6.85±4.3 and compared with control mean which equal to5.39±3.3. Regarding FSH, there was a normal level pre treatment compared with control with non significant decrement after treatment in both groups as shown in figure (4-18) and LH/FSH ratio figure (4-16) shows that there is significant increment in this ratio pre treatment compared with control with highly significant increment after treatment in group A $p<0.001$, and significant increment in group B $P<0.05$. This results were agrees with result of (Banaszewska ,*et al*.,2003.) who states that if you have PCOS your pituitary gland secretes higher levels of LH throughout the month instead of just before ovulation and ovulation may fail. Therefore LH/FSH ratios are increased to > 1 in women with PCOS.The ratio of LH to FSH is greater than 1:1, as tested on Day 3 of the menstrual cycle. The pattern is not very specific and was

present in less than 50% in one study. Still, finding a higher LH than FSH in the early part of the menstrual cycle is a hallmark of PCOS. It would seem that confirmation of an elevation in LH is very diagnostic of PCOS. The measurement of FSH will also permit the diagnosis of an occult ovarian failure where the FSH levels are particularly elevated. This is a diagnosis, which is been put in therapy planning.

5 .11.Testosterone:

In the present study there is an increment in total testosterone level in some patients figure(4-19) ,and it remains normal in others so, the mean value of total testosterone pre treatment in group A and B was equal to (1.25±0.75, 1.38±0.72) while post treatment it equal to (1.05±0.6, 1.05±0.56) respectively, compared with control group (0.67±0.26), this result may be due to the hypothesis that says free testosterone is more specific than total testosterone as the results of(Harborne ,*et al*.,2003)who report that in five of seven randomized studies ,in which insulin metabolism, SHBG, and androgens improved. This discrepancy can be explained by the fact that severe hyperandrogenism was not considered as a necessary inclusion criterion in our study. There are two studies to mention (Attia, *et al*., 2001and Mansfied, *et al*., 2003) that show a direct effect of metformin in reducing androgen production in theca cells.

Free testosterone provides a better diagnostic yield for ovarian hyperandrogenism because levels of SHBG are decreased thus increasing free hormone levels (Hull, 1987). There is a new evidence suggests that metformin may also have a direct activity on androgen production by the ovary. Metformin use, in some women with PCOS has shown weight loss, improved lipid profiles, lowering of blood pressure, lowered androgen levels, increased sensitivity to clomiphene, restoration or improvement in menstruation, pregnancy and improved pregnancy

outcome.The OCP(Diane) was superior to metformin in reducing serum total testosterone levels when compared with metformin alone, Morin-Papunen *et al*., (2000, 2003). Normal testosterone determinations in the hirsute patient can be misleading, partially because of inherent variation in commercial testing methods. However, bioavailable (free) testosterone levels may support the diagnosis, especially in a non hirsute woman with other signs and symptoms of PCOS (Boots, *et al*., 1998).

While Diane significantly decreases serum androgen levels. The combination of OCP s with metformin thus appears to further decrease androgen levels, without any additional effect on insulin sensitivity .By contrast, it is important to outline that patients often complain of hirsutism and other hyperandrogenic features, oral contraceptives have demonstrated their efficacy beyond those on insulin sensitivity. Therefore, the use of OCP s with or without metformin should be carefully evaluated according to individual requirements that include the need for contraception and improvement of metabolic derangements. (Morin-Papunen, *et al*., 2000). OCP s are the most efficient means of androgen suppression (ovarian as well as adrenal), and nearly any combination OCP s is effective in treating PCOS. This therapy results in lower and static LH levels without surges. The estrogen component stimulates hepatic production of sex hormone-binding globulin that reduces bioavailable androgen and can reduce hirsutism and acne. The progestin component provides competitive antagonism to androgen at its receptors, reducing the action of testosterone at the target organ (Richardson, 2003).

Treatment with antiandrogens is also effective in PCOS. Notably, antiandrogens markedly reduce androgens and improve gonadotropin secretion and hirsutism. (Diamanti-Kandarakis, *et al*. ,1995; De Leo ,*et al*.,1998; Eagleson ,*et al*.,2000 and Ibánez ,*et al*.,2000.).

5 .12 .Prolactin :

The mean level of prolactin hormone in group A pre treatment is 13±8.3, and it is 11.15±5.5 in group B, while in control it is 9.52±4.8 while post treatment it was 12.26±5.7, 14.14±8.8 respectively as in figure (4-21). Although it is slightly higher than control but it still within normal range. This result may goes parallel with study of (Angie, 2000.) who reveals that Some women with PCOS also have hyperprolactinemia. All women with reproductive difficulties should have a hormone screening done. Prolactin levels should be a part of that testing. More over; hyperprolactinaemia is a relatively frequent condition which affects almost half the patients suffering from poly cystic ovary. (Usually higher than 200 ng/mL)(Angelique ,*et al*., 2006.), while (Angela Hywood & Kerry Bone , 2006.) found out that about 25% of PCOS patients exhibit elevated prolactin .Other studies differ from those results and suggest normal level of prolactin .These results may be like that because many of our women had been complained of hyperprolactinaemia previously and was on regular course of treatment(Bromocriptine,Dostinex).Also there is positive relation ship between prolactin level and testosterone ,figure (4-22)this may be contributed to the theory which says that hyperprolactinemia may associated with PCOS. After treatment with cyproterone acetate +ethinyloestradiol the prolactin level may be increased and this agrees with most studies as (Ismail, *et al*., 1998.) who reports that there is a positive relationship between serum prolactin level and the duration of oral contraceptive pills intake and their steroid content, and this relationship is not related to the age and parity of the women. And with (Zacur, 1989.) who states that Prolactin levels have been reported to rise during use of oral contraceptives.

5.13. Liver enzymes (ALTand AST):

The liver enzymes in this study show high level pre treatment in comparism with control group, with significant decrement after treatment in both groups, table (4-4). And this result was agrees with most reports of other researchers like (Moghetti ,*et al*. ,2000.) who states that Renal and hepatic function should be checked before starting metformin because one of the major side effect of metformin was lactic acidosis. Other studies state that increased circulating concentrations of ALT are a specific marker of liver pathology and ALT has been established as a marker of liver fat accumulation in obese women (Tiikkainen ,*et al*. ,2003) .Small studies have reveals a decrease in ALT concentrations, after metformin treatment although improved liver histology has been a less consistent finding (Uygun ,*et al*. ,2004). This study thus expand an evidence to support a metformin-induced reduction in liver enzymes(Bugianesi ,et al .,2005).Other studies suggest that the most serious potential side effect of antiandrogens is liver toxicity, and patients should be monitored for changes in liver enzymes, especially if taking a high dose (200-300 mg/day). Toxicity is dose-dependent and the low doses used in oral contraceptive pills (2 mg) do not appear to represent a significant risk. Abnormalities of liver enzymes and the induction of liver tumors have been reported in animals treated with cyproterone acetate, but little is known about potential liver complications in humans taking this drug (Blake, *et al*., 1990).

5.14. Total Serum Protein, Serum Globulin, and Serum Albumin (TSP, S.G, S.ALB.) :

There were no significant changes in total serum protein after treatment and remains within normal range compared with control, with decrement in total globulin, and albumin as shown in table (4-5). These

previous three parameters done as one of assessment and follow up tests for those with autoimmune disease like (PCOS) .Most of the results were about SHBG as Singh ,*et al*. ,(1990.) who referred that Hyperinsulinaemia suppresses production of SHBG by the liver leading to higher concentrations of free testosterone, but total testosterone is also positively correlated with BMI, probably reflecting the effect of insulin on theca cell androgen production (Conway ,*et al*. ,1989.and Conway and Jacobs,1993). There are often low levels of sex hormone binding globulin, particularly among obese women Banaszewska ,*et al*.,(2003). The contribution of Testosterone to albumin is relatively small, and it undoubtedly does not undergo large changes with weight loss(Rosner,1997). Other studies suggest a decrement in albumin level after treatment with metformin was because of its side effects in loss of appetite and nausea and vomiting so decreases dietary protein.

5.15 .Blood groups:

The distribution of blood groups among PCOS patients and compared with control group shows that the blood group O+ ,(figure 4-26) was the most common one ,followed by blood group A+,B+,AB+. respectively and this result is in agreement with most researchers of PCOS ,such as (Elise, 2003.) who says that ,I believe that a large number of PCOS sufferers are of the O+/- group especially those with hirsutism and weight problems. Usually they have an imbalance in hormone and insulin levels. I believe that their life long diets are the culprits. 40% of Americans have an O blood type. Since the original hunter-and-gatherers had this blood type. Also there may be a genetic predisposition to this result with an association to ABO antigen in those infertile PCOS women.

5.16. PCV:

There is normal PCV level pretreatment in patients compared with control with a mean level equal to (0.4±0.03, 0.41±0.03, 0.39±0.03) respectively, while there was significant decrement in PCV level after treatment in both groups A, and B)with a mean of (0.38±0.027 , 0.38± 0.02) respectively , figure (4-27) this may consistent with the result of (Callaghan, et al.,1980.) who finds out that one of the side effects of metformin was Anaemia .By preventing optimal absorption of vitamins B12 and folic acid, metformin could induce or contribute to megaloblastic anemia. Megaloblastic anemia occurs when the bone marrow doesn't have enough B vitamins to manufacture red blood cells. The bone marrow then releases immature and dysfunctional red blood cells into circulation.

5.17 .WBC s, Differential WBC s count:

In affected patients with PCOS, WBC s count reveals non significant increment before treatment from control group with significant decrement after treatment $p<0.05$ as shown in figure (4-28) that reveals the mean value of WBCs in both patient's groups was 7.7±1.4, 7.18±1.6 and post treatment was 6.36±1, 6.34±1 respectively compared with control group 6.7±1.1. This result agrees with (Francesco, *et al*., 2007.) who states that a six-month course of a treatment with metformin reduces WBC count in PCOS women. Regarding differential WBC s count there is normal level of neutrophile with increment in lymphocyte count as shown in table(4-6) that suggests low grade chronic inflammation and this may be in agreement with (Orio ,*et al*.,2005.) who found out that in the leukocyte differential count a significant increase in lymphocyte and monocyte was observed in women with PCOS compared with control group and might be expected considering that they may play a key role in

pathophysiological mechanism of atherosclerosis .And was not consistent with (Jardena, *et al*. ,2005.) who states that WBC, neutrophil count, were higher in patients with PCOS compared with controls despite similar total fat mass. This may be because of presence of other pathology or hidden diseases that may accompany PCOS at the same time, in infertile women.

5.18. Ultrasound (U.S.) finding:

The ovarian diagnosis is confirmed by ultrasound, with findings of bilaterally enlarged polycystic ovaries. The ovary is usually greater than 9cm³ with more than 8cm³ peripherally oriented cystic structures in a sonographic plane by an increased stromal mass (>25% of the ovarian volume). Classification of polycystic suggests there are eight or more follicles present, with the follicles less than 10 mm in diameter (Hunter and Sterrett,2000).In our study 77% of total PCOS patients had U.S findings, as shown in table (4-7): (73%of group A and 80%of group B) 59% of them had large ovary and the other shows normal ovarian size, 44% shows presence of tinny follicles with or with out large ovary, while the rest had other ovarian changes like absence of mature follicle, and dense stroma, and 33% of patients shows normal U.S findings .In general (Transvaginal Ultrasound) shows:

1. General features
    1. Multiple small follicles in various stages
    2. Thick ovarian capsule
    3. String of pearls appearance
2. Criteria: Multiple Ovarian Cysts (seen in 80% of cases)
    1. Ten or more cysts in a single plane
    2. Each cyst <10 mm in diameter
    3. Dense stroma (Zawadzki and Dunaif ,1992).

3. Interpretation
    1. Polycystic appearance is seen in up to 33% of women
    2. Polycystic appearance does not diagnose PCOS
    3. PCOS clinical features must be present for diagnosis

US evidence of polycystic ovaries occurs in 16% of asymptomatic women, and 66-82% have the classic ultrasound appearance expected of polycystic ovaries (Abdel-Gadir ,*et al* .,1992). These ultrasound findings appear to be present in more than 90 percent of women with PCOS; however, they are also present in up to 25 percent of "normal" women. Ultrasonography alone is not sufficient to diagnosis PCOS (Franks, 1992).

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