بسم الله الرحمن الرحيم

Sudan University of Science and Technology (SUST)

College of Graduate Studies

Testosterone to Estradiol Ratio among Female with Polycystic Ovarian Syndrome in Khartoum State

نسبة التستوستيرون الى الاستراديول بين الإناث المصابات بمتلازمة تكيس المبايض في ولاية الخرطوم

A dissertation submitted in partial fulfillment for the requirement of M.Sc. degree in medical laboratory science -Clinical Chemistry

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June 2017

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قال تعالي:

(شَهْرُ رَمَضَانَ الَّذِيَ أُنزِلَ فِيهِ الْقُرْآنُ هُدًى لِّلنَّاسِ وَبَيِّنَاتٍ مِّنَ الْهُدَى وَالْفُرْقَانِ فَمَن شَهِدَ مِنكُمُ الشَّهْرَ فَلْيَصُمْهُ وَمَن كَانَ مَرِيضًا أَوْ عَلَى سَفَرٍ فَعِدَّةٌ مِّنْ أَيَّامٍ أُخَرَ يُرِيدُ اللّهُ بِكُمُ الْيُسْرَ وَلاَ يُرِيدُ بِكُمُ الْعُسْرَ وَلِتُكْمِلُواْ الْعِدَّةَ وَلِتُكَبِّرُواْ اللّهَ عَلَى مَا هَدَاكُمْ وَلَعَلَّكُمْ تَشْكُرُونَ)

صدق الله العظيم

سورة البقرة (الآية رقم 185)

Dedication

To …the masses of the Sudanese people, who are holding the burden of suffering and the cause…
To… the martyrs who sacrificed their souls cheaply to build a democratic homeland for us...!
To all the mothers, the tea and the marginal works that are struggling for a decent living.
To … the Makers of life "Aleomati "pulse of Poetry, Music & Painting …

To My Father; who taught me Patriotic love & the meaning of Humanity…

To My Mother who taught me the meaning of life; faithful and honesty, who sacrificed, overloaded for my Education …

To my brothers, sisters and friends …
To… Alsir Abu Hassan center for fertility ... Especially colleagues in the laboratory…..

Sincere gratitude extending any person who assisted me in one way or another.

Acknowledgments

All and first I thank Allah for giving me the strength and compassion for achieving my goals.

Secondly, I would like to express my gratitude and appreciation to my supervisor **Dr**: **Abdelgadir Elmugadam** for guidance, support, and utmost care.

Nevertheless, my appreciation is extended to **Dr. Mohamed karar.**

Also special thanks to all members of Sudan university of science and technology (SUST) Collage of graduate studies specially to staff members of clinical chemistry, I am really do not find the words that express my thanks and gratitude to him .

Finally, I am grateful to thank all patients participate in this study.

Abstract

Polycystic ovary syndrome (PCOS) is a common endocrine disease in women, characterized by heterogeneous presentation of hyperandrogenism, ovulatory dysfunction, and polycystic ovarian morphology (PCOM).

This study was carried out to assess plasma levels of testosterone and estradiol among female with polycystic ovarian syndrome (PCOs). Forty clinically diagnosed polycystic ovarian syndrome (PCOs) in the period between February to May 2017, chosen randomly from Alsir Abu Hassan center for fertility in Khartoum State, and forty apparently healthy individuals as control group, to evaluate the of effect polycystic ovarian syndrome (PCOs) on plasma levels of testosterone to estradiol ratio.

Plasma testosterone and estradiol measured by using ELISA, and results were analyzed using statistical package for social science (SPSS), computer programmed version 21.

Anti-Müllerian hormone hormones (AMH) used for the diagnosis of PCOs and when it is levels in the patients compared to the control group. (Mean±SD: 10.6±5.92 versus 1.4±0.22) ng/ml respectively with *P.Value* 0.000.

The study showed that, the mean plasma levels of testosterone and T/E_2 ratio were significantly increase in PCOs female patients. For testosterone (1.4±1.1 versus 0.87±0.65ng/ml, *p-Value* =0.011). For T/E_2 ratio (0.019±0.015 versus 0.01±0.008 ng/pg, *p-Value* =0.022). Also the finding of this study showed that, estradiol levels were insignificant difference in PCOs compared with control group. (Mean±SD: 86±39versus 101±38.6 pg/ml) respectively with *P.Value 0.086.*

Person correlation showed that, there was insignificant negative correlation between age of PCOs female and the level of testosterone and T/E_2 ratio(r= -.057- P.*Value* = 0.617) and (r= -.148 P.*Value* = 0.190) respectively and insignificant positive correlation between age of PCOs female and the level of estradiol (r= 0.193 P.*Value* = 0.927). There were insignificant negative correlation between the levels of testosterone and estradiol ((r= -.073 P.*Value* = 0.522). Also There were insignificant positive correlation between AMH and testosterone (r= 0.216 P.*Value* = 0.055), and insignificant negative correlation between AMH and estradiol (r= -0.010- P.*Value* = 0.927).

It is concluded that: the plasma levels of testosterone and T/E_2 ratio are higher in PCOs female patients.

مستخلص الدراسة

متلازمة تكيس المبايض (PCOs) هو المرض الاكثر شيوعا في النساء يصيب الغدد الصماء ، وتتميز باظهار اعراض غير متجانسة من فرط الأندروجين،اختلال التبويض واشكال تكيس فى المبيض (PCOs).

وقد أجريت هذه الدراسة لقياس مستويات البلازما من هرمون التستوستيرون واستراديول بين النساء المصابات بمتلازمة المبيض عديد التكيس (PCOs).

تم جمع 40 عينة مشخصة بمتلازمة تكيس المبايض خلال الفترة من فبراير إلى مايو 2017م، وتم اختيارهن عشوائيا من مركز السر أبو حسن للخصوبة في ولاية الخرطوم، وأربعين من الأفراد الأصحاء كمجموعة تحكم، لتقييم وتقدير (PCOs) على مستويات البلازما من هرمون التستوستيرون إلى نسبة استراديول.

تم تحليل هرمون تستوستيرون البلازما واستراديول قياس باستخدام إليزا والنتائج باستخدام حزمة إحصائية للعلوم الاجتماعية (SPSS)، الكمبيوتر المبرمج النسخة 21.

(AMH) استخدمت لتشخيص (PCOs) وعندما قارنت مستوياته في المرضى مع مجموعة التحكم (10.6 ± 5.92 مقابل 1.4 ± 0.22) نج / مل وكان الاحتمال الاحصائى للمقارنة 0.000

وأظهرت الدراسة أن مستويات البلازما من هرمون التستوستيرون و نسبة هرمون التستوستيرون إلى استراديول زيادة كبيرة في المرضى الذين يعانون من متلازمة تكيس المبايض. بالنسبة لهرمون التستوستيرون (1.4 ± 1.1 مقابل 0.87 ± 0.65نج / مل وكان الاحتمال الاحصائى للمقارنة 0.011).بالنسبة لهرمون التستوستيرون إلى استراديول (019.± 0.015مقابل 0.01 ± 0.008 نج/ بج وكان الاحتمال الاحصائى للمقارنة 0.022).

كما اظهرت نتائج الدراسة ان مستويات استراديول كانت ضعيفة في متلازمة تكيس المبايض مقارنة مع مجموعة التحكم. (86 ± 39 مقابل 101 ± 38.6 بج / مل وكان الاحتمال الاحصائى للمقارنة 0.086).

أظهرت علاقة الارتباط وجود علاقة سالبة غير معنوية بين اعمار الإناث المتضرر من متلازمة تكيس المبايض ومستوى هرمون تستوستيرون ونسبة التستوستيرون الى الاستراديول (معامل بيرسون للارتباط=- 0.057 , مستوى المعنوية=0.617) , (معامل بيرسون للارتباط=- 0.148 , مستوى المعنوية=0.190) على التوالي. ايضا هنالك ارتباطا إيجابيا غير معنوي بين اعمار النساء اللواتي يعانين من متلازمة تكيس المبايض ومستوى استراديول (معامل بيرسون للارتباط= 0.193 , مستوى المعنوية=0.927). كان هنالك ايضا ارتباط سلبي غير معنوي بين مستويات التستوستيرون واستراديول (معامل بيرسون للارتباط=- 0.073 , مستوى المعنوية=0.522) كذلك كان هناك ارتباط إيجابي غير معنوي بين(AMH) وهرمون التستوستيرون (معامل

بيرسون للارتباط=0.216 , مستوى المعنوية=0.055) ، وغير معنوية الترابط بين (AMH) واستراديول معامل بيرسون للارتباط=- 0.010 , مستوى المعنوية=0.927) .

وخلصت الدراسة إلى أن مستويات البلازما من هرمون التستوستيرون قد زادت بشكل ملحوظ في المرضى الذين يعانون من متلازمة تكيس المبايض.

List of contents

No.	Topic	Page
	Verse from Holly Quran	I
	Dedication	II
	Acknowledgements	III
	English abstract	IV
	Arabic abstract	VI
	List of contents	VIII
	List of tables	X
	List of figures	X I
	List of abbreviations	X II
	Chapter one	
	Introduction	
1.1	Introduction	1
1.2	Rational	2
1.3	Objectives	3
	General objective	3
	Specific objectives	3
	Chapter two	
	Literature review	
2.1	Polycystic ovary syndrome	4
2.1.1	Androgens excess	6
2.1.1.1	Hirsutism	6
2.1.1.2	Acne	8
2.1.1.3	Androgenic alopecia	9
2.1.1.4	Amenorrhoea	10
2.1.1.5	Infertility	12
2.2	Estradiol	14
2.3	Testosterone	17
2.4	Genetic of Polycystic Ovarian Syndrome	19
2.5	Obesity and Complication in Polycystic Ovarian Syndrome	22
2.6	Diagnostic criteria	25
	Chapter three	
	Materials and methods	
3.1	Study approach	28
3.2	Study design and Study area	28

3.3	Study population and Sample size	28
3.4	Inclusion criteria	28
3.5	Exclusion criteria	28
3.6	Ethical consideration	28
3.7	Data collection	28
3.8	Sample collection and processing	28
3.9	Estimation of Testosterone and Estradiol by ELISA	29
3.10	Principle of method	29
3.11	Procedure of testosterone and estradiol measurement. (Appendix II)	29
3.12	Quality control	29
3.13	Statistical analysis	29
	Chapter four	
	Results	
4.	Results	30
	Chapter five	
5.1	Discussion	39
5.2	Conclusion	40
5.3	Recommendations	40
	Reference	
	Reference	41
	Appendices	
	Appendix I	44
	Appendix II	45

List of table:

No.	Title	Page
Table (4-1)	Mean concentration of testosterone and estradiol and ratio between them in polycystic ovarian syndrome patients and control group.	32

Lists of figures

No.	Title	page
Figure (4-1)	Correlation between testosterone concentration and estradiol	33
Figure (4-2)	correlation between age and testosterone	34
Figure (4-3)	correlation between age and estradiol	35
Figure (4-4)	correlation between age and T/E_2ratio	36
Figure (4-5)	correlation between AMH and estradiol	37
Figure (4-6)	correlation between AMH and Testosterone	38

List of abbreviations

ACTH	Adrenocorticotropic hormone
AE	Androgen Excess
AES	Androgen Excess Society
ASRM	American Society for Reproductive Medicine
ASNs	Androgen-secreting neoplasms
AMH	Anti-Müllerian hormone hormones
BMI	Body mass index
CAH	Classic congenital adrenal hyperplasia
CYP	Cytochrome P
DHEA	Dehydroepiandrosterone
DHEAS	Dehydroepiandrostenedione sulfate
DHT	Dihydrotestosterone
ESHRE	European Society for Human Reproduction and Embryology
FSH	Follicle-stimulating hormone
GnRH	Gonadotropin-releasing hormone
HDL	High density lipoprotein
IH	Iatrogenic hyperandrogenism
LH	Luteinizing hormone
mRNA	Massager Ribonucleic acid
NADPH	Nicotinamide adenine dinucleotide phosphate
NCAH	Non-classic congenital adrenal hyperplasia
NICHD	National Institute of Child Health and Human Development
NIH	National Institutes of Health
PCOM	Polycystic ovarian morphology
PCOS	Polycystic ovary syndrome
P450	Protein 450
SHBG	Sex hormone binding globulin
SNP	Single nucleotide polymorphism
STRAW	Stages of Reproductive Aging Workshop
TBG	Thyroxine-binding globulin
TSH	Thyroid-stimulating hormone
T/C	Thymine /Cytosine
T/E_2	Testosterone to estradiol ratio
UK	United Kingdom
UTR	Untranslated region
Δ4A	Δ4-Androstendione

Chapter one

Introduction

Rationale

Objectives

1.1-Introduction

Polycystic ovary syndrome (PCOS) is a common endocrine disease in women, characterized by heterogeneous presentation of hyperandrogenism, ovulatory dysfunction, and polycystic ovarian morphology (PCOM). Despite PCOS being considered the most common female endocrinopathy during the reproductive life, the prevalence varies depending on the diagnostic criteria used and on the multiple faces with which this complex syndrome occurs (Palomba *et al*., 2015).

The polycystic ovary (or ovarian) syndrome (PCOS) was initially described by Stein and Leventhal in 1935. Despite the difficulty in ascertaining the prevalence of this disorder among women there are convincing data today to suggest that it affects between 6% and 8% of women worldwide, using the National Institutes of Health (NIH) 1990 criteria, such that it can be considered one of the most common disorders of humans, and the single most common endocrine abnormality of women of reproductive age (Azziz *et al*., 2009).

Features of hyperandrogenism associated with PCOS are hirsutism, acne, and female androgenetic alopecia. Enlargement of the clitoris (clitoromegaly), male pattern balding, and development of a muscular body habitus are evidence of severe androgen excess and are described as virilism. The 2003 consensus definition produced under the auspices of the American Society for Reproductive Medicine (ASRM) and the European Society for Human Reproduction and Embryology (ESHRE), although a topic for continuing debate, is gaining cautious acceptance. According to the consensus definition, PCOS is indicated when two of three of the following criteria are present, and when other causes of the clinical abnormalities are excluded: the presence of hyperandrogenism and/or hyperandrogenemia**,** irregular or absent ovulation and the presence of polycystic ovaries on ultrasound scanning (Nessar, 2011).

Adolescent girls with PCOS, similar to adult women with the condition, are at increased risk for the development of type 2 diabetes mellitus and the metabolic syndrome (glucose intolerance, dyslipidemia, hypertension and central obesity) as compared to the general adolescent population (O'Brien and Emans, 2008).

Women with high serum androgen concentrations tend to have increases in both serum total and free testosterone concentrations. Testosterone-bound sex hormone binding globulin (SHBG) is considered a biologically inactive measurement, so the measurement of free testosterone is the most sensitive method of assessing hyperandrogenemia (Khalifa *et al.*, 2007).

1.2-Rationale

Polycystic ovary syndrome (PCOS) is the most common endocrine disease in women worldwide in reproductive age. Hyperandrogenism is one of the diagnostic criteria for the polycystic ovary syndrome (PCOS) despite no agreed definition of hyperandrogenism.

Almost all scientific papers or researches agree there is excess androgen in PCOS patients but not determining the testosterone and estradiol level exactly and the ratio between them.

Ability to measure either total or free testosterone level accurately is essential for establishing the diagnosis of true androgen excess but the free testosterone level represents the most sensitive biochemical marker supporting the diagnosis of PCOS.

The measurement of testosterone to estradiol ratio may be used as biochemical marker for strength and supporting the diagnosis of polycystic ovary syndrome (PCOS).

There is no research conducted or scientific papers published in the Sudan in this subject exactly and very little at the level of world.

1.3-Objectives

General objective

To evaluate the plasma testosterone and estradiol levels among female with polycystic ovarian syndrome.

Specific objectives

1. To estimate the plasma level of testosterone and estradiol among female with polycystic ovarian syndrome compared to apparently healthy female.
2. To compare plasma level of testosterone /estradiol ratio in female patients with polycystic ovarian syndrome.
3. To correlate between plasma level of testosterone and estradiol in female patients with polycystic ovarian syndrome.
4. To correlate between age and testosterone /estradiol ratio in female patients with polycystic ovarian syndrome.
5. To correlate between AMH and testosterone and estradiol in female patients with polycystic ovarian syndrome.

Chapter two

Literature review

2. Literature review

2.1-Polycystic ovary syndrome

An international consensus definition of pcos has defined patients with pcos at least 2 of the following criteria: Oligomenorrhoea or amenorrhoea. Clinical and/or biochemical signs of excessive androgen secretion. Presence of at least 12 follicles measuring 2-9mm in diameter, an ovarian volume >10ml, or both. Only one ovary needs to meet this criterion. Although ultrasound scan is therefore not essential to make the diagnosis. Pcos is very common having prevalence in women of child bearing age 5-10% and may be higher in women of South Asian origin. There is no single diagnostic criterion to confirm the clinical diagnosis. clinical manifestation include infrequent or absent menses, an ovulatory infertility, signs of androgen excess (hirsutism ,acne or amenorrhea) although the classical profile of pcos is that of hyper secretion of LH and androgens with normal concentrations of FSH, a wide spectrum of findings are seen and abnormalities in LH are not always present. In addition to establishing the diagnosis, it is also important to exclude disorders with similar presenting features such as CAH, Cushing's syndrome and androgen-secreting tumours. many women with pcos have and increased risk of insulin resistance which, with the prevalence of obesity, is a powerful risk factor for progression to type 2 diabetes .they also have and increased long-term risk of endometrial hyperplasia /cancer (Simon *et al.*, 2013).

Polycystic ovary syndrome (PCOS) is a common hormone disorder that affects approximately 5 million women of reproductive age in the United States. Women with PCOS have difficulty becoming pregnant (i.e., are infertile) and may have high levels of androgen hormones from the ovary and adrenal gland. In addition to fertility impairment, a woman with PCOS may have some of the following symptoms and findings:- Irregular or no menstrual periods in women of reproductive

age (ovulatory dysfunction), Acne, Weight gain, Excess hair growth on the face and body, Thinning scalp hair, Ovarian cysts (polycystic ovarian morphology) and Mental health problems. Women with PCOS are often resistant to the biological effects of insulin and, as a consequence, may have high insulin levels. Women with PCOS are at risk for type 2 diabetes, high cholesterol, and high blood pressure. Obesity also appears to worsen the condition. The degree of obesity may vary by ethnicity. In 1990, the National Institutes of Health (NIH) held a conference on PCOS to create both a working definition of the disorder and diagnostic criteria. The outcome of this conference, the NIH Criteria, served as a standard for researchers and clinicians for more than a decade. In 2003, a consensus workshop in Rotterdam in the Netherlands developed new diagnostic criteria, the Rotterdam Criteria. The Androgen Excess (AE) and PCOS Society proposed the AE-PCOS Criteria in 2006 (David *et al.,* 2012).

Hyperandrogenism is very frequent in adolescent girls and is a source of concern for the girl herself, her family, and the clinician. Androgen excess during puberty produces a variety of clinical signs and symptoms that must be appropriately recognized, evaluated, and treated. Unfortunately for the pediatric endocrinologist, the criteria are so broad that many adolescents are presenting with transitory functional hyperandrogenism and menstrual disorders during puberty risk being misdiagnosed. The well known long-term sequelae of PCOS now present a challenge for pediatric endocrinologists to make an early diagnosis (in the pubertal period) and to treat these teenagers both symptomatically and prophylactically. The striking trend toward adolescent obesity should reinforce our responsibilities. We therefore propose to screen for PCOS all adolescents presenting oligo-amenorrhea within two years after menarche, particularly if hyperandrogenism is associated with low birth weight, family history of PCOS, abdominal obesity, and/or insulin resistance (Charles *et al.,* 2006).

This is a condition showing features of hyperandrogenism with anovulation and abnormal ovarian morphology and is the most common cause of anovulatory infertility. Presenting clinical symptoms may also include hirsutism, menstrual disturbances, enlarged polycystic ovaries and infertility. Plasma testosterone and androstenedione concentrations are often increased. The plasma LH may be elevated with normal FSH. Because plasma SHBG concentrations are reduced in obese individuals, the plasma concentration of free testosterone is often increased. The plasma prolactin concentrations may also be high. Multiple small sub capsular ovarian cysts may be demonstrated on ultrasound scanning of the ovaries. Polycystic ovary syndrome is also associated with insulin resistance, obesity and elevated plasma insulin concentrations, which may stimulate androgen production from the ovarian theca interna cells. Individuals may also have hyperlipidaemia, glucose intolerance and hypertension (Martin, 2012).

2.1.1-Androgens excess

A patient with androgen excess has variable degrees of excess hair on the face, chest, abdomen and thighs, acne, and obesity. Pcos is clinically defined by hyperadrogenism with chronic an ovulation without underlying disease of the adrenal or pituitary glands. This syndrome characterized by infertility, Hirsutism and obesity (in approximately half of those affected), and various menstrual disturbances from amenorrhea to irregular vaginal bleeding. Relatively low FSH concentrations and disproportionately high LH concentrations are common in pcos. Serum androstenedione and testosterone (total and free concentrations) are elevated with mean concentration 50%to150% higher than normal. PCOS patient have substantial estrogen production because of the peripheral conversion of androgens to estrogen. The anovulation is caused by continuous estrogen stimulation of the endometrium **(**Carl *et al*., 2006).

2.1.1.1-Hirsutism

Hirsutism is defined as the excessive growth of terminal hair in women and child in distribution similar to that occurring in post pubertal men **(**Carl *et al.,* 2006).

Clinical features of hyperandrogenism frequently seen in PCOS include hirsutism, acne, and androgenic alopecia. Here, we review the prevalence of these features in this disorder Hirsutism is the presence of terminal hairs on the face and/or body in a female in a male-type pattern. The most common method of determining the presence of hirsutism uses a visual score. Various methods have been proposed. The most commonly used method is a modification of a method originally reported by Ferriman and Gallwey. Nine body areas, including the upper lip, chin, chest, upper back, lower back, upper and lower abdomen, upper arm, and thigh, are assigned a score of 0–4 based on the density of terminal hairs. A score of 0 represented the absence of terminal hairs, a score of 1 minimally evident terminal hair growth, and a score of 4 extensive terminal hair growths. The cutoff value should be established after the study of a large population of unselected women. Using this approach, cutoff values for defining hirsutism have been variously reported to be a score of 6 or greater, 7 or more, and 8 or more. However, we should note that the prevalence of hirsutism in PCOS will vary according to the race and ethnicity of the population being studied. These data suggest that the degree of body and terminal hair growth and the prevalence of hirsutism are not significantly different between unselected White and Black women. Consequently, it is likely that there will be little difference in the prevalence of hirsutism between Black and White PCOS women, although this remains to be confirmed. Consistent with the lower population prevalence of hirsutism observed in East Asian women, a comparative study of patients with PCOS from the United States (primarily Mexican Americans), Italy, and Japan noted that Japanese women had a significantly lower mean hirsutism score than their non-Asian counterparts. However, the lesser prevalence of hirsutism among East Asian

PCOS patients may not extend to all groups in the region. For example, Wijeyaratne and colleagues observed that hirsutism was more prevalent and more severe among PCOS patients of Southern Asian extraction (Pakistani, Bengali, Gujarati, or Dravidian Indian) than Whites. Likewise, among women of Indian descent in New Zealand, about two thirds of women with PCOS presented with clinical evidence of hirsutism, similar to the prevalence found in women of European, Maori, and Pacific Island descent. Although it is clear that there is racial variation in hair growth patterns, race-specific normative ranges have not been well established, which is required to determine whether a particular woman has excessive amounts of body of facial hair. Overall, hirsutism is an important feature of PCOS, affecting approximately 65% to 75% of patients with PCOS, including women of White, Black, and Southeast Asian race. The prevalence of hirsutism in PCOS is likely to be less among women of East Asian extraction (Azziz *et al*., 2009).

2.1.1.2-Acne

Acne affects approximately 12% to 14% of White PCOS patients although the prevalence of this dermatologic abnormality varies with ethnicity: it is reportedly higher in Asian Indians and lower in Pacific Islanders. In a study of 248 women with PCOS in Italy, acne alone in the absence of other pilosebaceous features was present in 23.4%. Among 716 patients with PCOS, 14.5% presented with acne, either alone or in combination with hirsutism. In a prospective study of women presenting for blood donation, Asuncion and colleagues noted that of the 10 women diagnosed with PCOS, four (40%) had acne, three without associated hirsutism. However, various surveys have noted a relatively high prevalence of acne in the general population, particularly among younger women. Approximately 20% of individuals in their midteens and 15% of those in their early 20s complain of acne; even 10% of women in their 30s and 5% of women 40 to 60 years old will complaint of, albeit mild, acne.

Consequently, the degree to which PCOS increases the risk of acne above the general population prevalence is unclear. The variability in the prevalence of acne is compounded by the fact that there is no single scoring system used. Overall, although acne affects 15% to 25% of PCOS patients, it is unclear whether the prevalence of acne is significantly increased in these patients over that observed in the general population (Azziz *et al.,* 2009).

2.1.1.3-Androgenic alopecia

Scalp hair loss in women is a distressing complaint with significant psychologic morbidity. It usually represents the pilosebaceous unit response to endogenous androgens and may be associated with acne and hirsutism. Androgen sensitivity of the pilosebaceous unit varies, and there is poor correlation between clinical features and evidence of biochemical hyperandrogenism. The presence of DHT, formed from the 5a-reduction of Tin the dermal papilla, is associated with a higher 5a-reductase activity in the hairs plucked from a scalp presenting with androgenic alopecia. In addition to androgen excess, other potential etiologies of alopecia or diffuse scalp hair loss in any woman may be genetic (i.e., familial premature scalp follicular loss), environmental (e.g., damage following the use or abuse of hair cosmetics), and nutritional (e.g., poor protein intake, zinc deficiency, iron-deficient anemia). Androgenic alopecia is a recognized sign of PCOS. However, the prevalence of this abnormality in PCOS is unclear. Although we previously noted that PCOS patients may account for _10% to 40% of all women with alopecia, literature defining the incidence of alopecia in either normal women or women with PCOS is sparse. The pattern of hair loss in PCOS generally involves thinning of the crown with preservation of the anterior hairline. Androgenic related alopecia in women with PCOS tends to be seen in the anterior midvertex area extending to the crown. The anterior hairline remains intact in women with PCOS and significant a bitemporal

scalp hair recession is unusual except in virilizing syndromes. Unfortunately, a loss of at least 25% of scalp hair is needed before a woman becomes aware of thinning of her scalp hair. The sole presence of alopecia or diffuse scalp hair loss in women may be the sole dermatologic sign of PCOS (Azziz *et al.*, 2009).

2.1.1.4-Amenorrhoea

Amenorrhoea can be primary (menstruation has never occurred) or secondary. Oligomenorrhoea is sparse or infrequent menstruation; it can be due to less severe forms of some of the causes of amenorrhoea. Primary amenorrhoea can occur as part of the syndrome of female hypogonadism, but can also be present in normally feminized women. The commonest cause of amenorrhoea in women of child-bearing age is pregnancy, and this possibility, however unlikely, must always be excluded. The finding of an apparently high plasma LH concentration may suggest pregnancy before a pregnancy test is performed: chorionic gonadotrophins cross-reacts in some assays for LH. Pregnancy apart, amenorrhoea in normally feminized women is most frequently due to a hormonal disturbance that results in a failure of ovulation. Causes include: disordered hypothalamo-pituitary function, related to weight loss (30–35% of cases in most series) or hyperprolactinemia (10–12%), but idiopathic in some 10% of cases, ovarian dysfunction (e.g. autoimmune disease leading to premature menopause) (10–12%), increased androgen production (particularly polycystic ovary syndrome (PCOS) and late-onset congenital adrenal hyperplasia) (30–35%). Weight loss can lead to a decrease in the frequency of the pulsatility of GnRH secretion and thus decreased secretion of LH and FSH. Menstruation almost always ceases if weight falls below 75% of the ideal, and may do so with smaller losses. Regular menstruation returns if weight is regained. Severe stress and intensive exercise regimens, such as are adopted by elite long-distance runners, ballet dancers and gymnasts, can also lead to amenorrhoea, probably for complex

neuroendocrinological reasons in addition to any effect of decreased body weight. Amenorrhoea due to excessive androgen secretion is often associated with hirsutism or even virilism. Uterine dysfunction is an uncommon cause of amenorrhoea. It can be excluded, if necessary, by the progestogen challenge test. If medroxyprogesterone acetate is given orally (10 mg daily for 5 days), the occurrence of vaginal bleeding 5–7 days later signifies that the uterus was adequately oestrogenized. If bleeding does not occur, the test is repeated, giving oestrogen (ethinyloestradiol, 50 mg daily for 21 days, with progestogen on the last 5 days). Absence of bleeding indicates uterine disease. If bleeding occurs, oestrogen deficiency is present. The diagnosis of hormonal causes of amenorrhoea requires basal measurements of plasma FSH, LH and prolactin concentrations. A high FSH concentration is indicative of ovarian failure (and is more sensitive in this respect than LH). If LH, but not FSH, is elevated, and the patient is not pregnant, the most likely diagnosis is PCOS, and pelvic ultrasonography should be performed. If LH and FSH concentrations are normal or low, a pituitary or hypothalamic disorder should be sought, by anatomical studies and dynamic testing of the hypothalamo-pituitary axis in a manner similar to that described for male hypogonadism. As in males, however, the results of such tests do not always distinguish between pituitary and hypothalamic disorders. The management of amenorrhoea depends on the cause, and whether fertility is required. In hyperprolactinemia, the treatment is directed to the underlying cause wherever possible (e.g. withdrawal of drugs, treatment of hypothyroidism). In ovarian, pituitary or hypothalamic disease, when fertility is not required, cyclical oestrogen and (if the patient has a uterus) progestogen replacement is given. In established ovarian failure, pregnancy is only possible using donated ova. If fertility is required in pituitary failure, treatment is with human FSH and LH; HCG may be required to mimic the mid-cycle LH peak and stimulate ovulation. Careful monitoring of plasma oestradiol concentrations is necessary to detect hyper stimulation, which carries a

risk of multiple pregnancies and the production of ovarian cysts. Patients with hypothalamic disease may respond to clomiphene. This substance blocks oestradiol receptors in the hypothalamus and may stimulate GnRH (and thus LH and FSH) secretion. Nonresponders are treated with pulsatile GnRH. Clomiphene is also useful in inducing ovulation in patients with PCOS. When it has not been possible to distinguish between hypothalamic and pituitary disease, a failure to respond to pulsatile GnRH suggests that amenorrhoea is due to pituitary dysfunction (William *et al*., 2012).

Amenorrhea due to androgen excess can be due to adult onset CAH, corticotropin-dependent Cushing syndrome, or polycystic ovary syndrome (PCOS). Some individuals with 21-hydroxylase deficiency do not manifest any developmental abnormalities or salt wasting, but they present with signs of androgen excess. This clinical syndrome, referred to as nonclassic, adult-onset, or late-onset CAH, may be clinically indistinguishable from PCOS. Serum androstenedione and testosterone concentrations (total and free concentrations) are elevated, with mean concentrations 50 to 150% higher than normal. Abnormal bleeding patterns seen in PCOS are due to chronic anovulation and lack of progesterone stimulation and withdrawal. Chronic estrogen exposure without progesterone may predispose patients to endometrial cancer. Some attempt has been made to link PCOS to leptin, a hormone that is secreted by adipocytes and is thought to play a role in regulating food intake and metabolism. Animals that lack leptin are infertile; leptin injection increases gonadotropin secretion and restores fertility. For women with PCOS who wish to conceive, treatment is aimed at ovulation induction. Weight reduction should be attempted first in those women who are overweight, as it often helps to promote ovulation. If ovulation does not occur, then medications such as clomiphene citrate, metformin, and aromatase inhibitors may be useful. Ovarian hyperthecosis, a non-neoplastic lesion of the ovary characterized by the presence of islands of luteinized

thecal cells in the ovarian stroma, is sometimes confused with PCOS **(**Carl *et al.*, 2006).

2.1.1.5-Infertility

Infertility is a common clinical problem, leading approximately one in six couples in the UK to seek professional advice. Investigation is usually considered appropriate when a couple has been unable to conceive after 12 months of trying, assuming regular, unprotected intercourse. It can be primary (conception has never occurred) or secondary, and due to problems affecting either the male or the female. Ovulatory failure, due most frequently to hyperprolactinemia or hypothalamic–pituitary dysfunction, is responsible in approximately 20% of cases, and defective sperm production in about one-quarter. Endocrine causes of infertility are rare in males. A couple in their late 20s was infertile in spite of regular intercourse over a two-year period. Each partner had a child by a previous marriage. The woman's periods had recently become irregular. A semen sample contained a normal count of motile sperm. Physical examination revealed no abnormality. The woman was on steroid replacement treatment for adrenal failure, which had been diagnosed in her late teens (William *et al.*, 2012).

Infertility can result from ovulatory or uterine problems; mechanical problems, including obstruction of the fallopian tubes; male fertility factors; or multiple factors in either sex or combined female and male factors. Ovulatory problems are the most common cause of female infertility. Polycystic ovarian syndrome (PCOS) affects up to 5% of reproductive-age women. It is the most common cause of ovulatory infertility. PCOS is a condition characterized by multiple ovarian cysts, often found in a row, resembling a "string of pearls." Ovarian cysts are fluid-filled sacs arising from follicles swollen with fluid that are prevented from producing mature oocytes. Patients with PCOS also have hormonal imbalances, including decreased levels of

LH, FSH, and progesterone and increased androgen production, including excess testosterone and DHEAS causing hirsutism or male facial patterns of hair growth. Insulin resistance is a common associated condition. PCOS is generally diagnosed when two of the following three criteria are present and other possible causes can be ruled out: clinical or laboratory results showing excess androgen secretion, decreased or absence of ovulation, and ovaries found by imaging techniques such as ultrasound to contain many cysts. Although the exact etiology of the problem is still unknown, genetic factors may be involved. Around the time of menopause, impairment of ovulation may cause infertility with adverse effect on follicle size and oocytes quality despite regular ovulation and normal gonadotropin levels. These factors are considered when treating older women with infertility. Serum levels of LH, FSH, and inhibin A and B may be helpful in assessing infertility and treatment options. Infertility diagnostic testing is as varied as treatment options. The patient workup for infertility includes a careful, detailed history, which can help to limit the number of laboratory tests required. Availability of tests varies from center to center, so availability is one of the considerations for infertility testing. Typical laboratory tests ordered are FSH on day three of the ovulatory cycle, LH, estradiol, prolactin, and TSH levels. Measurement of ovarian and adrenal androgens such as testosterone and DHEAS should be decided on the basis of ovulatory status of the patient and the clinical picture. (Wendy, 2007).

Infertility is defined as the inability to conceive after 1 year of unprotected intercourse. It has been estimated that 93% of healthy couples practicing unprotected intercourse should expect to conceive within 1 year, and 100% will be successful within 2 years. a specific cause of infertility is identified in ≈80% of couples: one third are due to female factors alone, one third to male factors alone, and one third to a combination of problems. Primary infertility refers to couples or patients who have had no previous successful pregnancies. Secondary infertility encompasses patients

who have previously conceived, but are currently unable to conceive. These types of infertility generally share common causes. Infertility problems often arise as a result of hormonal dysfunction of the hypothalamic-pituitary-gonadal axis. Measurements of peptide and steroid hormones in the serum are therefore essential aspects of the evaluation of infertility. This section focuses on hormonal and biochemical aspects of evaluating infertility **(**Carl *et al.*, 2006).

2.2-Estradiol

The principal ovarian hormone is 17β-oestradiol, but some oestrone is also produced by the ovaries. Oestrogens are also secreted by the corpus luteum and the placenta. Oestrogens are responsible for the development of many female secondary sexual characteristics. They also stimulate the growth of ovarian follicles and the proliferation of uterine endometrium during the first part of the menstrual cycle. They have important effects on cervical mucus and vaginal epithelium, and on other functions associated with reproduction. Plasma concentrations of oestrogens are low before puberty. During puberty, oestrogen synthesis increases and cyclical changes in concentration occur thereafter until the menopause, unless pregnancy occurs. After the menopause, the sole source of oestrogens is from the metabolism of adrenal androgens; plasma concentrations fall to very low values. In the plasma, oestrogens are transported bound to protein, 60% to albumin and the remainder to SHBG. Only 2–3% remains unbound. Oestrogens stimulate the synthesis of SHBG and also that of other transport proteins, notably thyroxine-binding globulin (TBG) and transcortin, and thus increase total thyroxine and total cortisol concentrations in the plasma. Slowly rising or sustained high concentrations of oestrogens, together with progesterone, inhibit pituitary gonadotrophin secretion by negative feedback, but the rapid rise in oestrogen concentration that occurs prior to ovulation stimulates LH secretion (positive feedback). Oestradiol is present in low concentrations in the plasma of normal men. Approximately one-third is secreted by the testes, the

remainder being derived from the metabolism of testosterone in the liver and in adipose tissue. The stimulated corpus luteum secretes large amounts of oestrogens and progesterone, but after six weeks the placenta becomes the major source of these hormones. There is a massive increase in the production of oestriol during pregnancy, but production of oestrone and oestradiol increases also. Oestradiol is synthesized in the placenta from androgens secreted by the fetal adrenal glands. Its measurement in maternal plasma or urine was formerly used to assess feto-placental function, but now has been superseded by ultrasonography, which can be used to provide direct measurements of fetal growth and placental blood flow. The same applies to measurements of other placental products, for example human placental lactogen and placental alkaline phosphatase (a heat-stable isoenzyme), which have been used in the past as indicators of placental function (William *et al.*, 2012).

Estrogens are secreted by the ovarian follicles and by the placenta in pregnancy (and to a much lesser extent by the adrenal glands and testes). Estrogen promotes development and maintains the female reproductive system, including the uterus, fallopian tubes, and vagina. It is responsible for development and maintenance of secondary female sex characteristics. Estrogen peaks at midcycle, causing a decrease in FSH but promoting the LH surge at midcycle. There are three primary estrogens: estradiol-17, estrone, and estradiol. Estradiol is the principal estrogen synthesized by the ovaries. Hyperprogesteronemia: Prevents menstrual cycle from occurring and Causes infertility, abortion of fetus (Anna *et al.*, 2010).

Dynamic changes in circulating estradiol level, including increase at menarche and decrease at menopause, occur in a woman’s lifetime. Circulating estradiol levels decrease drastically during the menopausal transition, though the levels differ among races. It has been reported that estradiol levels in both Japanese and Chinese women were lower than those in Caucasians, Hispanic and African- Americans. This dynamic decrease in estradiol level induces menopausal symptoms, such as hot

flashes and night sweat, urogenital symptoms, osteoporosis, coronary heart disease, stroke and possibly early onset of Alzheimer's disease in postmenopausal women. However, not only estrogen but also other endocrinological hormones may be involved in the occurrence of these diseases. Little attention has been paid to roles of endogenous androgens in women despite the results of studies suggesting that androgens may play important roles. Androgens are known to be important for normal physiology in women and to play key roles in the physical, sexual and emotional well-being of women. Therefore, it is necessary to take account of androgens as well as estrogen when considering women's health (Yasui *et al*., 2012).

2.3-Testosterone

Testosterone promotes development and maintains the male reproductive system. It is responsible for development and maintenance of secondary male sex characteristics (e.g., facial and body hair, muscle development). In female children, development of male secondary sex characteristics/virilization occurs (increased androgen production by ovaries or adrenals as androgens are estrogen precursors in females) (Anna *et al*., 2010).

Testosterone is a powerful anabolic hormone. It is essential both to the development of secondary sexual characteristics in the male and for spermatogenesis. It is secreted by the Leydig cells of the testes under the influence of luteinizing hormone (LH). Spermatogenesis is also dependent on the function of the Sertoli cells of the testicular seminiferous tubules. These cells are follicle-stimulating hormone (FSH) dependent; they secrete inhibin, which inhibits FSH secretion, and androgen-binding protein, the function of which is probably to ensure an adequate local testosterone concentration. Testosterone concentrations in the plasma are very low before puberty, but then rise rapidly to reach normal adult values. A slight decline in concentration may be seen in the elderly. In the circulation, approximately 97% of

testosterone is protein bound, principally to sex hormone-binding globulin (SHBG) and to a lesser extent to albumin. The free fraction is readily available to tissues; albumin binds testosterone more loosely than SHBG, and albumin-bound testosterone may be in part available. Free testosterone is considered a better indicator of effective androgen availability than total testosterone, but its measurement is technically difficult, and the ratio of testosterone/SHBG concentration may not accurately reflect free testosterone status. The biological activity of testosterone is mainly due to dihydrotestosterone (DHT). This is formed from testosterone in target tissues in a reaction catalysed by the enzyme 5α-reductase. In a rare condition in which there is deficiency of this enzyme, DHT cannot be formed; male internal genitalia develop normally (Wolffian duct development in the fetus is testosterone dependent) but masculinization, which requires DHT, is incomplete. In states of androgen insensitivity, defects of the receptors for either testosterone or DHT, or both, can cause a spectrum of clinical abnormalities ranging from gynaecomastia to disorders of sex development. Testosterone is also present in females, at a much lower concentration, about one-third being derived from the ovaries and the remainder from the metabolism of adrenal androgens (William *et al.*, 2012).

In women of reproductive age, daily production of testosterone is shared equally between the ovaries and adrenal glands and accounts for approximately one-third of the testosterone in circulation. Peripheral conversion of androgen precursor steroids to testosterone in non-steroid producing tissues accounts for the remaining two-thirds of testosterone in circulation. These ratios change after menopause when the ovaries are in senescence. In women, there is controversy about the direction of circulating testosterone levels across the life span. It has been reported that total and free testosterone decreased with age between 15 and 60 years and that bioavailable testosterone decreased by approximately 28% between 25 and 85 years of age.

However, it has been shown that testosterone level did not vary during the menopausal transition from 45 to 55 years of age. Recent data indicate that total testosterone level increased from 43 to 50 years but not thereafter. In a previous study, we found that total testosterone level gradually decreased with age in women but that the change was not significant. However, levels of free and bioavailable testosterone showed significant decreases with age in women. Menopausal transition is characterized by variations in cycle length and elevation in follicle-stimulating hormone (FSH) level. Based on these characteristics, the American Society for Reproductive Medicine proposed the "Stages of Reproductive Aging Workshop (STRAW) staging system". Changes in free and bioavailable testosterone showed patterns similar to the pattern of changes in total testosterone. On the other hand, estradiol level was drastically decreased but showed a transient increase in the early menopausal transition, possibly due to an increase in FSH stimulation. The ratio of testosterone to estradiol (T/E), as an assessment of the balance of testosterone and estradiol gradually increased during the menopausal transition and increased significantly in postmenopausal stages. A relative testosterone excess was found in postmenopausal women. Torrens *et al.* reported that a relative androgen excess was found during the menopausal transition and both baseline total T/E ratio and its rate of change were associated with increased incident metabolic syndrome independent of ethnicity (Yasui *et al.*, 2012)

2.4-Genetic of Polycystic Ovarian Syndrome

The mode of inheritance of PCOS remains unknown, and recent studies indicate that this disorder could be a complex trait. This means that several genes are interacting with environmental factors to provoke the phenotype. In contrast, biochemical parameters, including fasting insulin levels or hyperandrogenemia, seem to be highly heritable parameters, suggesting that some clinical signs, symptoms, or biochemical

parameters of PCOS could be transmitted as Mendelian autosomal dominant or X-linked traits, but the genetic studies have not as yet concluded the pattern of heredity. While studies, so far, are unable to exclude an autosomal or X-linked dominant mode of inheritance, the heritability of PCOS is probably more complex, similar to that of type 2 diabetes mellitus or cardiovascular disease. However, a positive family history appears to be the most informative risk factor for the development of PCOS. Furthermore, environmental factors alter the clinical and biochemical presentation in those with genetic predisposition to PCOS. A relation between PCOS with the X chromosome aneuploidies and polyploidies in addition to other cytogenetic abnormalities has been confirmed. Some of the cases of PCOS may represent an intermediate condition in a spectrum that extends from the streak gonad of Turners syndrome to the normal ovary. The concept is that at least some cases of PCOS may be due to X chromosomal factors causing an abnormal follicular apparatus. In addition, large deletion of the long arm of chromosome 11 was seen in some of the PCOS cases. However, there is no large cytogenetic study to identify karyotype abnormalities. There are different candidate genes as a cause of PCOS; such as genes involved in steroid hormone synthesis and action, genes involved in carbohydrate metabolism and fuel homeostasis, genes involved in gonadotropin action and regulation; and genes in the major histocompatability region, which could account for certain PCOS features. Increased androgen secretion and insulin resistance persist in cultured theca cells and skin fibroblasts, respectively, from women with PCOS, which suggest that these are intrinsic, presumably genetic, defects. Different studies have indicated a genetic susceptibility to PCOS. It was shown that polycystic ovaries and hyperandrogenemia are present in 50% of sisters of affected women. Therefore genetic analyze of candidate genes have been performed. Both linkage and association studies have suggested that PCOS can be explained by the interaction of a small number of key genes with environmental,

particularly nutritional factors. Hyperandrogenemia is genetically determined and the result of familial studies indicating that hyperandrogenism clusters as a dominant genetic trait. The steroid synthesis gene CYP11a, coding for P450 cholesterol side chain cleavage and the insulin gene regulatory region may be involved. However, it is unlikely that the hyperandrogenemia of PCOS is principally determined by polymorphisms or mutations in the genes encoding a single steroidogenic enzyme activity, such as CYP17 or CYP11a. In addition, an increase of mRNA abundance in PCOS has been found in corresponding to the genes of aldehyde dehydrogenase-6 and retinol dehydrogenase-2, which both increases the expression of 17a-hydroxylase. Recent studies have found a significant prevalence of CYP21 mutation, gene encode the 21-hydroxylase enzyme mimic the PCOS phenotype, in the supposed PCOS population (Sheikhha *et al.*, 2007).

The first step in steroidogenesis is the conversion of cholesterol into progesterone, catalyzed by the P450 Cytochrome side chain cleavage enzyme encoded by CYP11a gene located at 15q. Investigation of CYP11A gene showed a significant association between serum testosterone levels and the alleles of the CYP11a with a 5' untranslated region (UTR) consisting of repeats of a (tttta) n pent nucleotide, a variable number tandem repeat (VNTR) polymorphis. Further investigation is required due to these controversial results in order to confirm a role in the a etiology of PCOS of this gene. Another part in steroidogenesis is the conversion of 17-hydroxyprogesterone into 11-deoxycortisol which is catalyzed by the 21-hydroxylase enzyme encoded by CYP21. The deficiency of this enzyme is responsible for most cases of congenital adrenal hyperplasia and increased serum 17-hydroxyprogesterone levels are correlated with its deficiency. It is a common finding among women with functional hyperandrogenism or PCOS an increased serum 17-hydroxyprogesterone response to ACTH stimulation. Furthermore, patients having both heterozygote CYP21 mutations and clinical symptoms exhibit a PCOS-like

phenotype. Accordingly, mutations of CYP21 have been investigated as a candidate gene in patients with PCOS. Two studies showed that children with premature pubarche and adolescent girls with hyperandrogenism were heterozygous for mutations in CYP21. On the other hand, there are other researchers that found no clear concordance between the CYP21 genotype and the functional origin of androgen excess. Overall, CYP21 and associated mutations do not seem to play a key role in the development of PCOS. The conversion of pregnenolone and progesterone into 17-hydroxypregnenolone and 17-hydroxyprogesterone, respectively and of these steroids into dehydroepiandrosterone (DHEA) and Δ4-Androstendione (Δ4A) is catalyzed by the P450c17α enzyme. This enzyme has both 17α-hydroxylase and 17, 20-lyase activities and is encoded by CYP17 located at 10q. It was reported increased P450c17α expression and enzymatic activity in ovarian theca cells from women with PCOS as well as increased transactivation of the CYP17 promoter. Moreover, it was showed that CYP17 expression is dysregulated at the level of mRNA stability in PCOS theca cells. Another study identified a rare T/C single nucleotide polymorphism (SNP) in the promoter region of CYP17 increasing the susceptibility to develop PCOS. Subsequently, more comprehensive studies have failed to detect a significant linkage between CYP17 and PCOS. Although CYP17 gene does not seem to be a candidate gene in the pathophysiology of PCOS, it should be noted that post-translational regulation of this gene product might play a role in the pathophysiology of PCOS. The enzyme complex aromatase converts androgens to estrogens. This enzyme complex is composed of the Cytochrome P450 aromatase and the NADPH Cytochrome P450 reductase, and P450arom is encoded by CYP19 located at 15p. Aromatase deficiency has been reported in a number of hyper androgenic patients. It has been demonstrated that granulosa cells obtained from medium-sized follicles of women with PCOS have little aromatase activity. Similarly, it has been showed that when

compared to the control follicles, all PCOS follicle contained low levels of P450arom mRNA, estradiol, and lower aromatase stimulating bioactivity. These findings indicate that the aromatase activity might be decreased in PCOS follicles, and that the possible androgen excess resulting might contribute to abnormal follicle development. Association studies utilizing SNPs and haplotypes showed association with PCOS symptoms and serum testosterone levels (Prapas *et al.,* 2009).

2.5-Obesity and Complication in Polycystic Ovarian Syndrome

Obesity is a feature of PCOS and about half of the patients presenting in secondary care have a body mass index of greater than 30. A further 25% are 'overweight', with a body mass index of greater than 25. Ideal body mass index is considered to be between 20 and 25. Body mass index (BMI) is calculated as the weight in kg divided by the height in meters squared (kg/m2). All features of PCOS are made worse by increased weight and are ameliorated by weight loss. Insulin resistance is considered to be present when the body requires more insulin than normal to regulate glucose homeostasis. In the general population, a relationship between obesity and insulin resistance has been recognized for many years. The more obese a patient is the more insulin resistant they are likely to be. Not all women with PCOS can be shown to be insulin resistant. However, insulin resistance, when present in PCOS is greater than can be accounted for by obesity alone. Insulin resistance proceeds, and is a strong predictor for, type 2 diabetes, which is more common in young women with PCOS. In PCOS the incidence of type 2 diabetes and hypertension also increases with age. Insulin resistance is also associated with specific atherogenic abnormalities of lipoprotein metabolism which are raised triacylglycerols and low HDL cholesterol. Altered endothelial function is also recognized. These abnormalities are important features of the metabolic syndrome, which predicts higher risk of later diabetes and cardiovascular disease in middle age. In spite of many studies showing increased

cardiovascular risk markers in PCOS, strong evidence for increased cardiovascular mortality, a hard end point, is lacking. Pregnancy outcomes in polycystic ovary syndrome Patients with PCOS have an increased risk of gestational diabetes. There is also an increased risk of pregnancy associated hypertension and pre-eclampsia. Babies tend to be delivered early and are more likely to be admitted to a neonatal intensive care unit. Perinatal mortality is higher. Whether these abnormalities are specifically associated with PCOS remains to be established as similar problems are found in obese women without PCOS. Insulin and androgen action Insulin, in addition to its essential role in regulating glucose homeostasis, is a hormone of many actions. Some of these are particularly relevant to the abnormalities found in PCOS. Insulin, together with LH, acts on the ovary to increase the ovarian secretion of androstenedione and testosterone. Insulin directly increases androstenedione secretion by ovarian theca cells acting via its own receptor. Measures which decrease insulin secretion by reducing insulin resistance, such as dietary manipulation, and treatment with insulin sensitizing agents such as metformin and thiazolidenedione drugs, which reduce circulating insulin, also reduce circulating androgens. Sex hormone binding globulin, which is lower in patients with PCOS, is secreted by the liver, a major target of insulin action. Insulin down-regulates the hepatic production of SHBG, independent of its actions on glucose regulation. Therefore SHBG is a marker of insulin action on the liver and low SHBG is a marker for insulin resistance. In the circulation, SHBG has a direct role in controlling the concentrations of circulating non-protein bound or free testosterone and DHT. These are regarded as the biologically active fraction. As testosterone and DHT are not involved in a feedback control with the hypothalamus and pituitary in women, rising SHBG levels reduce their biological availability and vice versa. Thus SHBG is an important controlling factor in the expression of androgen action, especially in women. Therefore insulin has two main effects on androgen action.

First, it increases ovarian androgen secretion and second, it enhances androgen bioavailability. Disordered follicular maturation in PCOS The principal cause of infertility due to PCOS is abnormal development of ovarian follicles with the accumulation of small follicles ‘the cysts (Nessar, 2011).

Women with PCOS present an adverse reproductive profile, including a high risk of pregnancy-induced hypertension, preeclampsia, and gestational diabetes mellitus. Patients with PCOS present not only a higher prevalence of classic cardiovascular risk factors, such as hypertension, dyslipidemia, and type-2 diabetes mellitus, but also of nonclassic cardiovascular risk factors, including mood disorders, such as depression and anxiety. Moreover, at the moment, clinical data on cardiovascular morbidity and mortality in women with PCOS are controversial. Women with PCOS show an increased risk of endometrial cancer compared to non-PCOS healthy women, particularly during premenopausal period. Currently, we are unable to clarify if the increased PCOS early- and long-term risks are totally due to PCOS or mostly due to obesity, in particular visceral obesity that characterized the majority of PCOS patients. In any case, the main endocrine and gynecological scientific societies agree to consider women with PCOS at increased risk of obstetric, cardio metabolic, oncology, and psychological complications throughout life, and it is recommended that these women be accurately assessed with periodic follow-up(Palomba *et al.*, 2015).

2.6-Diagnositic Criteria of Polycystic Ovarian Syndrome

In 1990, the first formal attempt to consolidate a clinical definition of PCOS by the National Institute of Child Health and Human Development resulted in PCOS being defined as the combined presence of Clinical and/or biochemical signs of hyperandrogenism and Oligo- or chronic anovulation in the absence of all other reasons for anovulatory infertility. The NICHD criteria were deliberately listed in

order of perceived importance. The use of these criteria defined PCOS as a syndrome whose primary determinant was a derangement in androgen homeostasis with consequent effects on menstrual cyclicity. Ultrasonographic evidence of polycystic ovaries was concluded to be “suggestive” of PCOS but not necessarily diagnostic. This prevailing opinion reflected the paucity of British and European attendees at the meeting to define the NICHD criteria, because Ultrasonographic evidence of polycystic ovaries had long been considered definitive evidence of PCOS in the UK and most of Europe. The NICHD criteria represented a very important first step towards establishing a universally accepted clinical definition for PCOS. However, it is important to recognize that the criteria were based on majority opinion and not clinical trial evidence. In the years that followed, it became apparent that the clinical presentation of PCOS was much more variable than that described by the NICHD criteria, and that polycystic morphology of the ovaries was a consistent finding in women demonstrating biochemical and clinical evidence of the syndrome (Marla *et al*., 2008).

In 2003, the European Society for Human Reproduction and Embryology and the American Society for Reproductive Medicine amended the consensus criteria to include polycystic ovaries as a third diagnostic marker and to allow for a diagnosis of PCOS if two of three criteria were met Oligo- or chronic anovulation, Clinical and/or biochemical signs of hyperandrogenism and Polycystic ovaries, in exclusion of other etiologies of androgen excess and anovulatory infertility is necessary. These “Rotterdam criteria” were intended to broaden the phenotypic expression of the syndrome and to redefine PCOS as primarily a syndrome of ovarian dysfunction. The Rotterdam criteria are controversial. Fulfilling two of three diagnostic criteria implies that PCOS can be diagnosed in the absence of androgen excess or menstrual irregularity the very factors that were once considered absolute requisites for the

syndrome. While most agree that PCOS exists as a spectrum, it has been difficult to reconcile the absence of androgen excess in the diagnosis (Marla *et al*., 2008).

In 2006, the Androgen Excess Society formed a task force to review existing data on the phenotypic expression of PCOS and Patient demonstrates both: Hirsutism and/or hyperandrogenemia and Oligo-anovulation and/or polycystic ovaries and exclusion of other etiologies of androgen excess and anovulatory infertility are necessary. The AES concluded that although there was good evidence for features of PCOS (e.g., mild insulin resistance and mild ovarian dysfunction) in women with polycystic ovaries, androgen excess, and regular menstrual cycles, there was conflicting evidence supporting the presence of such features of PCOS in women with polycystic ovaries and ovulatory dysfunction but without clinical or biochemical signs of hyperandrogenism. The AES has proposed a new set of diagnostic criteria that acknowledge the wide prevalence of morphologic polycystic ovaries and the wide heterogeneity of PCOS. They do not, however, recognize a mild variant of the syndrome in which little is known about metabolic status or long-term health risks (Marla *et al*., 2008).

All current definitions of PCOS require that other disorders of androgen excess or ovulatory function be excluded. Principally, the former includes 21-hydroxylase-deficient non-classic congenital adrenal hyperplasia (NCAH), Cushing's syndrome, androgen-secreting neoplasms (ASNs), and drug-induced or iatrogenic hyperandrogenism (IH): the latter includes thyroid dysfunction and hyperprolactinemia. NCAH is excluded by the measurement of a follicular phase (pre-ovulatory) basal 17ahydroxyprogesterone (17-HP) level, which if >2–4ng/ml mandates an acute adrenocorticotropic hormone (ACTH) stimulation test. Alternatively, evaluation for Cushing's syndrome, ASNs, or IH should be instituted if the history and physical exam suggests their possibility.Thyroid dysfunction and hyperprolactinemia can be excluded by the routine measurement of thyroid-

stimulating hormone (TSH) and prolactin levels, although the prevalence of these disorders in women with overt hyperandrogenism is relatively low. Overall, patients being evaluated for PCOS should, at a minimum, have NCAH excluded by a basal 17-HP level, and possibly thyroid dysfunction and hyperprolactinemia, by TSH and prolactin levels (Bradley *et al*., 2007).

Chapter three

Materials and methods

3. Materials and Methods

3.1-Study approach

A quantitative method was used to evaluate the plasma testosterone and estradiol levels among female with polycystic ovarian syndrome during the period from February to May 2017.

3.2-Study design and Study area

This cross sectional study was conducted in Khartoum state, the capital of Sudan.

3.3- Study population and Sample size

The study included eighty volunteered to participate in this study, 40 with polycystic ovarian syndrome, 40 control group healthy subjects without any diseases.

3.4-Inclusion criteria

Sudanese with polycystic ovarian syndrome, and healthy volunteer were included in this study.

3.5-Exclusion criteria

The criteria of exclusion based on excluding any menopausal and women receiving contraceptives.

3.6-Ethical consedration

Consent was taken regarding acceptance to participate in the study and re-assurance of confidentialty. Before the specimen was collected, the donors knew that this specimen was collected for research purpose.

3.7-Data collection

Data were collected using a structural interviewing questionnaire, which was designed to collect and maintain all valuable information concering each case examined.

3.8-Sample collection and processing

About 2.5 ml of venous blood were collected from each participant (both case and control). The sample collected under aseptic conditions and placed in sterile lithium heparin containers and centrifuged for 5 minutes at 3000 RPM to obtain plasma then they obtained sample were kept in plain containers at 2-8 C^0 until the time of analysis.

3.9-Estimation of testosterone and estradiol by using ELISA mehod:

3.10-Principle of method:

In which one of the reaction components is bound to a solid-phase surface. In this Technique, an aliquot of sample is allowed to interact with the solid-phase antibody. After washing, a second antibody labeled with enzyme is added to form an Ab–Ag–Ab–enzyme complex. Excess free enzyme–labeled antibody then is washed away, and the substrate is added; the conversion of substrate is proportional to the quantity of antigen **(**Carl *et al.*, 2006).

3.11-Procedure of testosterone and estradiol measurement (Appendix II)

3.12-Quality control

The precision and accuracy of all methods used in this study were checked by commercially prepared control sample before it is application for the measurement of test and control samples.

3.13-Statistical analysis

Data was analyzed to obtain means standard deviation and correlation of the sampling using statistical package for social science (SPSS) computer Programmed version 21, t test and person correlation were used for comparison and correlation.

Chapter four

Results

4. Results

The biochemical results of serum testosterone and estradiol in patients with polycystic ovarian syndrome are given in tables and figures:

Table (4-1): Illustrate the mean concentration of testosterone and estradiol and ratio between them in polycystic ovarian syndrome patients and control group. The levels of testosterone and T/E_2 ratio were significantly increased in PCOs compared with control groups. (Mean±SD: 1.4±1.1 versus 0.87±0.65ng/ml: 0.019±0.015 versus 0.01±0.008 ng/ml/pg/ml).with *P.Value* 0.011 and 0.022 respectively. The estradiol levels were insignificant in PCOs compared with control group. (Mean±SD: 86±39versus 101±38.6 pg/ml) respectively with *P.Value 0.086.* AMH were significantly increased in PCOs compared with control groups. Minimum (6.2 versus 0.01) ng/ml and maximum (28.6 versus 5.0) ng/ml. (Mean±SD: 10.6±5.92 versus 1.4±0.22) ng/ml respectively with *P.Value* 0.000.

Figure (4-1): Show correlation between testosterone concentration and estradiol (r= -.073 *P.Value* = 0.522).

Figure (4-2): Show correlation between age and testosterone (r= -.057- *P.Value* = 0.617).

Figure (4-3): Show correlation between age and estradiol (r= 0.193 *P.Value* = 0.927).

Figure (4-4): Show correlation between AMH and estradiol (r= -0.010- *P.Value* = 0.927).

Figure (4-5): Show correlation between AMH and Testosterone (r= 0.216 *P.Value* = 0.055).

Figure (4-6): Show correlation between T/E_2ratio and age (r= -.148 *P.Value* = 0.190).

Table (4-1): Illustrate the mean concentration of testosterone and estradiol and ratio between them in polycystic ovarian syndrome patients and control group.

Variable	PCOS N=40 Mean±SD	Control N=40 Mean±SD	*P.Value*
Estradiol pg/ml	86±39	101±38.6	0.086
Testosterone ng/ml	1.4±1.1	0.87±0.65	0.011
T/E_2 ratio	0.019±0.015	0.01±0.008	0.022
AMH	10.6±5.92	1.4±0.22	0.000

*Result given in mean ± SD, *P-Value* ≤ 0.05 Consider significant.

* Independent sample T test was used for comparison.

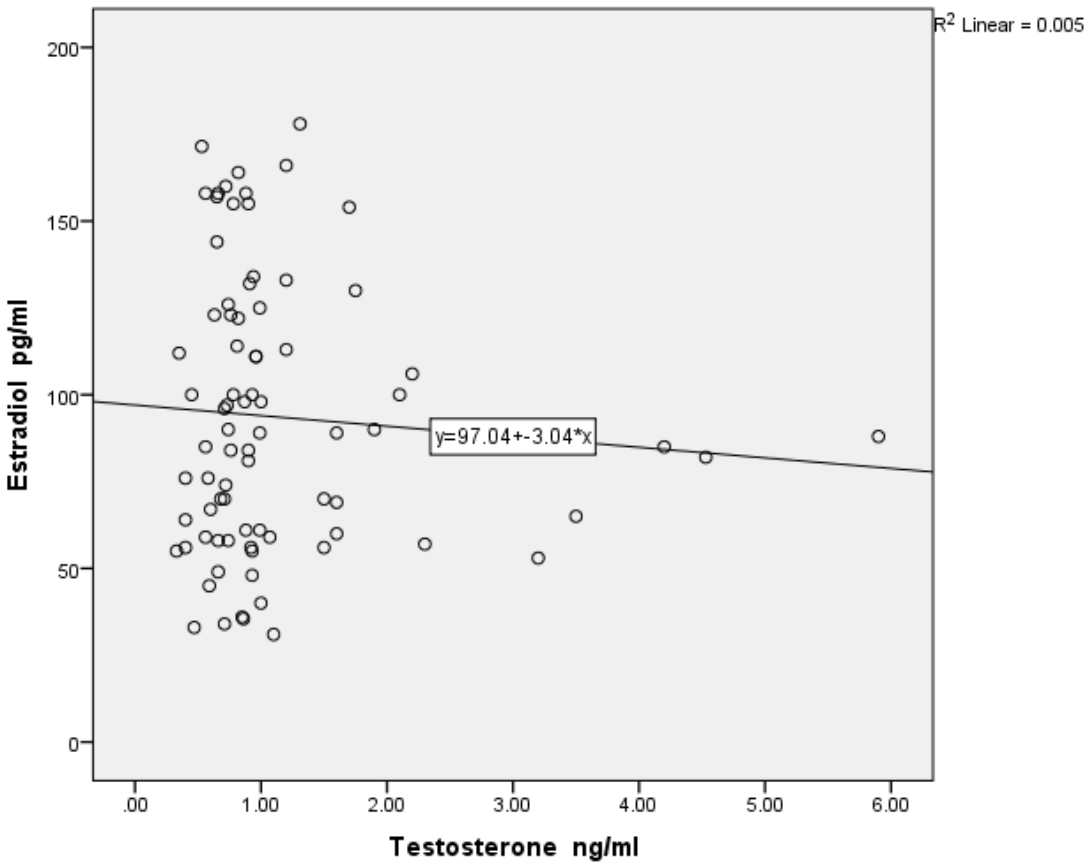

Figure (4-1): Show correlation between testosterone concentration and estradiol(r= -.073, P.Value = 0.522).

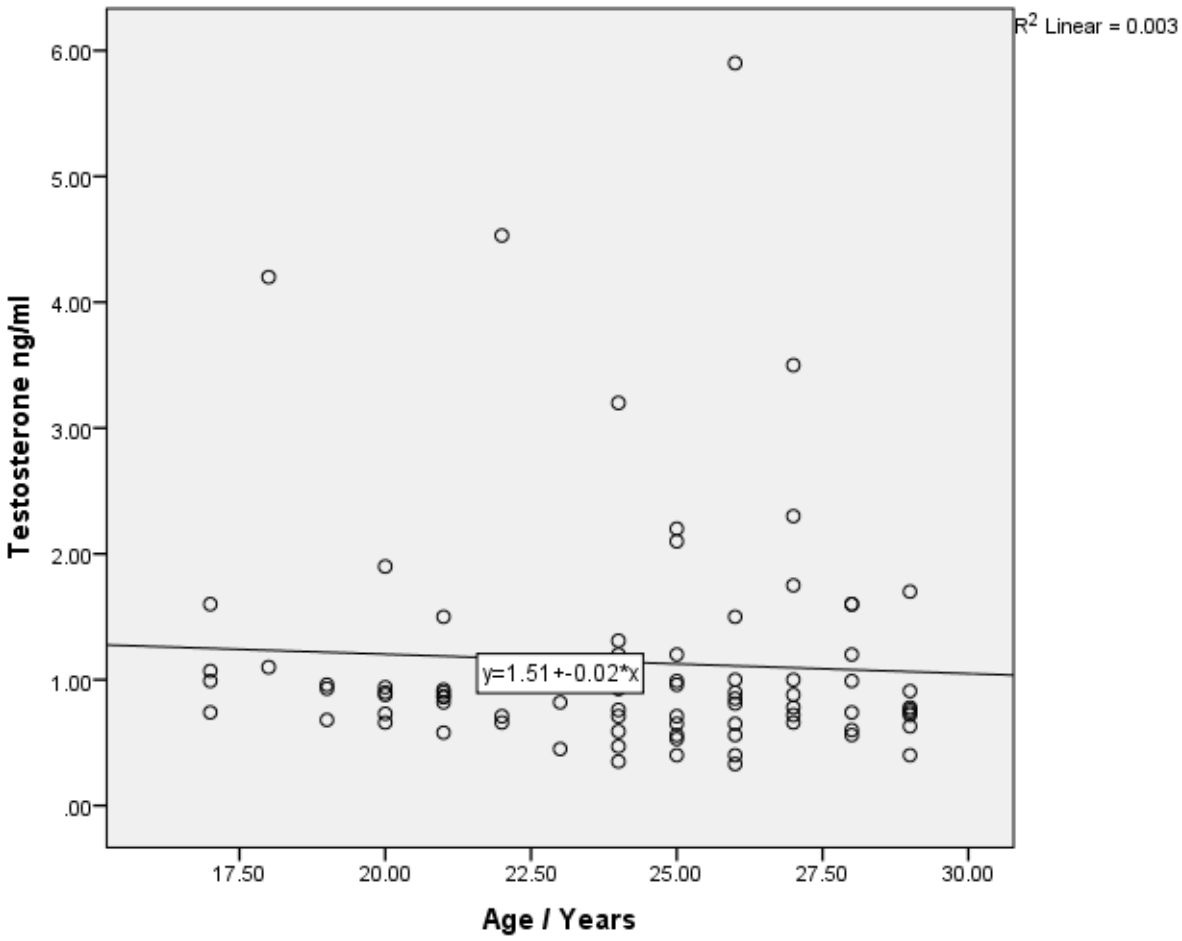

Figure (4-2): Show correlation between age and testosterone (r= -.057, *P.Value* = 0.617).

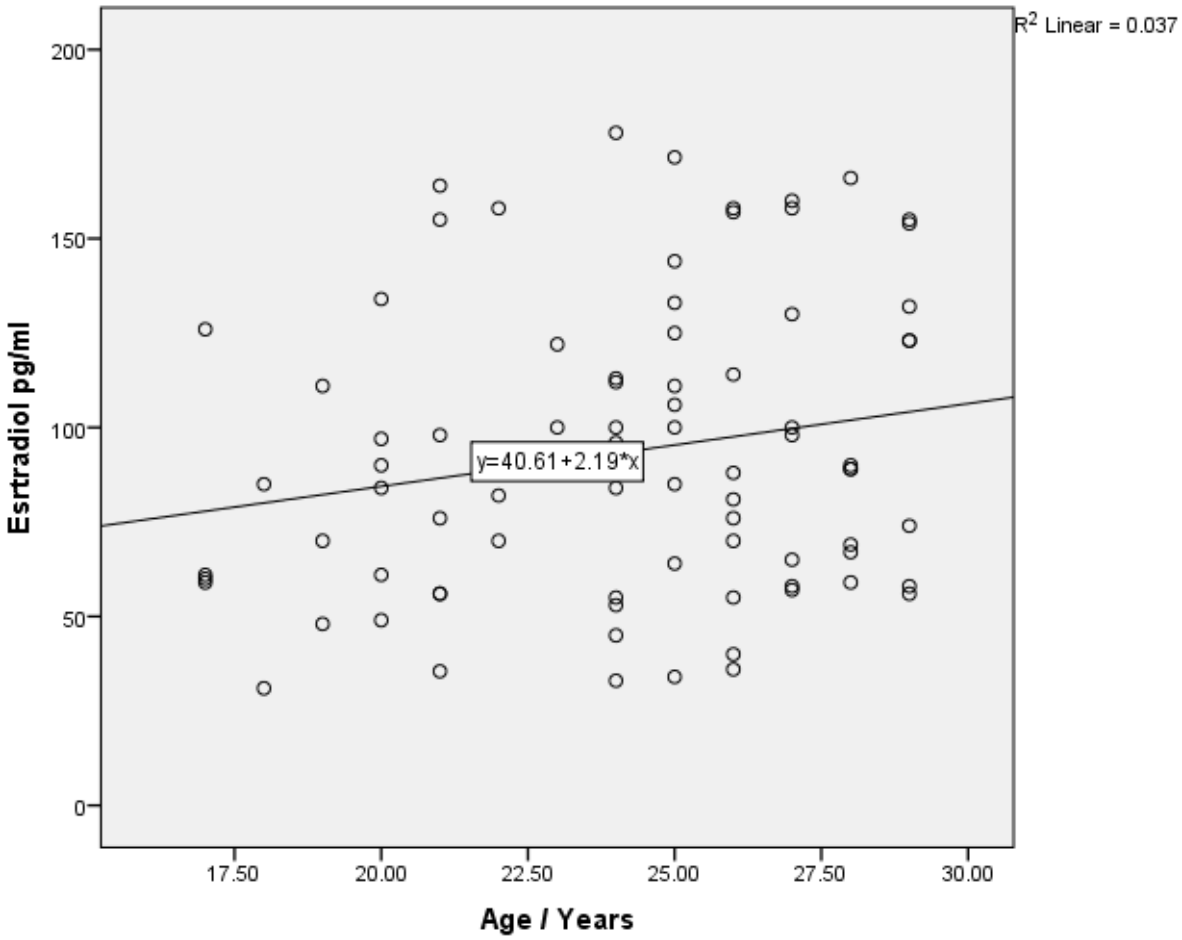

Figure (4-3): Show correlation between age and estradiol (r= 0.193, *P.Value* = 0.927).

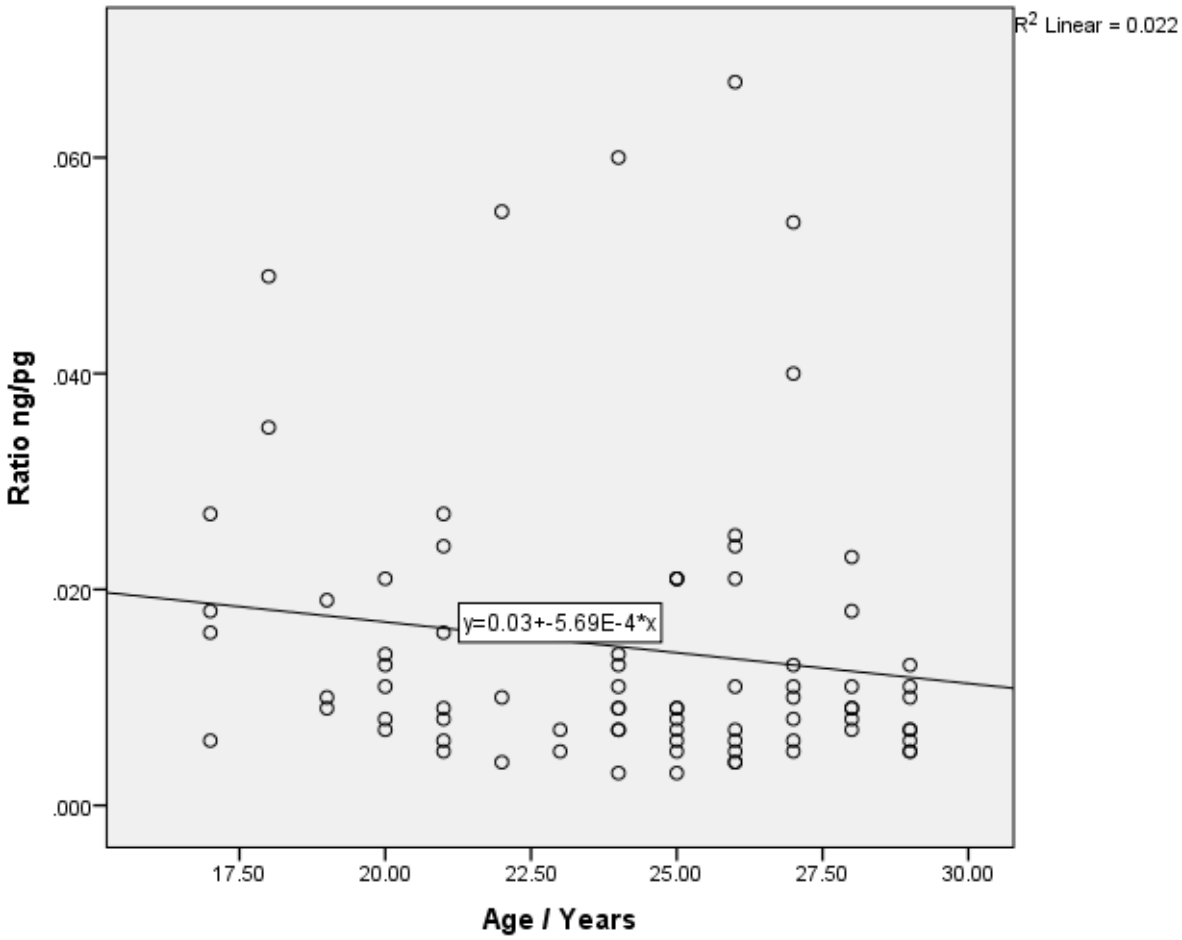

Figure (4-4): Show correlation between T/E_2ratio and age (r= -.148, *P.Value* = 0.190).

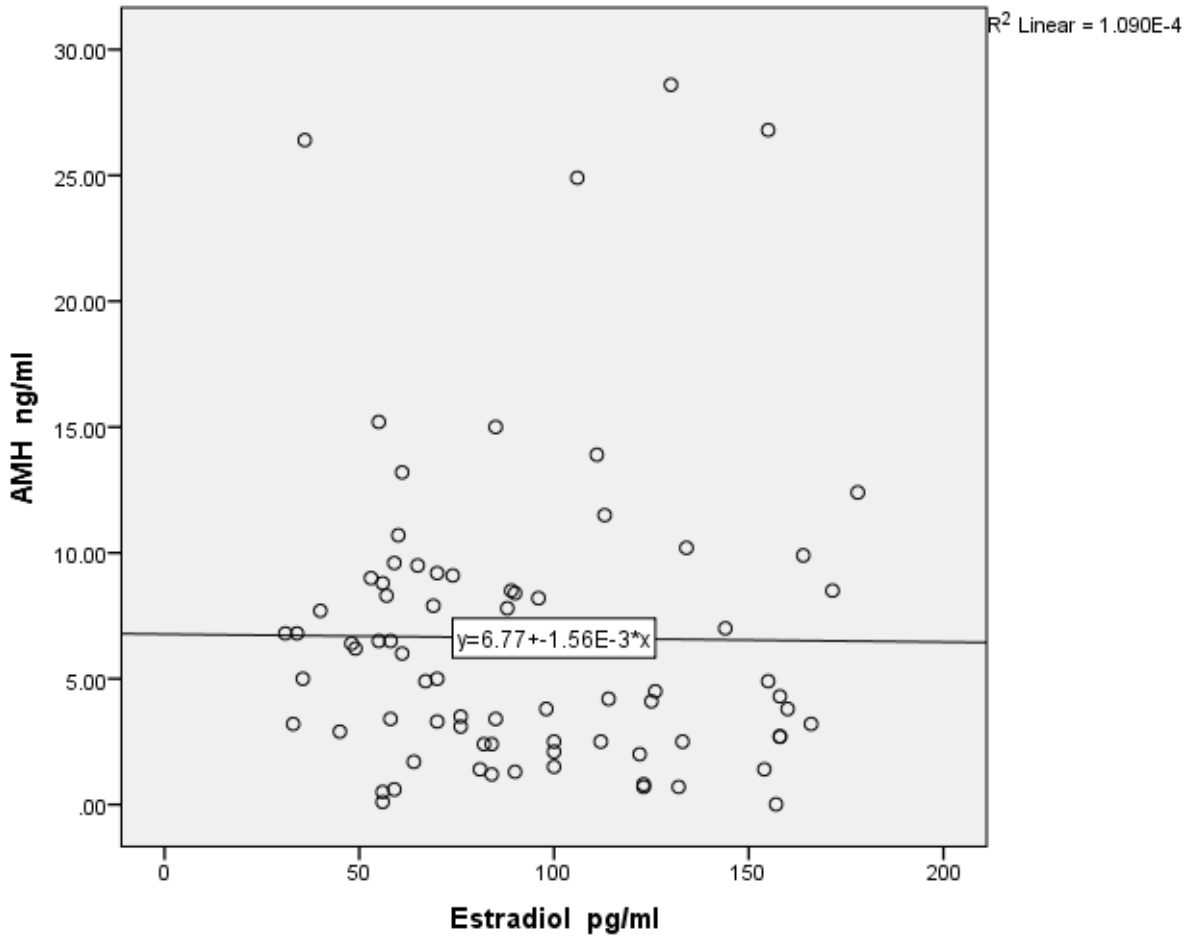

Figure (4-5): Show correlation between AMH and estradiol (r= -0.010- , *P.Value* = 0.927).

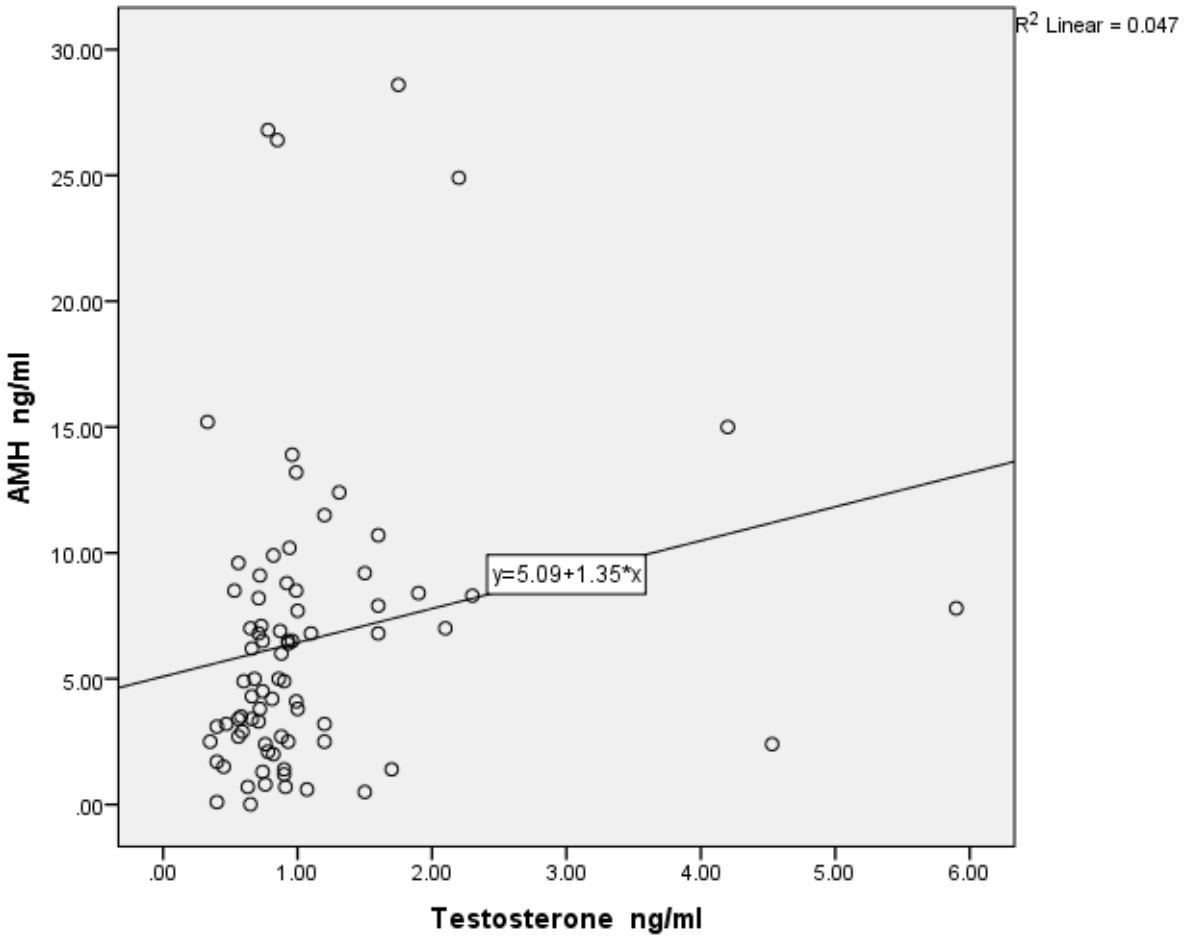

Figure (4-6): Show correlation between AMH and Testosterone (r= 0.216, *P.Value* = 0.055).

Chapter five

Discussion, conclusion and recommendations

5.1 Discussion

Polycystic ovary syndrome (PCOS) is a common endocrine disease in women, characterized by heterogeneous presentation of hyperandrogenism, ovulatory dysfunction, and polycystic ovarian morphology (PCOM) (Palomba *et al*., 2015).

Polycystic ovary syndrome (PCOS) disrupt the fertility hormones in females by increasing or decreasing them, this study conducted to get the disrupt of testosterone / estradiol ratio among pcos female patients.

In research, the ages ranged from 17 to 29 years. In the past they thought PCOs presents during the reproductive years only, but now can be diagnosed from fetal life.

Although PCOS classically presents during the reproductive years with menstrual irregularities, hyperandrogenism and metabolic complications, we now understand that the origin of the disorder probably occurs very early starting from fetal life. In utero exposure to elevated testosterone levels coupled with gestational hyperglycemia may contribute to early differentiation of PCOS or may lead to amplification of the phenotype in genetically predisposed individuals. The spectrum of presentation of PCOS phenotype changes across the life span of a given individual. Improved understanding of the disease spectrum has allowed us to identify endocrine and metabolic changes in the very young subject with high risk of developing PCOS (Belinda George and M Ganapathi, 2016).

The present study revealed significant increase in mean testosterone and T/E_2 ratio levels among case when compared to control group with *p-value* 0.011 and 0.022 respectively, while estradiol showed insignificant difference with *p-value* 0.086. This finding agreed with studies done by (Shang-Gwo *et al.,* 2008) and (Amato *et al., 2*010). Who reported that polycystic ovary syndrome (PCOS) had higher levels of T/E_2 ratio as compared to control group. Also similar to study done on by

(Lerchbaum et al., 2014) who approved that (PCOS) had higher levels of testosterone as compared to control and estradiol not significant difference in PCOs compared to control. Because the PCOs female produce excess LH and AMH hormone which can inhibit the ovarian production of estradiol, also inhibit the aromatase enzyme that can lead to prevent conversion of testosterone to estradiol in peripheral tissues.

In the present study of PCOs, there are no correlations were found between plasma levels of testosterone and estradiol. This finding was agreed with studies done by (Lerchbaum *et al.*, 2014).

Also the result showed that, there was no correlations were found between AMH and plasma levels of testosterone and estradiol .This finding was agreed with estradiol and disagreement with testosterone in studies done by (A.F. Begawy *et al.*, 2010).

Also the result showed that, there was no correlations were found between age and plasma levels of testosterone and estradiol and T/E_2 ratio.

5.2- Conclusion

From the results and finding of this study, it is concluded that:

The plasma levels of testosterone and T/E_2 ratio are higher in PCOs female patients. No correlations were found between AMH and plasma levels of testosterone and estradiol. No correlations were found between age and plasma levels of testosterone and estradiol and T/E_2 ratio.

5.3-Recommendations

1-Therefore, we should consider plasma testosterone as a very sensitive indicator of biochemical diagnosis hyperandrogenemia, especially when there is no evidence of clinical hyperandrogenemia in an oligo or anovulatory women.

2- The measurement of T/E_2 ratio can be used as biochemical marker for diagnosis of polycystic ovary syndrome (PCOS).

3- More studies should be carried out on the T/E_2 ratio.

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Appendices

Appendix I

Questionnaire

Sudan University of science and Technology

College of graduate studies

Testosterone to Estradiol ratio among Female with Polycystic Ovarian Syndrome

Patient name or code:-……

Age:…….Years

Methods of diagnosis:-

……

……

……

……

……

……

Duration of disease: Months ( ) Years ( )

Laboratory investigations:-

Test name	Concentration
Testosterone	**ng/ml**
Estradiol	**pg/ml**

Appendix II

Estradiol Sheet.pdf

Testosterone Sheet.pdf