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Study of Some Hormonal and Biochemical Parameters Associated with Polycystic Ovary Syndrome in Type 2 Diabetic Women in Misan Province

A Thesis

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Dedication

To my Father and Mother

To my Husband who supported me along the time

To my lovely Kids: Ruqayah and Massooma

To my Brothers and Sisters

To all who helped me dedicate this work.

Faten khudhair

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List of Abbreviations

Abb.	Meaning
ADP	Adenosine-5-diphosphate
ACTH	Adrenocorticotrophic hormone
ACR	Albumin creatinine ratio
ADA	American Diabetes Association
ASRM	American Society of Reproductive Medicine
4-AP	4 – Aminophenazone
AES	Androgen Excess and PCOS Society
A4	Androstenedione
Anov	Anovulation
BMI	body mass index
CVD	Cardiovascular disease
CHE	Cholesterol esterase
CHOD	Cholesterol oxidase
CRP	C-Reactive Protein
CYP19A1	C Enzyme Aromatase
CYP 19	Cytochrome p-450 c19
CYP17	Cytochrome P450 17
DAP	dihydroxyacetone phosphate
DHEA	Dehydroepiandrosterone
DHEA-S	Dehydroepiandrosterone sulfate
DHT	Dihydrotestosterone
ELISA	Enzyme-linked immunosorbent Assays
ESHR	European Society for Human Reproduction and Embryology
FTO	fat mass–and obesity-associated gene

E1	Estrone
E2	Estradiol
FBG	Fasting Blood Glucose
FSH	Follicle Stimulating Hormone
FSHR	Follicle Stimulating Hormone Receptor
FFA	Free Fatty Acids
GDM	Gestational Diabetes Mellitus
GOD	Glucose oxidase
G3P	Glycerol-3-phosphate
HBA1c	Glycated hemoglobin
GH	Growth Hormone
GnRH	Gonadotropin Releasing Hormone
HDL	High density lipoprotein
HPLC	High –Performance Liquid Chromatographic
HOMA	Homeostasis Model Assessment
HA	Hyperandrogenism
H2O2	Hydrogen Peroxide
HSD17B	17β-Hydroxysteroid dehydrogenase
HPA	Hypothalamic-Pituitary Axis
IGF-1	Insulin-like growth factor 1
IGT	Impaired glucose tolerance
IL-6	Interleukin-6
INSR	Insulin Receptor
IR	Insulin resistance
LPL	lipoprotein lipase
LDL	Low density lipoproteins
LH	Luteinizing Hormone
LHR	Luteinizing Hormone Receptor gene
Mes	Metabolic syndrome
4MUP	4-methylumbelliferyl phosphate

MAU	Microalbuminuria
MAPK	Mitogen-activated protein kinases
NCEP	National Cholesterol Education Program
NIH	National Institutes Health
NKF	National Kidney Foundation
NAFLD	Non-alcoholic fatty liver disease
OD	Ovulatory dysfunction
OS	Oxidative stress
POD	Peroxidase
GPO	phosphate dehydrogenase
PI3K	phosphoinositide 3-kinase
PCO	Polycystic Ovary
PCOM	Polycystic ovarian morphology
PCOS	Polycystic ovary syndrome
PRL	Prolactin
QUICKI	quantitative insulin sensitivity checks index
SHBG	Sex Hormone Binding Protein
SD	Standard Division
SPSS	Statistical Package for the Social Sciences
T	Testosterone
TG	Triglyceride
TZDs	Thiazolidinediones
TRH	Thyrotropin releasing hormone
TNF- α	Tumor Necrosis Factor – α
T2DM	Type 2diabetes mellitus
VLDL	Very low density lipoprotein
WHO	World Health Organization

1. Introduction

Infertility is one of the major challenge affecting the lives of every men and women among the worldwide. It is defined as the inability of the couple to realize pregnancy during an average stage of one year despite regular competence (3-4 times per a week) unprotected intercourse (Cooper *et al.* ,2010). Approximately 10-15% of young couples worldwide suffer from this problem (Boivin *et al.,* 2007; Novak, 2007). Besides many environmental factors there are many causes leading to this phenomenon in women like endometriosis, early ovarian failure, pelvic inflammatory disease, fibroids and polycystic ovary syndrome (Eniola *et al.*,2017).

Polycystic ovary syndrome (PCOS) is one of the common causes of ovarian infertility, this syndrome first described by Stein and Leventhal in 1935, additionally referred to as Stein-Leventhal syndrome, PCOS is a complicated endocrine and metabolic disorder that affects between 5−17% of women international (Dumesic *et al.*,2015; Azziz ,2016). Clinical and/or biochemical hyperandrogenism, chronic an oligomenorrhea (anovulation) and the found of polycystic ovaries on transvaginal ultrasound (Sohrevardi *et al.*,2016). In PCOS, endocrine abnormalities may be leading to increased free testosterone levels, a high luteinizing hormone/follicle-stimulating hormone (LH/FSH) ratio and a low sex hormone binding globulin (SHBG) (Homburg,2002; Barber *et al.*,2007).

In addition, about 60–80% of women with PCOS have elevated androgen levels and this lead to the clinical signs involve: hirsutism, menstrual disturbances, oligomenorrhea/amenorrhea, obesity, infertility/first trimester miscarriage, acanthosis nigricans, male pattern alopecia, acne and development of ovarian cysts (Sheehan,2004; Vrbikova *et al.*,2004; Azziz *et al.*,2006). Also, it has a high level of LH with normal levels of FSH (Speroff and Fritz ,2005).

This syndrome is also related with glucose intolerance, hyperinsulinemia, insulin resistance (IR), obesity, infertility and blood lipid levels abnormalities, and that constitute a metabolic syndrome (Dokras *et al.*,2005; Fauser *et al.*, 2012).

Recent epidemiological data observed a strongly relationship between PCOS and metabolic syndrome that 75% of PCOS females have insulin resistance, practically, the symptoms of PCOS can be recognized in all fertility women with IR and metabolic syndrome (Jukic *et al.*2016; Pal *et al.*2016). Furthermore, the occurrence of metabolic syndrome in PCOS women's is 2-4 times greater in comparison with the common population, and this occurrence be high by 50% during 30-40 years old age (Apridonidze *et al.*,2005).

Metabolic syndrome includes the following diseases: cardiovascular risk, hypertension, endothelial dysfunction and insulin resistance, and about 50% - 60% of women with PCOS infects with IR that its prevalence in the general public between (10- 25) % depending on method of evaluation and mean body weight (Jensterle *et al*,.2008; Baptiste *et al.*,2010).

Teede *et al.,* (2011) refers to PCOS women are usually obese, contributing to the formation of an external component of IR that lead to development of hyperglycemia which drives the hyperandrogenemia in these women.

The Aims of Study

The current study aimed to investigate some hormonal and biochemical parameters associated with PCOS women with type 2 diabetes.

1.The hormonal parameters include:

- Androgens: Dihydrotestosterone (DHT) and Testosterone (T)
- Follicle stimulating hormone (FSH)

- Luteinizing hormone (LH)
- LH/FSH ratio
- Prolactin (PRL)
- Insulin.

2. The biochemical parameters include:

- ❖ Fasting Blood Glucose (FBG)
- ❖ Insulin resistance (IR)
- ❖ Lipid profile include:
  - Cholesterol
  - Triglyceride (TG)
  - High density lipoprotein (HDL-C)
  - Low density lipoprotein (LDL-C)
  - Very low density lipoprotein(VLDL-C).
- ❖ Total protein
- ❖ C-Reactive Protein (CRP)
- ❖ Microalbuminuria(MAU)
- ❖ Glycated hemoglobin (HbA1c)

2.Literature Review

2.1. Polycystic Ovary Syndrome

Polycystic ovary syndrome (PCOS) is the most common endocrine and metabolic disorders in premenopausal women which is characterized by polycystic ovaries, hyperandrogenism and irregular menstrual cycles (March *et al.*,2009). Metabolic abnormalities and obesity are the most common in PCOS women's and about 50- 70% of them are insulin resistance (IR) (Diamanti-Kandarakis *et al.*,2008; Dumesic *et al.*,2015).

Mahabeer *et al.*, (1990); Dunaif *et al.*, (1996); Vrbikova *et al.*, (2008) reported that the most women with PCOS are able to recompense for their insulin resistance (IR), but a large ratio of them have changed beta-cell function. So, leading to glucose intolerance, that increase the risk of developing type2 diabetic mellitus (T2DM), independently of age and body mass index (BMI) (Legro *et al.*,1999).

Moreover, in PCOS women's have an increased risk of developing hypertension and dyslipidemia that lead to increase prevalence of metabolic syndrome (Legro *et al.*,2001; Glueck *et al.*,2003; Chen *et al.*,2007).

The etiology of PCOS is poorly understood and high prevalence would possibly be due to the environmental and the genetic predisposition, epigenetic factors, that mean up to 70% can be attributed to heredity and genetic factors (Sirmans and Pate,2014).

2.1.1. History of PCOS

Both Irving Stein and Michael Leventhal who they worked inObstetrics and Gynecology department, Michael Reese Hospital, Chicago, USA، described the clinical, the macroscopic and the histological features of PCOS for the first time in 1935, they had observed an association between amenorrhea, hirsutism and PCO (Stein,1935).

2.1.2. Definitions and Diagnostic Criteria of PCOS

The most recent PCOS definitions are the following:

1. Ovulatory dysfunction includes (amenorrhea or oligo menorrhea) and hyperandrogenism according to National Institutes of Health (NIH) (1990) (Zawadzski,1992).

2. Hyperandrogenism، PCO morphology on ultrasound and ovulatory dysfunction required at least two of the criteria listed by the European Society for Human Reproduction and Embryology (ESHRE) in association with the American Society of Reproductive Medicine (ASRM) (Rotterdam, 2004).

3. The presence of hyperandrogenism with ovarian dysfunction which included (ovulatory dysfunction or PCO morphology on ultrasound to diagnose the syndrome according to (AES) in 2006 (Azziz *et al.*, 2006).

In all previous definitions, the term hyperandrogenism refers to elevated level of the androgens clinically or biochemically, the clinical signs of hyperandrogenism is hirsutism, acne and alopecia, table (2.1) (Legro *et al.*, 1998; Rotterdam, 2004).

According to diagnostic criteria practical, the prevalence of PCOS changes from 5% to 10% according to NIH (1990), from 10% - 15% according to the AE-PCOS (2006) recommendations, and from 6% - 21% according to the Rotterdam criteria are applied (Lizneva *et al.*,2016). In other wise, the prevalence of PCOS elevated with the ESHRE/ASRM (2003) and AES (2006) criteria due to their broader definition with insertion of additional phenotypes among women with PCOS e.g. phenotype A (HA+OA+PCO) for 66%, phenotype B (HA+OA) for 13%, phenotype C (HA+PCO) for 11% and phenotype D (OA+PCO) for 9% (Lizneva *et al.*,2016).

Table 2.1. Diagnostic Criteria for PCOS

PCOS phenotype	NIH criteria 1990	Rotterdam criteria ESHRE 2003	AES criteria 2006
Anov + HA +PCO	✓	✓	✓
Anov + HA	✓	✓	✓
Anov + PCO		✓	
HA + PCO		✓	✓

Anov= Anovulation, HA=Hyperandrogenism, PCO=Polycystic Ovary

(Veltman-Verhulst ,2012)

2.1.3. Clinical Signs of PCOS

PCOS is a heterogeneous with multiple phenotypes that different in their severity, signs and symptoms, overall, PCOS is the systemic disorder that will be affect a woman during her life, until beyond the reproductive years (Delitala *et al.*, 2017). These clinical signs (disorders) are included:

2.1.3.1. Hyperandrogenism

The term androgen refers to a family of hormones: testosterone (T), dihydrotestosterone (DHT), dehydroepiandrosterone (DHEA) and androstenedione, T is one of the most androgen used as biomarker of hyperandrogenemia in women with PCOS (Münzker *et al.*, 2015).

Hyperandrogenism (HA) is the most common diagnostic signs of PCOS women, that was described in women with PCOS by Stein and Leventhal in 1935. It is characterized by increasing level of serum androgens (hyperandrogenemia) or the clinical manifestations linked to androgen function, approximately 60-80% of this occurs in PCOS women (Franks ,2006). In early studies of the syndrome mentioned to the hyperandrogenism related with irregular menstrual cycles, infertility, obesity and IR (Smith *et al.*, 1979, Wild *et al.*, 1983).

Barber *et al.*, (2016) noted that a positive relationship between hyperandrogenism and insulin resistance (IR). Wang *et al.*, (2012); Münzker *et al.*, (2015) explained and identified a deferent forms of T including elevated free testosterone, bioavailable testosterone or total testosterone, also they observed that androstenedione and testosterone are an ovarian derived androgen approximately represent for half of a total androgen production in women, and androgens also derived from adrenal glands (DHEA) and a small amount of adipose tissue.

A metabolite of androgen such as DHT or stanolone, produced by the enzyme 5α-reductase, is one of the four principle androgens in humans, may be elevated in PCOS (Lerchbaum *et al.*, 2012). DHT and testosterone bind to the same androgen receptor, but DHT does so with greater receptor affinity, DHT cannot be aromatized, DHT and testosterone primarily bind to SHBG, only one third of estrogen binds to SHBG (Saartok *et al.*,1984).

Normally, ovaries and adrenal glands are contributed to the synthesis of sex steroids hormones (Yen, 1977; Arrais and Dib,2005; Handa and Weiser,2014). Figure (2-1).

In women with PCOS, the recreation of enzyme Cytochrome P450 c17 (CYP17), which converts progesterone to 17-hydroxyprogesterone and from 17-hydroxyprogesterone to androstenedione (A4) is exaggerated and a lowered activity of CYP19A1 favors androgen manufacturing in these women (Yang *et al.*,2015).

Moran *et al.*, (2015) mentioned that the high levels of androgen precursors DHEA, and A4 in non-obese women sufferers with PCOS compared to overweight women with PCOS. Moreover, adrenal hyperandrogenism in PCOS women has been associated with elevated blood pressure and decreased insulin sensitivity (Alpañés *et al.*,2015).

Burger, (2002) explained that 50% of the T comes from the conversion of A4 in the liver, 25% is synthesized in the adrenal.

In some peripheral tissues, DHEA is converted to androstenedione (A4) in the liver and directly to DHT from A4, and except previous T formation, the ovaries are produced 20-30% of DHEA and 50% of A4 and almost all the circulating DHEAS is produced in the adrenal cortex because of the DHEAS is the best marker of adrenal androgen precursor production, it is about 20-30% of

PCOS women have androgen extra of adrenal starting place (Yildiz and Azziz, 2007).

Dunaif and Book, (1997) refer to the Insulin resistance with compensatory hyperinsulinemia is frequent in women with PCOS. Insulin increases the recreation of CYP17 that additionally favors the conversion of progestogen precursors to androgen manufacturing in ovaries and adrenals (Schiffer *et al.*,2017). Fan *et al.*, (2013) found that another parameter used to determine the hyperandrogenemia is SHBG.

Zhao and Qiao, (2013) reported that the clinical signs associated with hyperandrogenism principally include: a hirsutism that occurs in 70% of PCOS women's and defined it as it is the excessive growth of hair androgen dependent, on the other hand, evidence of hyperandrogenism can also be seen in male pattern baldness (alopecia) and acne. Perhaps, these manifestations related to increased activity of the enzyme 5-α reductase in hair follicles and sebaceous glands and that converts T to DHT (Metwally, 2012).

These clinical results in the women with PCOS may be related, at least in part, to the increased prevalence of anxiety and depressive disorders due to hand down self-esteem (Cooney *et al.*, 2017).

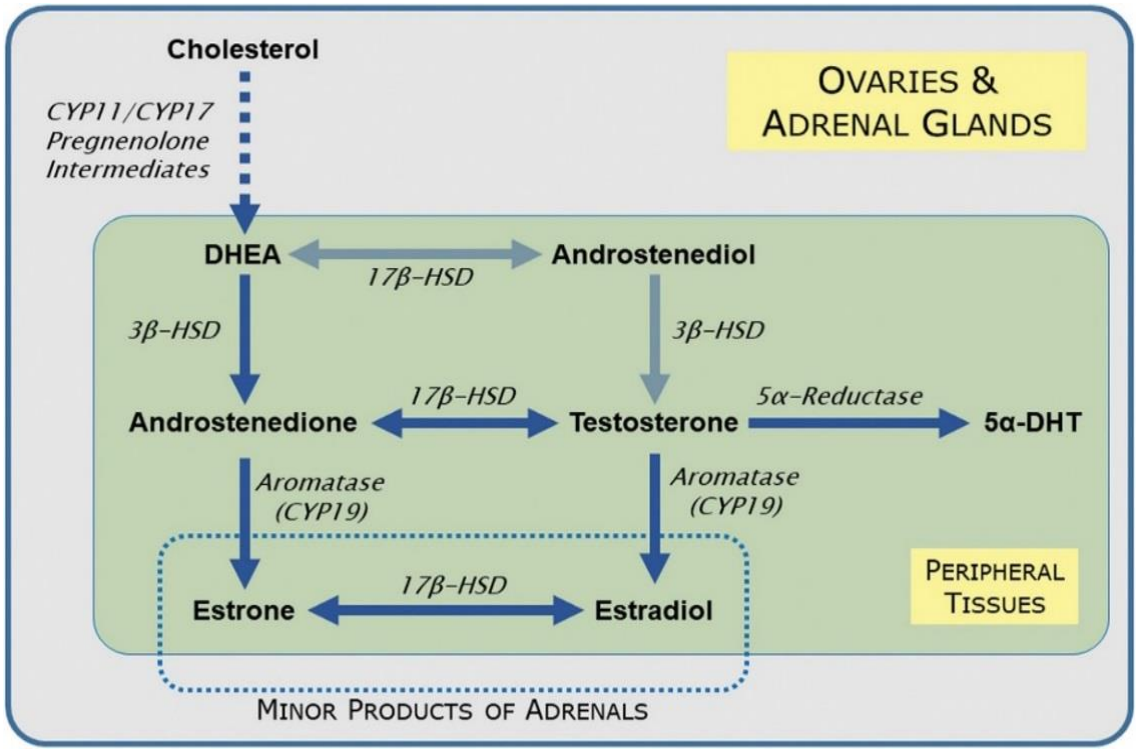


Figure 2.1. Synthesis of androgens.

(Traish *et al.*,2018)

2.1.3.2. Ovulatory Dysfunction (OD)

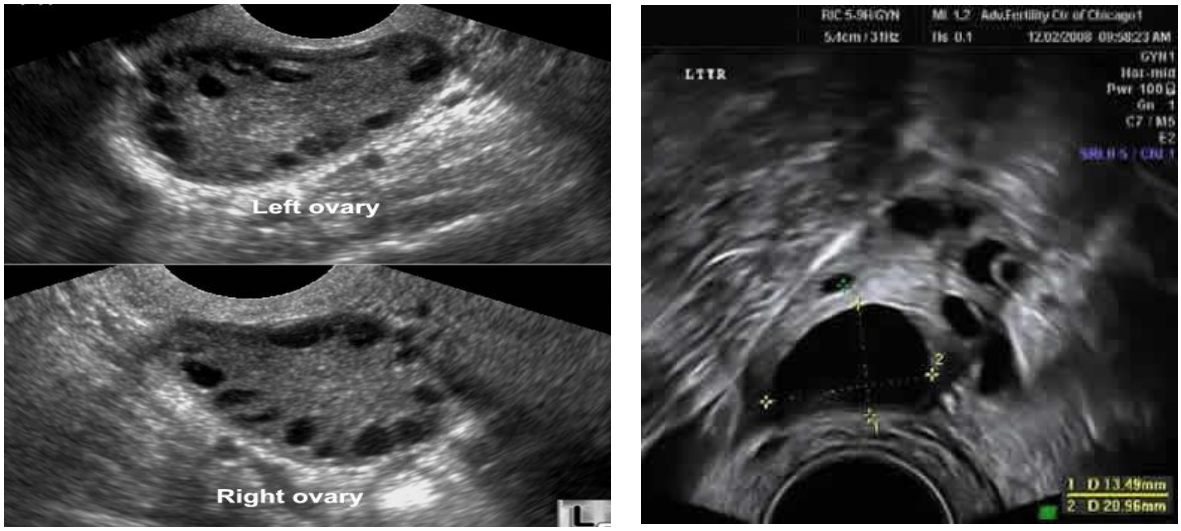
Ovulatory dysfunction (OD) is one of the most common diagnostic sign well-known in women with PCOS and found in over 95% of PCOS women (Barthelmess *et al.*, 2014).

This term is as anovulation, oligo menorrhea (irregular menstrual cycles less than 9 menses per year) or amenorrhea (lack of menstrual cycle for at least three months), OD is the most common cause of an ovulatory infertility, which represents 40% of PCOS women's affected that leading to infertility (Legro *et al.*, 2007, Sirmans and Pate, 2014).

2.1.3.3. The Polycystic Ovary Morphology (PCOM)

The PCO morphology definition has varied in excess of the years, the first definition was by Stein and Leventhal in 1935 who they described the macroscopic form of PCO ovaries as enlarged, bilateral, stressed ovaries that were frequently clearly globular in shape (Stein,1935). The histological description was included the presence of multiple cysts, often larger than 15 mm and a hypertrophic theca cell layer was lined these cysts, it was also noted that the tunica albuginea was much wider than in normal ovaries and the ovaries were empty of corpus luteum (Crum *et al.*, 2003; Schmidt,2011).

Goodman *et al.*, (2015) mentioned that the enlargement the ovary and increased in volume more than 10 mL, the presence of more than 25 cysts is considered to be a diagnostic of PCOS by using more sensitive ultrasound technology. The scan should be repeated if a follicle is >10 mm in diameter, figure (2-2) (Rotterdam, 2004).



A **B**

Figure 2.2. Ovarian ultrasound

A: Ultrasound image of left and right ovaries showing ovarian pap smears in women with PCOS **B:** Ultrasound image of normal. (Schmidt, 2011).

2.1.3.4. Other Clinical Signs of PCOS

Moore and Campbell, (2017) reported that the changes in hypothalamic and pituitary hormones are associated with PCOS, especially, elevated in LH or LH: FSH ratios via the follicular arrested. In other wise, many authors reported that the clinical features of PCOS also lead to metabolic disturbances such as impaired glucose tolerance, IR, dyslipidemia, non-alcoholic fatty liver disease (NAFLD), obesity or central adiposity (El Hayek *et al.*, 2016).

Copps and White, (2012) mentioned that the IR is impaired response to insulin in target organ tissues and it is observed about 60-80% of women with PCOS (Barthelmess *et al.*, 2014). In PCOS insulin resistance also lead to the risk of other cardio metabolic disorders such as hypertension and type 2 diabetes mellitus (T2DM) in later life (Ranasinha *et al.*, 2015).

Nevertheless, hyperprolactinemia, congenital adrenal hyperplasia, androgen-producing tumors and Cushing syndrome are various disorders affected the ovarian volume and the reproductive hormones and must to be excluded from PCOS diagnosis (Schmidt, 2011; Brzana *et al.*, 2014, Singla *et al*., 2015، Williams *et al.*, 2016).

2.1.4. Prevalence of PCOS

Polycystic ovary syndrome (PCOS) is an endocrine female disorder during reproductive age and have many different phenotypes differs greatly according to ethnicity and geographical location, therefore the diagnostic criteria used worldwide ranging between (4% to 21%) (Bozdag *et al.*,2016). In the other hand, many authors' observed that the prevalence of PCOS is varying according to different communities as table (2-2).

Table 2.2. Prevalence of PCOS

Name of country	Prevalence rate	Criteria	Source
Australia	21%	----------	Davis *et al.*, 2002, Boyle *et al.*, 2012
Iran	7.1% and 14.6%	NIH, the Rotterdam criteria	Tehrani *et al.*, 2014
Southern China	2.2%	the Rotterdam criteria	Chen *et al.*, 2008
South-eastern United States	4.0%	NIH	Knochenhauer *et al.*, 1998
SriLankan population	6.3%	the Rotterdam criteria	Kumarapeli *et al.*, 2008
Spain	6.5%	NIH	Asuncion *et al.*, 2000
Greek population	6.8%	----------	Diamanti-Kandarakis *et al.*, 1999
United Kingdom	26%	----------	Michelmore *et al.*, 1999

2.1.5. Pathophysiology

Despite the fact of the precise pathogenesis of PCOS is still unknown, it is agreed that the disorder arises from a culmination of signs and that they adversely affect multiple body systems, across the hypothalamus-pituitary-ovarian axis (Ong, 2018).

González, (2012) noted that the varying degrees of disrupted insulin signaling, impaired ovarian hormone production (steroidogenesis) and chronic low grade inflammation may be contributing and combine to the developmentof PCOS. Yarak *et al.,* (2005) suggested that four theories:

2.1.5.1. Hyperinsulinemia and Insulin Resistance

Obesity and metabolic syndrome recurrently coincide with PCOS and then IR has been recognized as the link with a studies that showing to over 90% of PCOS women are insulin resistant **(**Barber *et al.,* 2016, Zhu *et al.,* 2016).

Lewy *et al.,* (2001) suggested that IR central to the pathophysiology of PCOS, the ways in which hyperinsulinemia and IR contributed to PCOS are summarized in figure (2-3). Many authors observed that IR is positively linked with hyperandrogenism with most severe phenotypic appearance of the PCOS in women (Palomba *et al.,* 2010, Barber *et al.,* 2016).

Cadagan *et al.,* (2016) observed that insulin in ovarian tissues acts as a co-gonadotrophin with synergistic effects via LH by starting steroidogenesis . Therefore, it has been hypothesized that hyperinsulinemia and IR in peripheral tissues undesirably affect insulin-sensitive ovaries lead to increase androgen production and mess up the menstrual cycle, leading to invented the ‘selective insulin resistance’ theory (Rojs *et al.,* 2014).

Rojas *et al.,* (2014) found that the hyperinsulinemia may increase the function of pituitary gland to change the frequency and amplitude of LH release to increase hyperandrogenism in PCOS.

Abu-Hijleh *et al.*, (2016) observed that IR has been associated with decreased levels of sex-hormone binding globulin (SHBG) which is also one of the most common clinical finding in PCOS women and SHBG is responsible for the metabolism and binding of sex steroid hormones, especially with testosterone. So, decreased SHBG lead to increased free androgens and may be link PCOS (Vassilatou,2014).

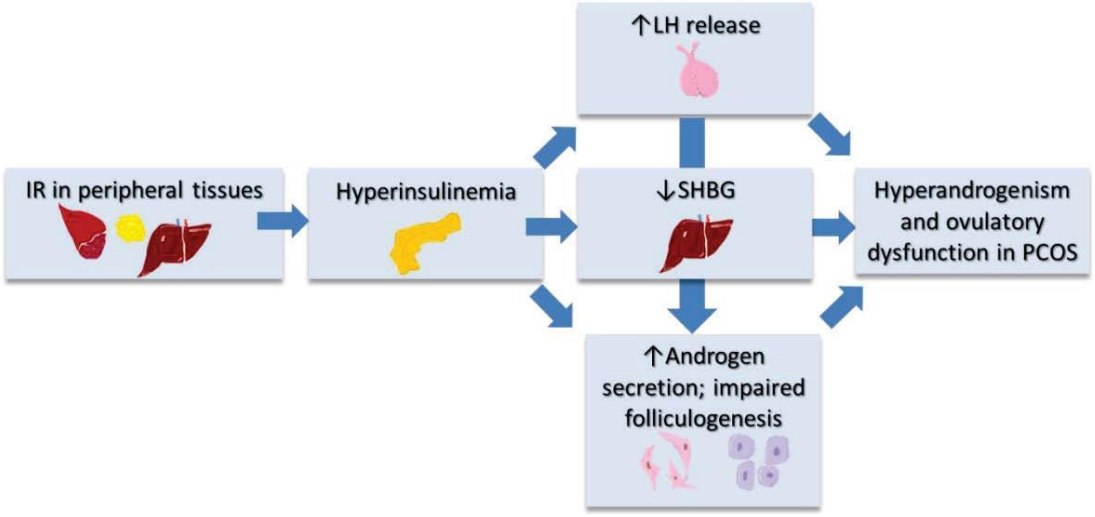


Figure 2.3. Schematic insulin resistance in the pathogenesis of PCOS. (Ong, 2018).

2.1.5.2. Defect in the Neuroendocrine System

Women with PCOS haveahigh LH secretion with a low FSH secretion because of inappropriate gonadotropin secretion that considered as a main characteristic of PCOS, the increased secretions of gonadotropins is linked with increased activity of gonadotropin GnRH pulse generator and to pituitary response to GnRH (Panda *et al.*,2016).

Sheikhha *et al.*, (2007) reported that the LH/FSH ratio used for indicate abnormal gonadotropin secretion is normally 2–3/1.

Daniels and Berga, (1997) found that the hyperandrogenemia itself may be occurred due to hypothalamic desensitization to progesterone and estrogen negative feedback that increased gonadotropin secretion and hence forth ovarian androgen production, producing a self-driven viscous circle.

Rojas *et al.,* (2014) found that the hyperinsulinemia may increase the function of pituitary gland to change the frequency and amplitude of LH release to increase hyperandrogenism in PCOS. Hyperinsulinemia lead to inhibit a follicular development and ovulation as a result of high androgen and by changing gonadotropin (Barbieri,1986).

2.1.5.3. Defect in Ovarian Steroid Synthesis

The high secretion of GnRH in women with PCOS is still unclear, whether this secretion caused by the low levels of progesterone (Nelson *et al.,* 2001).

Nelson *et al.,* (2001) suggested that the thecal cells in PCOS, are more efficient to converted androgen precursors to a testosterone than the normal cells.

In PCOS, the peripheral metabolism of steroids hormone is altered, principally in muscular, adipose tissues and the pilosebaceous unit by increased the activity of 5á-reductase which converting testosterone into dihydrotestosterone, and this 5á-reductase activity mediated by IGF-1 and may be increased by hyperinsulinemia (Richardson, 2003).

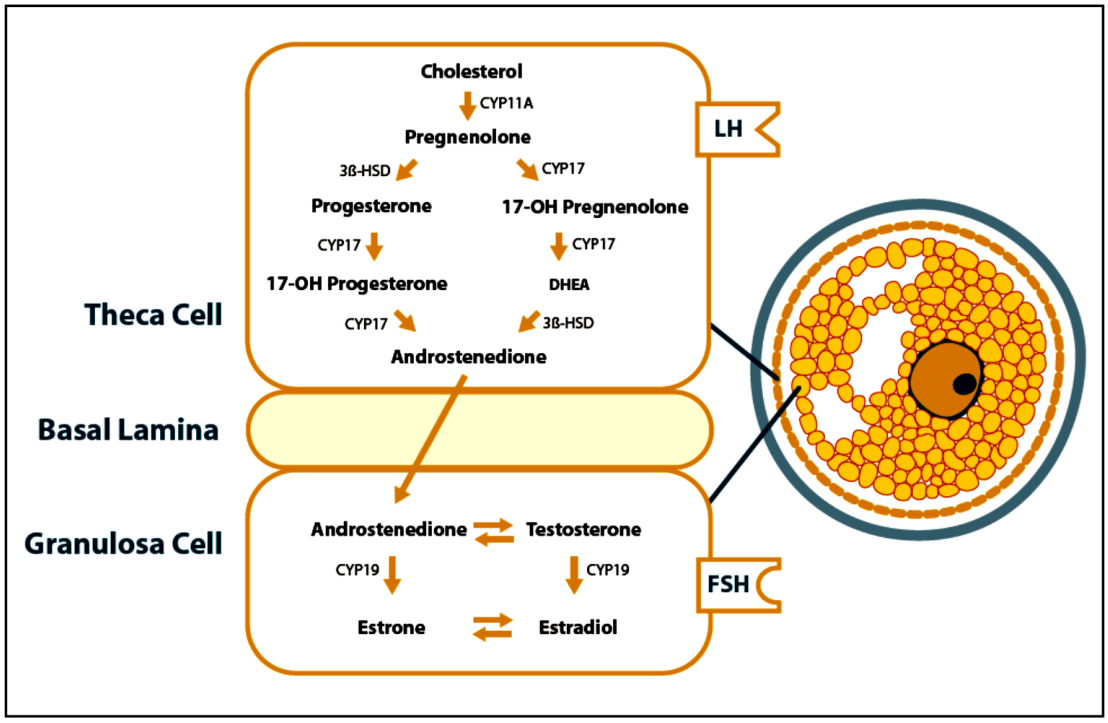
Taylor *et al.,* (1997); García-Rudaz *et al.,* (1998) and Balen, (2004) observed that PCOS women's have elevated LH serum concentration and increased LH pulse amplitude and frequency but then normal FSH levels, that lead to LH: FSH imbalance and raised up androgen synthesis.

Gilling-Smith *et al.,* (1997) and Nelson *et al.,* (1999) demonstrated that the theca cells of women with PCOS have intrinsic steroidogenic defect, and are hyper-responsive to gonadotrophins, lead to secrete excess androgens even in

normal LH levels, which might be further potentiated via increased insulin and LH concentrations.

Many authors demonstrated that the androgens stimulate the growth of smaller antral follicles, on the other hand inhibit the development of larger antral follicles (Jonard and Dewailly ,2004; Maciel *et al.*,2004; Duncan, 2014).

The elevated level of insulin, as a result of insulin resistance, acts synergistically with LH to increase the androgens production from ovarian theca cells by activation of 17α-hydroxylase, is a key enzyme in ovarian androgen



biosynthesis (Nestler ,1991). Figure (2.4)

Figure 2.4. Mechanism of the synthesis of ovarian steroids (Schmidt, 2011)

2.1.5.4. Peripheral Increase in Cortisol Metabolism

Ehrmann, (2005) observed that about 25% of PCOS women increased androgen production by adrenal glands possibly as a result of a genetic influence or secondary of abnormal secretion ovarian androgens. Usually the main source of androgen is ovary in PCOS women, also its found another source of androgen is adrenal gland that is about 40-70% of adrenal androgen level elevated in this patients particularly DHEA-S (1-3) (Wild *et al.*,1983).

2.1.6. Etiology of PCOS

2.1.6.1. Genetic of PCOS

PCOS origin is still not fully understood despite numerous studies on PCOS, the phenotype of the PCOS family group appears to be intergenerational with hyper androgen and insulin resistance as they are the most common characteristics (Alberti *et al.*,2009; Gonzalez ,2012; Li *et al.*,2014). Women's PCOS sisters increased the frequency of PCO morphology and hyperandrogen, decreased insulin sensitivity, high insulin concentrations and LH an increase in the proportion of obesity of women in the general population, in addition to PCOS siblings were found to have increased metabolic and hormonal disturbances, including high levels of TG and cholesterol, LH and FSH responses are elevated to stimulate GnRH agonist, higher DHEAS concentrations, IR and Insulin hyperactivity (Azziz,2006; Ehrmann *et al.*,2006).

Vink *et al.*, (2006) refers to the genetic factors that play an essential role in the etiology of PCOS, with 65% an estimated heritability. Furthermore, many authors mentioned that evidence of genetic influence includes familial collecting of PCOS, increased prevalence of type 2 diabetes mellitus and hyperandrogenemia in first-degree relatives of PCOS women (Ehrmann *et al.*, 2005). Moreover, Azziz *et al.*, (2009) reported that 35% and 40% of the mothers

and sisters of PCOS women's have been affected respectively.The major groups of PCOS candidate genes be studied and these genes linked with the steroidogenic pathway, metabolism, obesity and genes associated with inflammatory cytokines including fat mass–and obesity-associated gene (FTO), insulin receptor (INSR) can be important gene for PCOS filter, luteinizing hormone receptor gene (LHR) and follicle stimulating hormone receptor (FSHR) (Carey *et al.*,1994; Chen *et al.*, 2011, Du *et al.*, 2014, Jones and Goodarzi, 2016).

2.1.6.2. Prenatal Androgen Excess

Overall, the excess androgen exposure intra uterine and prenatal environment may be play a critical role in the development of PCOS that contributing to metabolic and reproductive dysfunction in offspring (Abbott *et al.*, 2005). IR, hyperandrogenism, PCO, increased luteinizing hormone (LH) concentrations, hyperlipidemia, glucose intolerance, and increased risk of T2DM are involved in reproductive and metabolic dysfunctions (Dumesic *et al.*, 1997, Abbott *et al.*, 1998, Eisner *et al.*, 2002, Birch *et al.*, 2003, Manikkam *et al.*, 2004, Recabarren *et al.*, 2005).

Barker, (2004) suggested that the maternal and fetal environment play an important role in adult developmental programming through genetic modification.

Wickstrom, (2007); Symonds *et al.*, (2009); Alfaradhi and Ozanne, (2011) and Lakshmy, (2013) noted that undernutrition among mothers and children, smoking, stress, and hormonal imbalance are one of the most recognized developmental studies of prenatal programming of PCOS. In a rodent models the degree of reproductive or/and metabolic dysfunction reliant on testosterone exposure dose (Foecking *et al.*, 2005, Wu *et al.*, 2010).

During gestation PCOS women are described to have elevated androgen levels and have higher concentrations of enzymes that convert unconjugated steroids into androstenedione and afterward testosterone in the placenta (Sir-Petermann *et al.,* 2002; Maliqueo *et al.,* 2013).

Also, some studies reported that female fetuses born from mothers with PCOS revealed high testosterone levels than male fetus's levels in the fetus umbilical vein (Barry *et al.,* 2011).

Palomba *et al.,* (2013) mentioned that the prenatal acrogenic hypothesis has been questioned as an etiological agent in PCOS, where the human fetus is protected from the effects of excessive maternal androgens by a combination of a high level of placental aromatase activity, which metabolizes androgens to estrogen, and increases the concentrations of plasma binding proteins, which reduce the free testosterone in the circulatory system. These changes suggest an increased capacity to maintain the androgenic state (Maliqueo *et al.*, 2013).

Furthermore, Barbieri *et al.,* (1986) found that women with gestational diabetes have a higher significant concentrations of T and DHT in amniotic fluid of their embryos (both male and female).

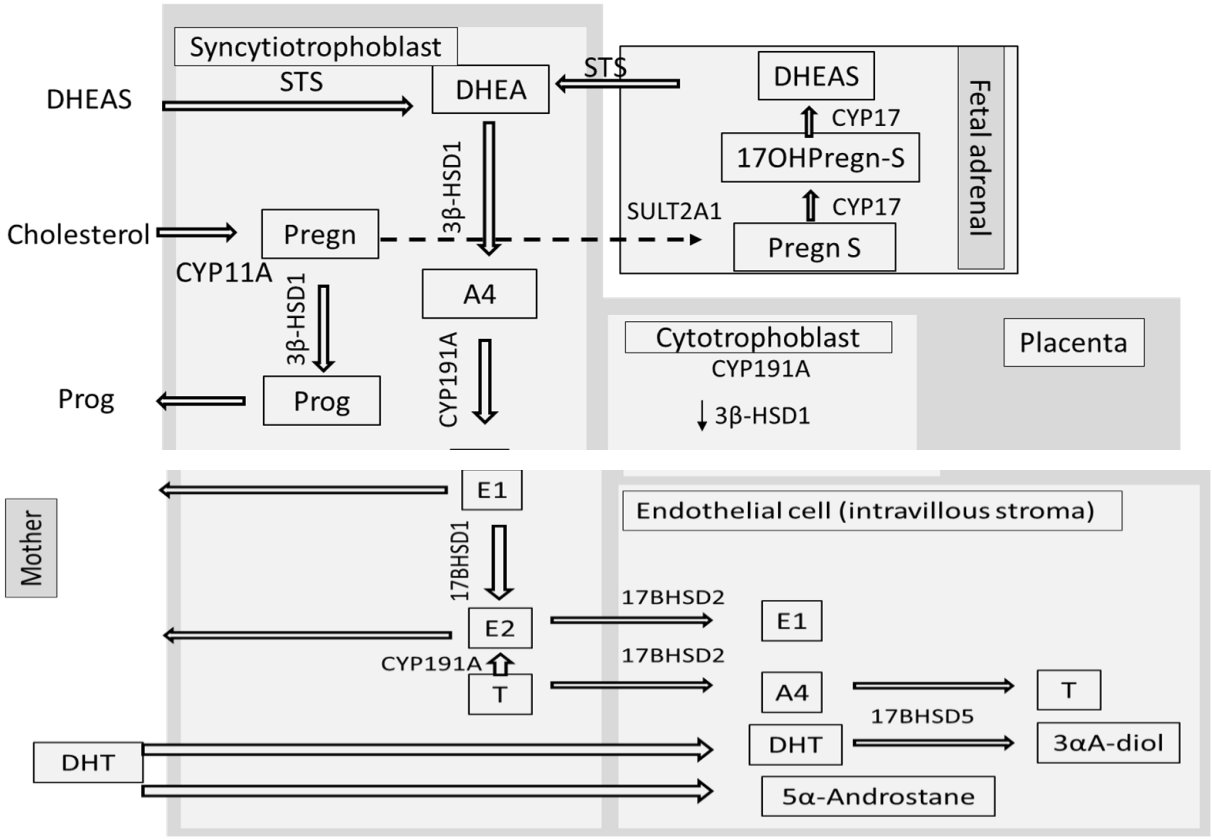


Figure 2.5. Simplified view of steroidogenesis pathways in human placenta.

(Fornes,2017)

2.1.6.3. Hypothalamic-Pituitary Ovarian Axis

Roland and Moenter, (2014) referred to the hypothalamic-pituitary axis (HPA) is a complex feedback circle containing of the hypothalamus (having gonadotropin releasing hormone (GnRH) neurons), pituitary gland (responsible for the secretion of FSH and LH) and the ovary, that responds to changes in gonadotropin concentrations by follicular maturation and ovulation. The abnormalities in the HPA occur in PCOS women's and these abnormalities lead to an increased GnRH pulse frequency and disruption in the release of FSH and LH leading to an increase in immature follicles on the ovary and dysfunction of menstrual cycle also, different studies have reported that an increase in GnRH, in PCOS women's (Moore and Campbell, 2017).

The defects in gonadotropin release are inherent to PCOS or secondary occurs under condition**,** there are two factors play important role in gonadotropin regulation are insulin and hyperandrogenism through hyperinsulinemia causes an elevated level of LH receptor expression and premature release of the follicle, which combine to cause follicular arrest and lead to subfertility or infertility (Diamanti-Kandarakis, 2008).

2.1.7. Metabolic Characteristics of PCOS

2.1.7.1. Insulin Resistance (IR)

Insulin resistance (IR) is the common metabolic disorder that supports the pathophysiology of PCOS, obesity, diabetes, metabolic syndrome, and the other various health complications (Peppa *et al.,* 2010). Insulin acts to stimulate the glucose uptake in peripheral tissues such as adipocytes and skeletal muscles, also acts to suppress hepatic glucose production to maintain blood glucose homeostasis (Janus *et al.,* 2016). Pancreatic β-cell insulin secretion is amplified as a result of IR to provide sufficient concentrations of insulin to stimulate action and realize glucose homeostasis, causing in compensatory hyperinsulinemia commonly detected in people with IR (Bergman *et al.,* 1985, Kahn, 1985). On other hand, Grundy, (2004) explained IR according to the (WHO) that glucose uptake (i.e. insulin sensitivity) less than quartile for population under hyperinsulinemic-euglycemic conditions. Some studies suggest that 10% of women with PCOS have diabetes, and 70% of them have been insulin-resistant (Ovalle and Azziz 2002; Azziz *et al.*, 2006; Majumdar and Singh, 2009). Munir *et al.,* (2004) referred that Insulin resistance is a common feature in PCOS women and in order to maintain glucose homeostasis are required a compensatory increase in circulating insulin concentration.

Perhaps, the increase in insulin secretion may be contributed to ovulatory cycles dysfunction, hyperandrogenism and changed follicular development in PCOS women see fig. (2.6) (Romualdi *et al.,* 2011). This hypothesis supported by many evidences studies by Dunaif *et al.*, (1996), Hasegawa *et al.*, (1999), La Marca *et al.,* (2000) concerning the role of insulin sensitizing medication, in women with PCOS that treated with thiazolidinediones (TZDs) or metformin, an improvement in peripheral insulin sensitivity is observed leading to restoration of an ovulatory cycles and reductions in androgen concentrations.

Metformin is one of the most common insulin-sensitizing agent used for the treatment of PCOS by suppressing hepatic gluconeogenesis and improving insulin sensitivity in peripheral tissues, reduces hyperandrogenemia and visceral adiposity by direct action on ovarian theca cells and also facilitates weight loss, (Moll *et al.*,2007; Palomba *et al.*,2008; Li *et al.*,2011; Tang *et al.*,2012). Also it is used for metabolic abnormalities in PCOS, the insulin sensitizer has also been clinically shown to improve hyperandrogenemia, reduce ovarian volume, and increase regularity of menstruation, TZDs namely rosiglitazone and pioglitazone, also it is used for decreasing IR and hyperandrogenemia, as well as restoring ovulation in PCOS (Sepilian and Nagamani ,2005; Sanoee *et al.*,2011, Suvarna *et al.,* 2016).Overall, the mechanisms by which insulin mediates in order to produced androgens in the ovary are unknown but, hyperinsulinemia lead to alterations in cytochrome P450c17(CYP-17), LH receptor, insulin receptor (INSR) and contributed to excess production of progesterone, testosterone and 17α-hydroxyprogesteroneas compared with the normal theca cells (Diamanti-Kandarakis and Papavassiliou, 2006; Diamanti-Kandarakis *et al.,* 2008).

Insulin resistance was defining as a decreased sensitivity of target organ tissues to the action of insulin, also IR is known as a reduced glucose response to a given amount of insulin, or IR is described as decreased insulin-mediated glucose uptake, hyperinsulinism is defined as a state of elevated insulin clinically or biochemically (hyperinsulinemia) (Essah and Nestler,2006). IR is measured by several tests, some of these measures are very dependable but a complex like the hyperinsulinemic glycemic glucose clamp and others less exact but easier like HOMA-IR, insulin resistance is a prevalent in PCOS women's independently of obesity and play critical role in the reproductive and metabolic complications of the syndrome, (Polak *et al.*,2017). The homeostasis model assessment (HOMA) determines IR and pancreatic β-cell function from basal glucose and insulin (or C-peptide) levels with a simple mathematically derived nonlinear equation (Matthews *et al;*1985)

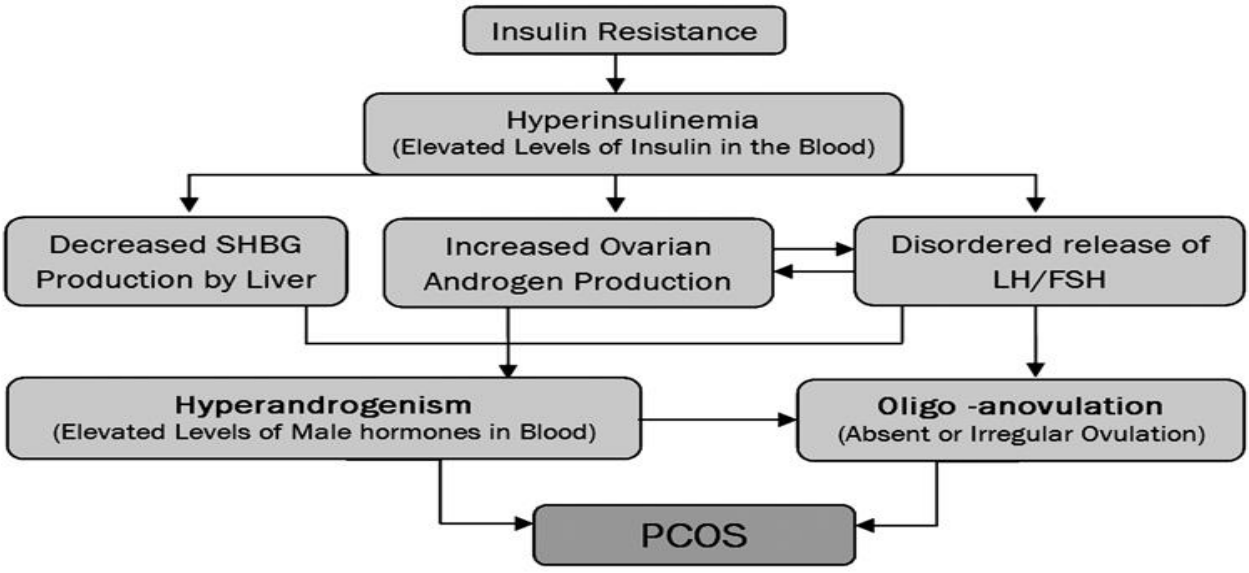


Figure 2.6. Schema of Insulin Resistance

(Savitha ,2015)

2.1.7.2. Diabetes Mellitus Type2 (T2DM)

Type 2 diabetic mellitus (T2DM) is a metabolic disorder characterized by distorted lipid profile and hyperglycemia caused by the pancreatic β cells being unable to secrete sufficient amounts of insulin to overcome insulin resistance (Nolan *et al.*,2011).

Legro *et al.*, (1999) estimated that PCOS patients in USA account about 40% of T2DM and 20% of IGT during the reproductive age. Furthermore, women with PCOS are a significant risk for developing gestational diabetes (Boomsma *et al.*, 2006; Ashrafi *et al.*, 2014).

A large-scale cohort study documented a significantly higher proportion (22%) of gestational diabetes mellitus(GDM) in women PCOS, compared to 7% in the general female population (De Wilde *et al.*, 2014; Minooee *et al.*, 2017). GDM, is a significant lifetime risk for developing T2DM, with 35% - 60% of women with GDM progressing to T2DM within 10 years after birth (Metzger *et al.*, 2007; Noctor and Dunne, 2015).

2.1.7.3. Metabolic Syndrome (Mes)

The National Cholesterol Education Program (NCEP)defines metabolic syndrome as many characteristics including fasting glucose, blood pressure, waist circumference, and lipid standards that are related to triglyceride and HDL- cholesterol (Expert Panel on Detection, 2001).

Metabolic syndrome (Mes) is also called insulin resistance syndrome or X syndrome inside the population diabetes (Reaven,1988; Moran *et al.*, 2010). Recent evidence submits that some confusing factors, including the study method and some characteristics of anthropometrics, e.g. age and body mass

index (BMI) have a serious impact on outcomes (Hart *et al.*, 2011; Tehrani *et al.*,2014). On the other wise, Eckel *et al.*, (2005) explained that Mes is a cluster of metabolic disorders include:

- Insulin resistance,
- T2DM
- Hypertension,
- Obesity,
- Dyslipidemia,
- CVD

2.1.7.4. Obesity

Women with PCOS are exposure to weight gain leading to increase the prevalence of PCOS due to the relationship between obesity and PCOS (Teede, 2013; Shorakae *et al.*, 2014,).

Lim *et al.*, (2012) found that a strong relationship between obesity and PCOS in spite of their different ethnicity nearly more than 61% of women with overweight or obese, however, the causes and their relationships are still unknown.

Borruel *et al.*, (2013) demonstrated that women with PCOS have increased visceral or abdominal fat accumulation. In the face of no known evidence of more visceral/abdominal fat accumulation in PCOS womens, many authors noted that the visceral fat has an increased lipolytic activity in response to catechol amines that lead to increase the release of free fatty acids (FFA) to the liver via portal circulation causing hepatic lipotoxicity and insulin resistance (Dicker *et al.*,2009; Samuel *et al.*,2010).

Elbers *et al.,* (1997) mentioned that a testosterone could have a role in visceral fat accumulation as demonstrated in iatrogenic hyperandrogenism in female-to- male transsexuals who were exposed to testosterone. Also, rodent models support a direct role for androgen excess in the accumulation of abdominal fat (Benrick *et al.*,2017). Previous study found that obesity-induced hyper androgenic anovulation is associated with 20 times greater levels of insulin and is reversed in transgenic littermate's mice lacking the insulin receptor in theca cell (Wu *et al.*,2014).

Obesity is associated with a reduction in infertility treatment (Pasquali *et al.,* 2006). Weight loss in obese women may be improving significantly the effects of PCOS and their characteristics (Barber *et al.*, 2007).

2.1.7.5. Dyslipidemia

Dyslipidemia is a type of qualitative and progressive lipid disorders that reflect structural disorders, metabolism, and biological activities of both arterial lipoproteins, which include decreased levels of anti-sclerosis, high-density lipoprotein (HDL) cholesterol and increased levels of lipoprotein B, TGs, VLDL, and LDL cholesterol (Kaur,2014).

Dyslipidemia is more common in PCOS, independent of insulin resistance, BMI and obesity that exacerbates fat disorders (Wild *et al.*,2011). It is estimated that 70% of PCOS patients exhibit abnormal serum fatty levels (Diamanti-Kandarakis *et al.,* 2007). The increase in TGs, LDL cholesterol levels and low HDL cholesterol levels are often associated with women with PCOS (Diamanti-Kandarakis *et al.*,2007; Wild *et al.*,2011).

Shamdeen and Mohammad, (2007) mentioned that women with hyperandrogenism have a high TG and VLDL cholesterol levels, but low HDL cholesterol levels. Excess androgen and insulin resistance lead to deposition of

fat in the abdominal region which facilitates production of androgen by their ovaries and adrenal glands (Escobar-Morreale and San Millán, 2007; Saleem, 2017).

Crespin *et al.,* (1973) mentioned that the main cause of dyslipidemia in women under 40 years of age may be PCOS. Wild, (2012) suggest that the causes of dyslipidemia in PCOS are hyperandrogenism and IR.

2.1.7.6. Cardiovascular Disease (CVD)

CVD is the most reasons of death in human, patients with clinical signs of PCOS suffer from early clinical signs of arteriosclerosis, increased hypertension and arterial vascular weakness, compared to general women, but that the PCOS phenotype, age, BMI and ethnicity may vary with cardiovascular risk profiles (Toulis *et al.*,2011; Gunning and Fauser, 2017; Pinola *et al.,* 2017; Roth *et al.*, 2017;). In addition, PCOS women were found to have elevated signs of low-grade chronic inflammation, including CRP, fibrinogen and white blood cells, which also implicated in cardiovascular disease (Orio *et al.*,2005). Other studies referred that the inflammatory biomarker included a high-sensitivity CRP have association with CVD risk (Ridker and Silvertown ,2008; Puri *et al.*,2013; Halcox *et al.*,2014; Quaglia *et al.*,2014). Obesity, IR, T2DM, and dyslipidemia are metabolic imbalances which they are occur in women with long period of PCOS leading to CVD (Gunning and Fauser, 2017).

Ouyang *et al.*,*(* 2009); Laughlin *et al.*,*(* 2010); Macut *et al.*,(2015) observed that increased levels of androgen can contribute to the risk of cardiovascular disease in PCOS women and stimulate androgen inflammation and oxidation in the lining of blood vessels, as well as stimulate the re-absorption water and sodium by the kidneys ,which contributes to the development of atherosclerosis and hypertension respectively ,also found that it has been shown that females at

high levels of testosterone contribute to atherosclerosis and the progress of cardiovascular disease.

2.2. Prolactin

Prolactin is a peptide hormone secreted and synthesis by pituitary gland, and suppressed by dopamine via the portal venous system (Melmed ,2003).

Frantz and Kleinberg, (1970) observed that estrogen, dopamine receptor antagonists, thyrotropin-releasing hormone and epidermal growth factor are factors inducing synthesis and secretion of prolactin.

Klibanski, (2010) observed that a prolactin negatively controls the secretion of pituitary hormones that responsible for gonadal function, including follicle stimulating hormone and luteinizing hormone.

Ben-Jonathan *et al.,* (1996) mentioned that a variety of etiological factors may be leading to hyperprolactinemia disorders including of hypothalamo-pituitary axis disorders, polycystic ovary syndrome, , stress, interruption of dopamine synthesis, primary hypothyroidism, pituitary tumors and different medications .Nevertheless, the etiology of hyperprolactinemia may be leading to infertility, hypogonadism and agalactorrhea, or it may remain asymptomatic (Schlechte, 2003; Klibanski, 2010).

Vallette-Kasic, (2002) found that asymptomatic patients with complete gonadal and reproductive function and moderately elevated prolactin levels may be had macroprolactinemia.

Prolactin is under dual control by hypothalamic hormones carried through the hypothalamic–pituitary portal circulation (fig.2.7).

Verhelst and Abs, (2003) mentioned that the major signal that preventing, inhibitory prolactin release is mediated by the neurotransmitter dopamine also, observed that the stimulatory signal is mediated by the hypothalamic thyrotropin

releasing hormone (TRH), therefore the amount of prolactin released from the anterior pituitary gland determined through, the balance between the two opposite signals.

Gillam *et al.*, (2006) mentioned that hyperprolactinemia may be developing as a result of pathological or pharmacological interruption of hypothalamic-pituitary dopaminergic pathways and is occasionally idiopathic.

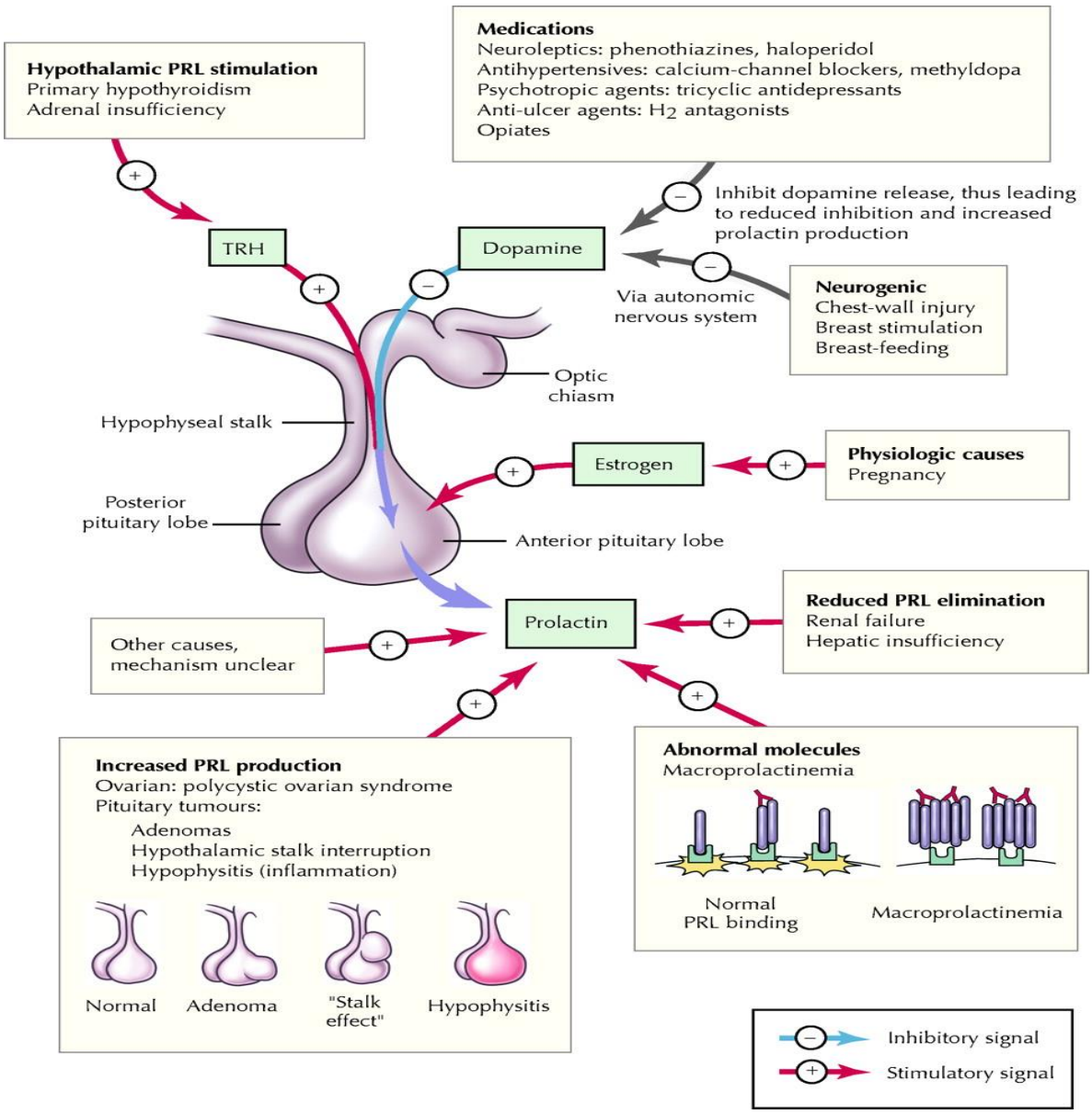


Figure 2.7. Causes of hyperprolactinemia.

(Serri *et al.*,2003)

2.3.C-Reactive Protein

CRP is an acute-phase protein released by the liver during chronic inflammatory disorders and in response to infection, CRP is in widespread clinical use as a marker of inflammation (Newling *et al.*,2018). In addition to, PCOS women were found to have elevated signs of low-grade chronic inflammation, including C-reactive protein (CRP), fibrinogen and white blood cells, which were also implicated in cardiovascular disease (Orio *et al.*,2005).

Many studies refer to the metabolic syndrome, T2DM and PCOS have similar profiles of inflammatory hallmarks, and as well as known to influence on insulin resistance (González, 2012). Beside a chronic low-grade inflammation, endothelial dysfunction may be contributing to the development of PCOS (Roby and Terranova, 1990, Päth *et al.*, 1997).

Nehir Aytan *et al.,* (2016) mentioned that in the clinical studies, PCOS women had significantly higher levels of plasma inflammatory markers like TNFα and CRP in comparison to women without PCOS also, anti-inflammatory cytokines including interleukin-37, interleukin-35 and interleukin-27, were all declined in these PCOS women.

González *et al.,* (2014), suggested that the diet may be also contribute to inflammation in women with PCOS, on the other hand glucose intake stimulates the oxidative stress and release of CRP, TNF-α and IL-6. So, inflammation could also explain why women with PCOS are a higher risk of cardiovascular disease and dyslipidemia (Maleedhu *et al.*, 2014).

2.4. Microalbuminuria (MAU)

Microalbuminuria is a urine albumin-to-creatinine ratio (UACR) which is range between (30–300) mg/dl and is used as an early marker of endothelial damage of renal glomeruli (Ekblad *et al.*,2018). Microalbuminuria (albumin level increased in urine) is one of the most key characteristics of diabetic nephropathy (Klausen *et al.*,2004).

Van de Wal *et al.*, (2005) observed that the prevalence of microalbuminuria is 6% -8%, in the general population, while this percentage increased in patients with diabetes and hypertension about 10% -15% and 15% -20%, respectively also, observed that the etiology of microalbuminuria remains unclear.

Xia *et al.*, (2015) mentioned that MAU is used to be a marker of early renal damage from hypertension, and it is related with the increased risk of cardiovascular disease. The clinical and epidemiologic evidence shows that the microalbuminuria is linked with an increased risk for all-causes and cardiovascular mortality (Gerstein *et al.*,2001). Overall, there is a paucity of data concerning the relationship between PCOS and microalbuminuria (Patel *et al.*,2008; Duleba and Ahmed,2010; Caglar *et al.*,2011). A microalbuminuria (MAU) test was determined the micro albumin in urine sample a randomly (Mathiesen *et al.*,1990).

3. Materials and Methods

3.1 Materials

3.1.1. Subjects

The current study involved 120 women at the age of (35-45) years, divided into four groups and each group has 30 women as the following:

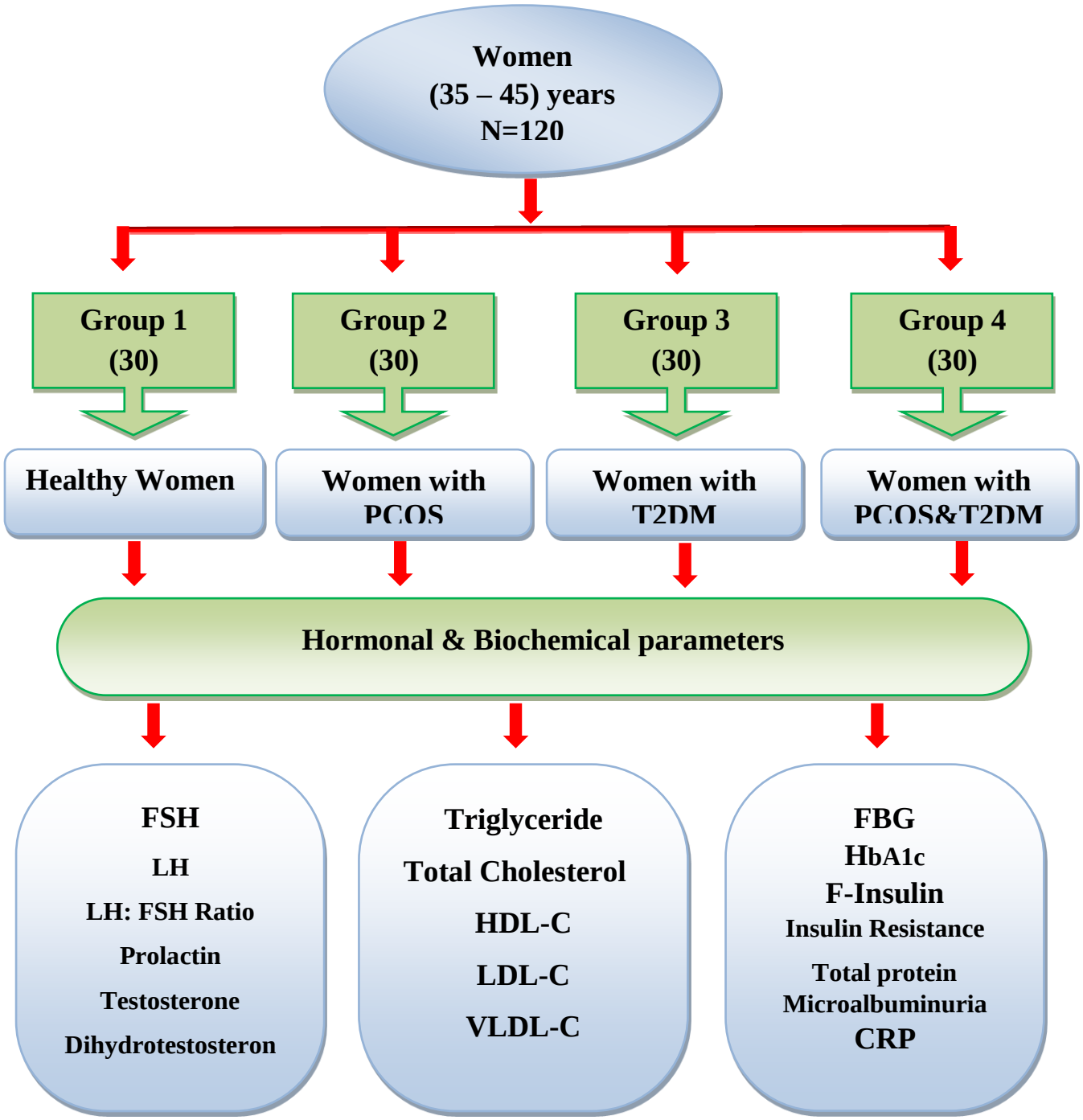
- First group (control group) healthy women.
- Second group women with polycystic ovary syndrome(PCOS).
- Third group women with type2 diabetes mellitus T2DM.
- Fourth group women with PCOS and T2DM.

Women with PCOS have been checked medically by ultrasound waves to confirm that they have PCOS by radiologist. In the other hand some women were excluded because they suffer from hypertension, thyroid disease, pituitary tumors, women taking oral contraceptives and other hormonal drugs. The practical part was carried out in diabetes and endocrines center and the central health laboratory, in addition to Dejla private laboratory of Misan province. A questionnaire was designed to obtain the actual information of women with PCOS, as set out in the appendix.

Women's with PCOS were diagnosed by using the Rotterdam ESHRE/ASRM criteria from 2003, that including at least two of the following:

- Polycystic ovaries on ultrasound,
- Oligo- or anovulation
- Biochemical and clinical signs of hyperandrogenism (Bremer and Miller ,2008).

3.1.2. Experimental design



3.1.3. Instruments and Equipment

The instruments and equipment that used in this study with their companies and countries of origin are listed in the table (3-1).

Table 3.1. The instruments and equipment's used in this study

No.	Instrument	Company/Origin
1.	AIA.360	TOSOH 15784011, Japan
2.	BLOOD ROLL MIXER KJMR-II	Shanghai, China
3.	Chest Freezer	BD-GL340LX,China
4.	Cotton	M.O.H ,Iraq
5.	D 10-HBA1c	BIO-RAD ,France
6.	Digital Camera	Sony/ Japan
7.	Digital Timer	USE
8.	EDTA tubes	M.O.H/ China
9.	Enzyme-linked Immunosorbent Assays (ELISA)	BioTek Instruments ,216270, U.S.A
10.	Eppendorf Tubes(1.5 ml)	Star Lab/ UK
11.	Gel tubes (SSGT tubes)	M.O.H/ China
12.	I chroma CRP Reader	Boditech, PFR10F181282,Korea
13.	Lab incubator	Gemmy Industrial Corp.705065,Taiwan
14.	Micro Pipetes volumes(0-10)ml,(1-20)ml,(20-100)ml and(100-1000)ml.	Eppendorf Gilson/ Germany
15.	Plain tubes	AFMH, England
16.	Power Spin ™ Centrifuge	UNICO,L0805152 ,USA
17.	Staining rakes	Meheco , China
18.	Syringe(10ml)	Zhejiang INI Medical Devices/China
19.	TG Gloves	Malaysia
20.	Tips(10ml,20ml,100ml and 200 ml)	Star Lab/ UK
21.	UV-VIS Spectrophotometer	APEL PD-303 ,306071,Japan
22.	Water Bath	Memmert, L-409-0938, Germany

3.1.4. Kits

The kits used in this study are listed in the table (3-2), with producing companies and countries:

Table 3.2. Kits and their supplies

No.	Material (Kits)	Company/Origin
1.	Cholesterol manual	Spinreact / Spain
2.	C-reactive protein (CRP) manual	Boditech, ,Korea
3.	CRP manual	Spinreact / Spain
4.	Follicle Stimulating Hormone (FSH) Enzyme test	Tosoh Bioscience / Japan
5.	Glucose manual	Spinreact / Spain
6.	High density lipoprotein (HDL-C) manual	Spinreact / Spain
7.	Human Dihydrotestosterone (DHT) ELISA	My BioSource,266535/USA.
8.	Insulin Enzyme test	Tosoh Bioscience / Japan
9.	Luteinizing Hormone (LH) Enzyme Immunoassay	Tosoh Bioscience / Japan
10.	Microalbuminurea (MAU) manual	Boditech, ,Korea
11.	Prolactin Hormone Enzyme Immunoassay	Tosoh Bioscience / Japan
12.	Testosterone Enzyme test	Tosoh Bioscience / Japan
13.	Total protein manual	Spinreact / Spain
14.	Triglyceride manual	Spinreact / Spain

3.1.5.1. Diagnostic Kits

3.1.5.2. TOSOH Kit

1. ST AIA-PACK Cup
2. Calibration Set
3. Sample Diluting Solution
4. Substrate Set II
5. Wash Concentrate
6. Diluent Concentrate

3.1.5.3. MAU Kit

The contents of MAU kit, are listed in the following table (3-4).

Table 3.4. The components of i chroma ™ Microalbuminuria

No.	Components	Numbers
1-	Cartridges	25
2-	Detection Buffer Tubes	25
3-	ID Chip	1
4-	Instruction For Use	1

3.1.5.4. CRP Kit

The contents of CRP kit, are listed in the following table (3-5).

Table 3.5. The components of ichroma ™ C-Reactive Protein (CRP)

No.	Components	Numbers
1-	Cartridges	**25**
2-	Detection Buffer Tubes	**25**
3-	ID Chip	**1**
4-	Instruction For Use	**1**
5-	Sample Collector	**25**

3.1.5.5. HbA1c Kit

The D-10 HbA1c Kit contains are listed in the following table (3-6).

Table 3.6. The components of HbA1c Kit

No.	Item	Specification
1-	Calibrator/Diluent Set، Hb A2/F/A1c	Calibrator Level 1: 3 vials (7 mL) Calibrator Level 2: 3 vials (7 mL) Calibrator Diluent: 1 bottle (100 mL)
2-	Elution Buffer1	2 bottles(2000 mL)
3-	Elution Buffer2	1 bottle (1000mL)
4-	Floppy Diskette	kit-specific D-10 Dual Program Parameter information.
5-	Sample Vials	Two packs (1.5ml)
6-	Thermal Paper	Box of 10 rolls
7-	Wash/ Diluent Solution	1 bottle(1600mL)
8-	Whole Blood Primer	4 vials (1.0 mL)

3.2. Methods

3.2.1. Sample Collections

3.2.1.1. Blood Samples

About 10 ml of venous blood was withdrawn by a medical syringe on the follicular phase (2-3) day of the menstrual cycle at 9 a.m. of each subject (patients and controls). The blood sample was divided into: (2 ml) put in EDTA tubes after being gently shaken to prevent blood clotting and used to analyze HbA1c and (8 ml) put in gel tube for 20 minutes at room temperature for clotting. Then, centrifuge at 3000 rpm for 10 minutes to collect the serum, some serum used for the purpose of biochemical tests including fasting blood sugar tests, lipid profile and CRP within 24 hours. Serum remained put in labeled Eppendorf tubes and it was given a serial number together with the patient's names and then stored in the freezer at (-20) ºC until using for measurement of hormones concentration.

Insulin resistance was measured through the HOMA-IR index, as follows: HOMA-IR = fasting Insulin (mg/dl) x fasting glucose (mg/dl) /405.

3.2.1.2. Urine Samples

Urine samples (5 ml) of all participants (patients and control) were obtained in a suitable plastic tube for urine examination. The urine was then centrifuged at 3000 rpm for 10 minutes after it was analyzed.

3.2.2. Evaluation of Reproductive Hormones Assay

The levels of reproductive hormones (FSH, LH, PRL and T) be measured by using immune enzymometric assay in Dejla private laboratory, while the DHT hormone level be measured by used the ELISA system in the central health laboratory in Misan province/Iraq.

3.2.2.1. Determination of Dihydrotestosterone (DHT) Hormone

DHT was determined by using enzyme-linked immunosorbent assay (ELISA) system, with human DHT ELISA kit.

3.2.2.1.1. Principle of Assay.

The ELIZA kit uses double-sandwich ELIZA technique and the ELISA Kit provided are typical. Fig. (3.1).

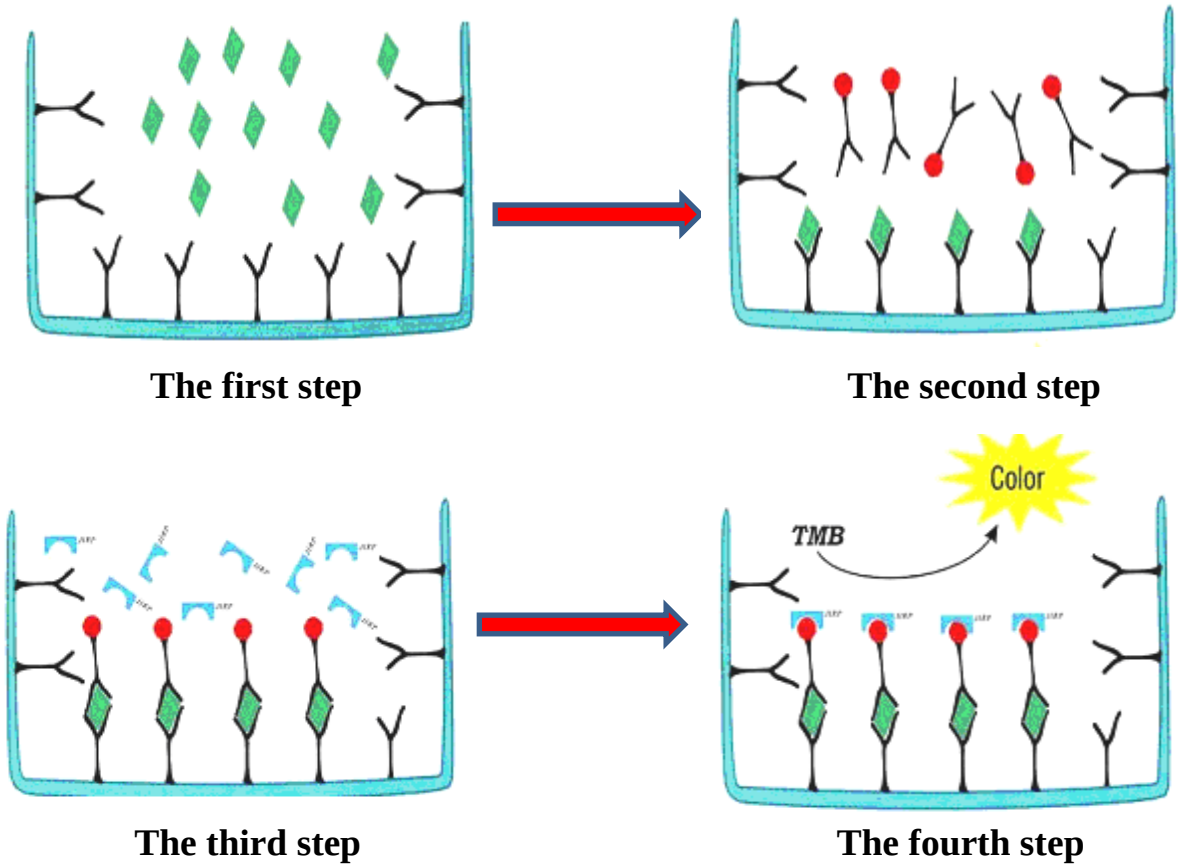


Fig. 3.1. Diagram of DHT ELISA kits.

3.2.2.1.2. Preparation of Reagents

- The Elisa Kit put out of refrigerator for 20 minutes.
- The concentrated washing solution diluted with double distilled water (1:25).
- Human DHT standard sample: The diluent 1.0ml added into Human DHT lyophilized standard sample and it was kept for 30 min. After the sample completely dissolved, it mixed slightly and label on the tube (1) was put , then worked dilution as needed.

  (It is recommended to use the following concentration value to standard curve: 2000,1000,500, 250, 125, 62.5, 31.2pg/ml). The lyophilized standard completely dissolved and well mixed.
- Legend of standard sample dilution method: Seven clean tubes were taken and label them with 2,3,4,5,6,7,8 respectively. A 300µl standard sample diluent added into each tube. Pipette out 300µl diluent from tube 2 to tube 3 and mix well. Further Pipette out 300µl diluent from tube 3 to tube 4, and mixed well. The above steps are repeated to tube 7. Standard sample dilution in tube 8 has negative control.

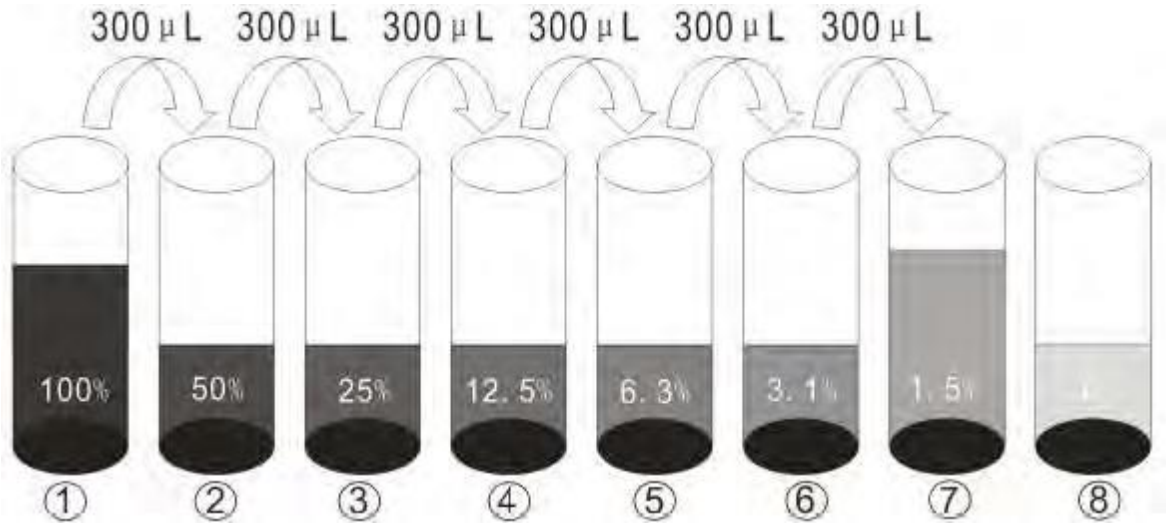


Figure 3.2. Show the steps of reagents preparation.

- Biotinylated Human DHT antibody liquid:

antibody diluent was employ to dilute the concentrated biotinylated antibody (1:100) to form biotinylated antibody liquid. The preparation should be done 30 min in advance. And it's only for use on that day

- Enzyme-conjugate liquid: Referring to needed amount, the concentrated enzyme-conjugate diluted by enzyme-conjugate diluent (1:100) to form enzyme-conjugate liquid. The preparation should be done 30 min in advance. And it's only for use on that day.
- Colour Reagent liquid: Colour Reagent liquid prepared for 30 min in advance with Colour Reagent A and Colour Reagent B by the proportion of 9:1. Fig. (3.2)

3.2.2.1.3. Procedure Assay of ELISA Method

- One hundred microliter (100 µl) sample and Human DHT standard samples was added to each well, and incubated at 37℃ for 90 min.
- Biotinylated Human DHT antibody liquid prepared in 30min in advance.
- The Elisa plate washed 3 times
- The biotinylated Human DHT antibody liquid (100µl) was added to each well and then incubated at 37℃ for 60min.
- The enzyme-conjugate liquid prepared 30min in advance.
- The Elisa plate 3 times washed
- The enzyme-conjugate liquid (100µl) was added to each well except blank wells then incubated at 37℃ for 30 min.
- The Elisa plate washed 5 times
- Colour Reagent liquid (100µl) was added to individual well (also into blank well), then incubated at 37℃.
- Colour Reagent C (100µl) was added then the results were read in OD（450nm）within 10 min, see fig. (3.3).

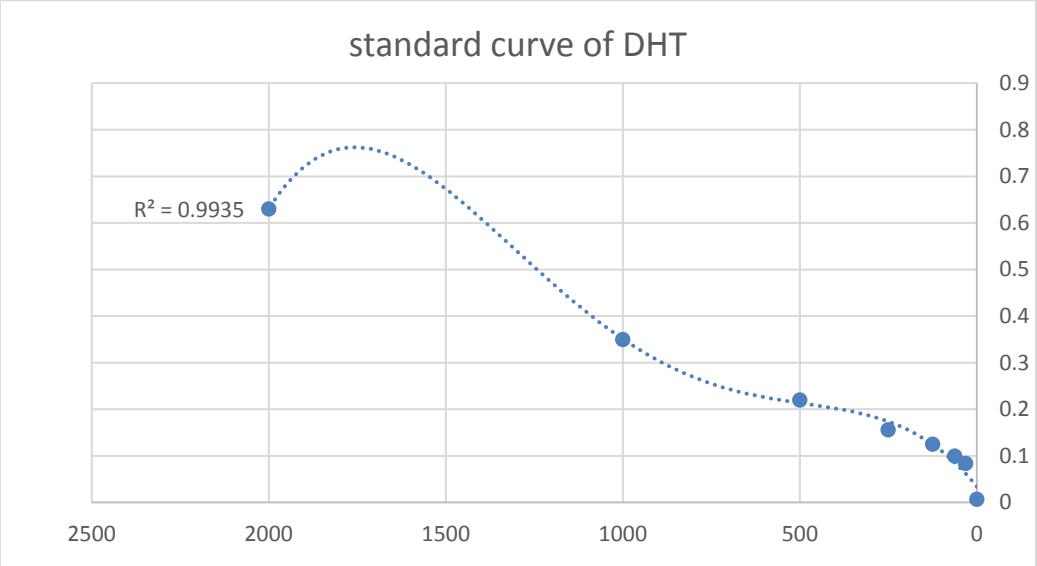


Figure 3.3. Show the standard curve to estimate the DHT

3.2.2.2. Determination of Testosterone (T) Hormone

Testosterone was performed in Dejla private laboratory. The assays were performed on an AIA-360 (TOSOH Bioscience, Japan) see fig. (3.5).

3.2.2.2.1. Principle of Testosterone Assay

ST AIA-PACK Testosterone is a competitive enzyme immunoassay which is executed entirely in the ST AIA –PACK Testosterone test cups, fig. (3.4). Testosterone presented in the test sample competes which enzyme –labeled testosterone for a limited number of binding sites on the testosterone specific monoclonal antibody immobilized on a magnetic solid phase. The magnetic beads are washed to remove unbound enzyme-labeled testosterone and then are incubated with a fluorogenic substrate,4-methylumbelliferyl phosphate(4MUP). The amount of enzyme-labeled testosterone concentration in the test sample (Fiet *et al.*,1994).

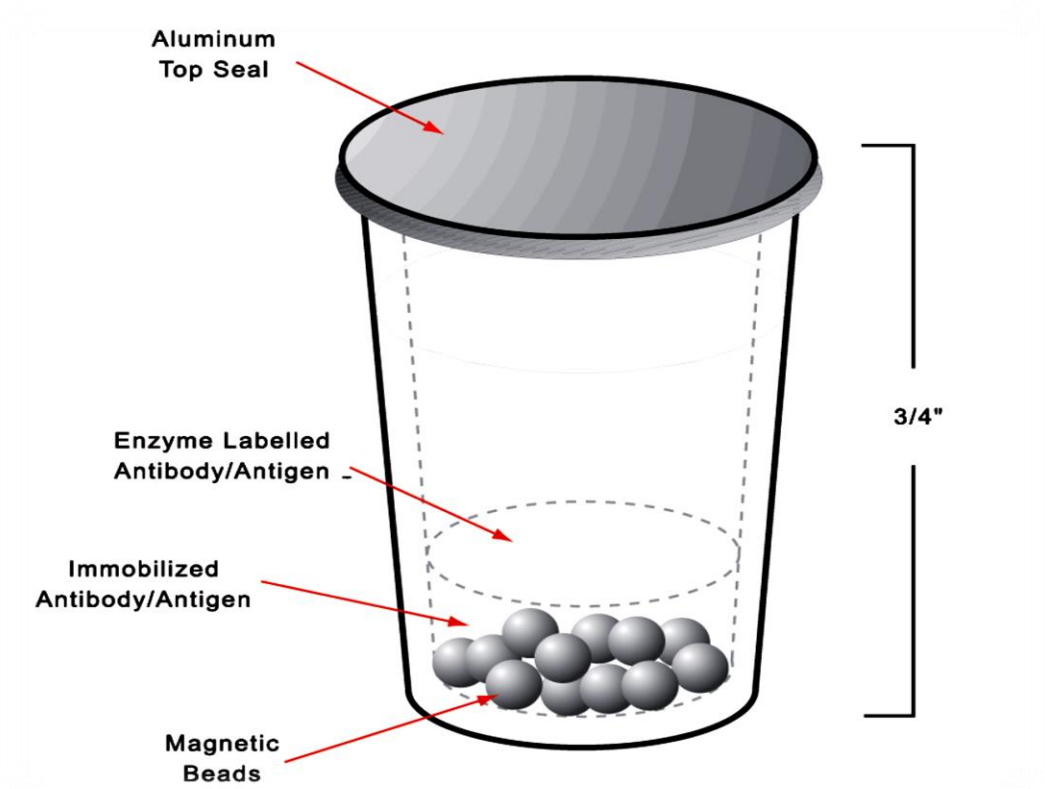


Figure 3.4. Test Cup Diagram.

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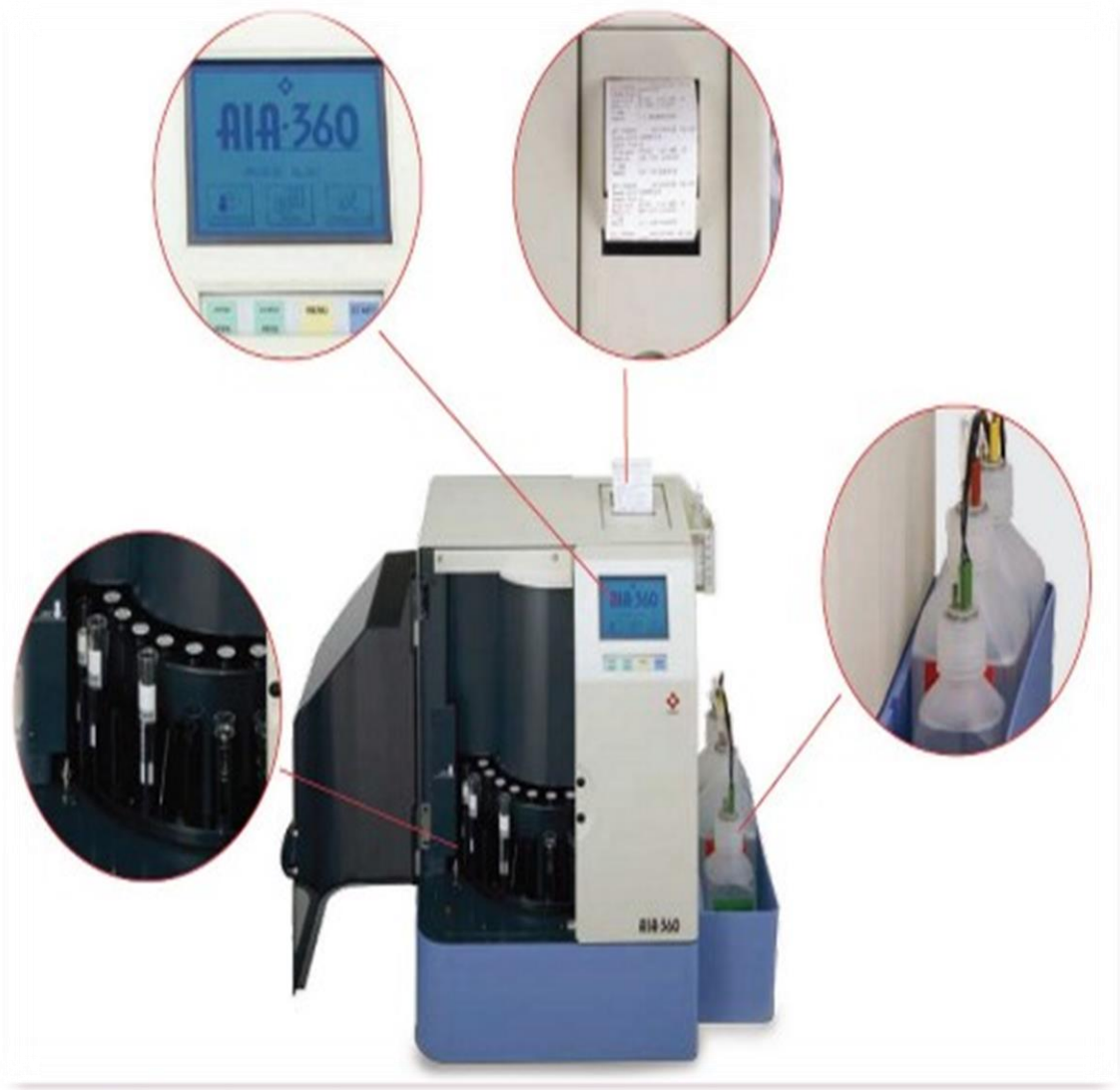


Advanced Sample Carousel Convenient Reagent Access

Figure 3.5.AIA-360.

3.2.2.2.3. Procedure

- The testosterone kit put out of refrigerator for 10 minutes.
- The reagents are placed in their place on the side of the external device.
- Five hundred (500 µl) of sample was taken by micro pipette and place it in the Hitachi sample cap and put it in the place known inside the device.
- ST AIA-PACK Testosterone was put in the place known within the device.
- Testosterone was choosing from the screen of the device.
- The steps for the device followed to start the calibration process automatically, which takes about an hour.
- When the examination is complete, both the test cup and the Hitachi sample cap are removed from the device.

3.2.2.2.4. Calculation of Result

The TOSOH AIA System Analyzers implement all reagent and sample handling operations automatically. The TOSOH AIA System Analyzers was read the rate of fluorescence formed by the reaction and automatically convert the rate to testosterone concentration in ng/dL.

3.2.2.3. Determination of Follicle-Stimulating Hormone (FSH)

FSH was performed in Dejla private laboratory. The assays were performed on an AIA-360.

3.2.2.4. Determination of Luteinizing Hormone (LH)

LH was performed in Dejla private laboratory. The assays were performed on an AIA-360.

3.2.2.5. Determination of prolactin (PRL)

PRL was performed in Dejla private laboratory. The assays were performed on an AIA-360.

3.2.3. Determination of Biochemical Parameters

3.2.3. 1. Determination of Insulin

Insulin was performed in Dejla private laboratory. The assays were performed on an AIA-360.

3.2.3.2. Determination of Glucose

Glucose was determined by spectrophotometric method.

3.2.3.3. Determination of Cholesterol

Cholesterol was determined by spectrophotometric method.

3.2.3.4. Determination of Triglyceride

Triglyceride was determined by spectrophotometric method.

3.2.3.5 Determination of HDL-Cholesterol

HDL was determined by spectrophotometric method (Naito, 1984.).

3.2.3.6. Determination of LDL- Cholesterol

The LDL was determined according to Friedewald formula

LDLc = Total cholesterol - HDLc - (TG/5) (Friedewald *et al.*,1972).

3.2.3.7. Determination of Total protein

Total protein was determined by spectrophotometric method by using a kit from SPINREACT company in Spain.

3.2.3.9.CRP and MAU

3.2.3.9.1. I-CHROMA™ CRP and MAU Principle

I-CHROMA™ CRP and MAU used a sandwich immunodetection method, such that in the test vial mixing the blood sample with the detection buffer, the CRP or MAU antigen in the blood sample binds to the fluorescence labeled detector anti-MAU or anti-CRP antibody in the buffer.

The sample mixture is loaded and transfers on the matrix of the test cartridge; the complexes of the detector antibody and CRP or MAU are captured to the anti-CRP or MAU sandwich pair antibody that has been immobilized on the test matrix. The fluorescence intensities are converted into the CRP or MAU concentration calculated by pre-programmed calibration process. The result of the tests is displayed on the reader as mg/dL for microalbuminuria and Mg/L for CRP (Waugh *et al.,* 2003, Oh *et al.*,2005).

3.2.3.9.2.MAU Test Procedure:

• Ten µl of test sample was draw.
• It into the detection buffer tube added.
• The tube shacked up and down 10 times or more.
• About 75 µl of sample mixture draw.
• The sample mixture load onto the test cartridge.
• The incubated for 12 minutes.
• The test cartridge inserted into reader and ‘select’ pressed
• The result was read.

3.2.3.9.3. CRP test procedure:

• Whole blood sample draw to use the sample collector.
• The sample collector assembled with detection buffer tube.
• the tube Shacked up and down 10 times.
• The two drops discarded.

• The two drops apply to the test cartridge.

• The test cartridge inserted into reader and press 'select'.

• The waited 3 minutes.

• The result was read.

3.2.3.10. HbA_{1c}

3.2.3. 10.1. Principle of the Procedure

The D-10 Dual Program is based on chromatographic separation of the analyses by ion-exchange high –performance liquid chromatographic (HPLC). The samples are automatically diluted on the D-10 and injected into the analytical cartridge. The D-10 delivers a programmed buffer gradient of increasing ionic strength to the cartridge, where the hemoglobin is separated based on their ionic interactions with the cartridge material. The separated hemoglobin then bass through the flow cell of the filter photometer, where changes in the absorbance at 415 nm are measured. A calibrator is analyzed with each run for adjustment of the calculation parameters for determination of HbA1c. the value recorded is in percent (Rohlfing *et al.*,2008).

3.2.3.10.2. PROCEDURE

- ✓ The HBA1c kit put out of refrigerator for 10 minutes.
- ✓ The reagents are placed in their place on the side of the external device.
- ✓ The two milliliter (2ml) of blood was taken by medical syringe and place it in the EDTA tube and then, the sample mixed gently by inverting the tube.
- ✓ The sample tubes allowed to reach the room temperature (25^0C) before performing the assay.
- ✓ The sample tubes are loaded into the D-10 sample rack and put it in the place known inside the device D-10.
- ✓ Patient/QC IDs was appearing on the screen after they have been scanned by the barcode reader.

- ✓ The **DONE** button was press after you have entered each patient ID.
- ✓ The **START** button was press to begin the analysis.
- ✓ The steps for the device followed to start the calibration process automatically.

3.3. Statistical Analysis

The results are expressed as Mean ± Standard Division (SD). Students ANOVA test and Duncan's were used to analyze results by using Statistical Package for the Social Sciences (SPSS) version 22.0. P-value ≤ 0.05 was considered significant.

4. Results

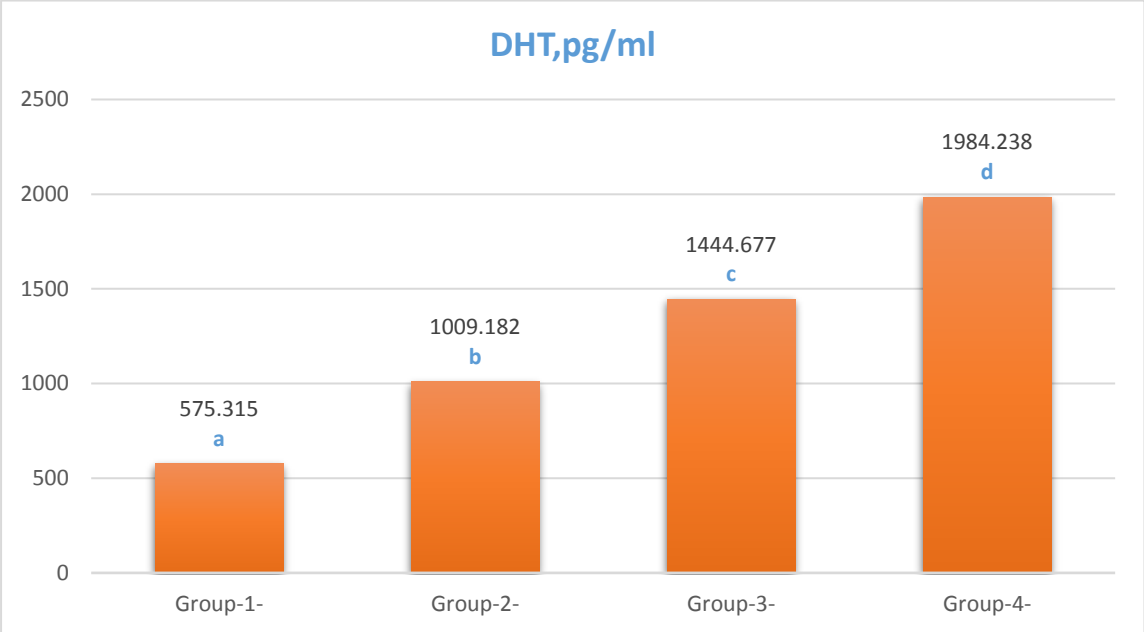
4.1. Hormonal Parameters:

4.1.1. Dihydrotestosterone (DHT)

The results revealed that the value of DHT in the second group (1009.182 ±0.369) pg/ml increased significantly in comparison with the control group (575.315±0.482) pg/ml.

In third group (1444.677 ±0.663) pg/ml a significant increase ($p \leq 0.05$) was observed in comparison with the control and second groups.

In fourth group (1984.238±0.556) pg/ml a significant increase ($p \leq 0.05$) was observed in comparison with the control, second and third groups, figure (4.1) and table (4.1).



- Values represent mean ± SD.
- Different letters refer to a significant difference at ($p \leq 0.05$).
- Similar letters refer to non-significant differences.

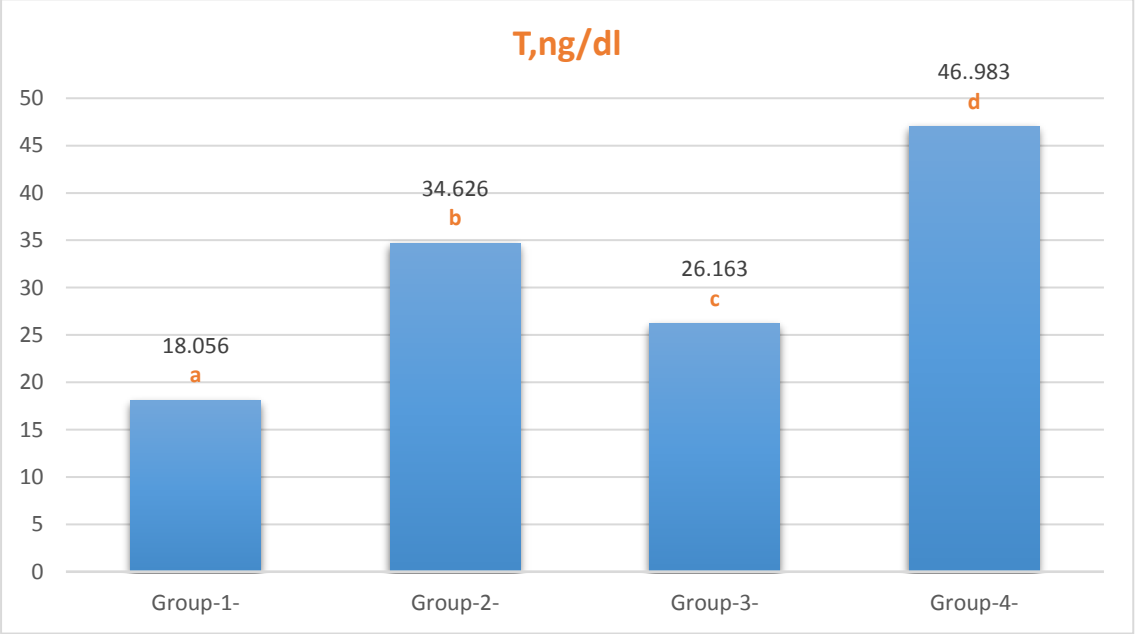
Figure 4.1. Dihydrotestosterone hormone concentration associated with PCOS in type2 diabetic women.

4.1.2. Testosterone

The result revealed that the value of testosterone in the second group (34.626 ±3.021) ng/d increased significantly ($p \leq 0.05$) in comparison with the control (18.056 ng/d ±1.519) ng/d.

In third group (26.163±2.547) ng/d a significant ($p \leq 0.05$) increase was observed in comparison with the control group, but a significant decrease was observed in comparison with second group.

In fourth group (46.983 ±2.393) ng/d a significant ($p \leq 0.05$) increase was observed in comparison with the control, second and third groups, figure (4.2) and table (4.1).



- Values represent mean ± SD.
- Different letters refer to a significant difference at ($p \leq 0.05$).
- Similar letters refer to non-significant differences.

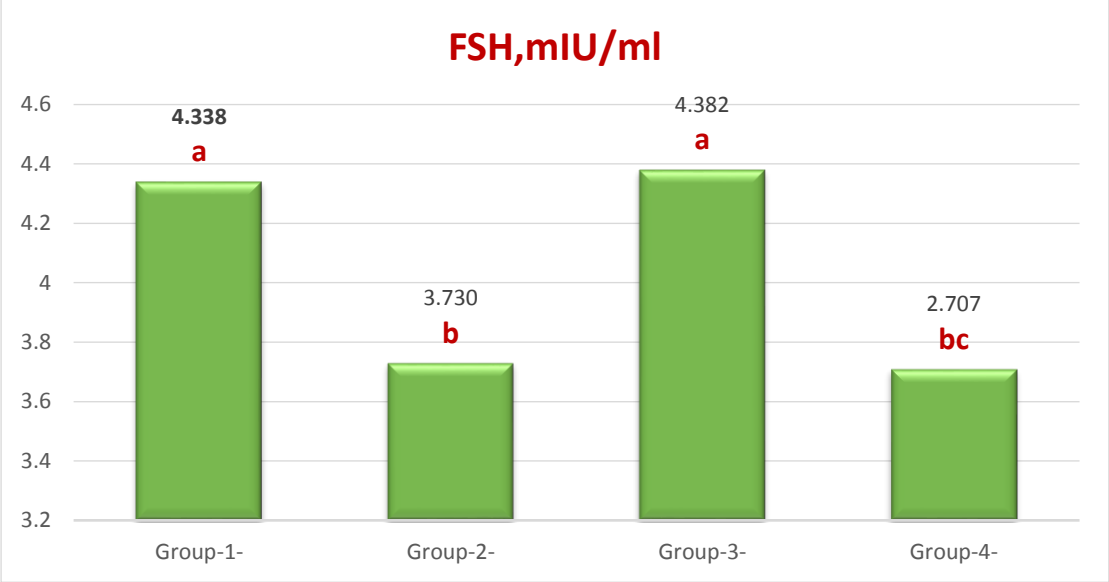
Figure 4.2. Testosterone hormone concentration associated with PCOS in type2 diabetic women.

4.1.3. Follicle Stimulating Hormone (FSH)

The results revealed that the value of FSH concentration in the second group (3.730 ±0.848) mIU/ml decreased significantly ($p \leq 0.05$) in comparison with the first group (control) (4.338 ±1.066) mIU/ml.

No significant differences were observed between the third group (4.382 ±1.029) mIU/ml and the first group (control), but there was a significant ($p \leq 0.05$) increase in comparison with the second group.

In fourth group (3.707 ±1.101) mIU/ml decreased significantly ($p \leq 0.05$) was observed in comparison with the first (control), and third groups, but no significant differences were observed in comparison with the second group, figure (4.3) and table (4.1).



- Values represent mean ± SD.
- Different letters refer to a significant difference at ($p \leq 0.05$).
- Similar letters refer to non-significant differences.

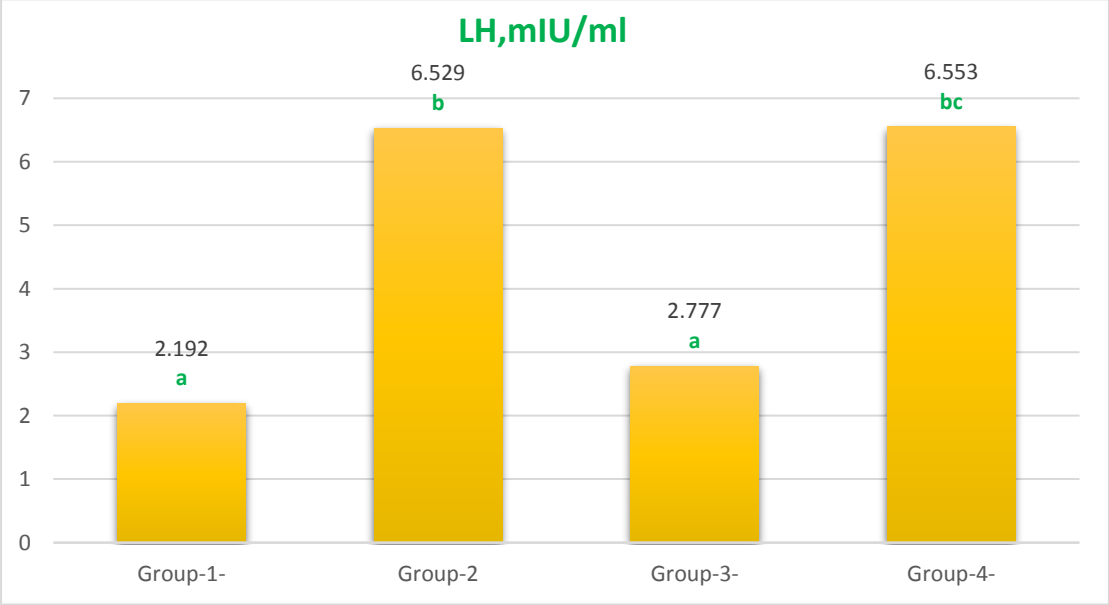
Figure 4.3. Follicle stimulating hormone concentration associated with PCOS in type2 diabetic women.

4.1.4. Luteinizing Hormone (LH)

The results in figure (4.4), table (4.1), revealed that the value of LH hormone concentration in the second group (6.529 ±0.830) mIU/ml increased significantly ($p \leq 0.05$) in comparison with the first group (control) (2.192 ±0.789) mIU/ml.

No significant differences were observed between the third group (2.777 ±1.274) mIU/ml and the control group, but there was a significant ($p \leq 0.05$) decrease in comparison with the second groups.

In fourth group (6.553 ±2.326) mIU/ml a significant ($p \leq 0.05$) increase in comparison with the first (control) and third groups, but no significant differences were observed in comparison with the second group.



- Values represent mean ± SD.
- Different letters refer to a significant difference at ($p \leq 0.05$).
- Similar letters refer to non-significant differences.

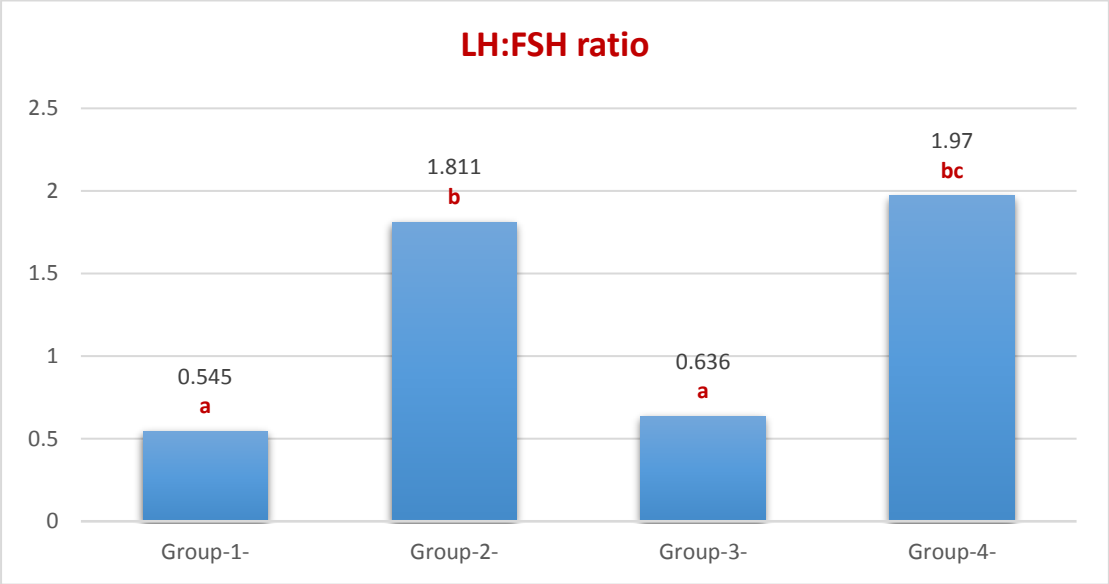
Figure 4.4. Luteinizing hormone concentration associated with PCOS in type2 diabetic women.

4.1.5. LH: FSH Ratio

The results revealed that the value of LH: FSH Ratio in the second group (1.811±0.332) increased significantly ($p \leq 0.05$) in comparison with the control group (0.545± 0.274).

No significant differences were observed between the third group **(**0.636±0.249**)** and the control group, but there was a significant ($p \leq 0.05$) decrease in comparison with the second group.

In fourth group (1.970±0.966) a significant ($p \leq 0.05$) increase was observed in comparison with the first and third groups, but no significant differences were observed in comparison with the second group, figure (4.5) and table (4.1).



- Values represent mean ± SD.
- Different letters refer to a significant difference at ($p \leq 0.05$).
- Similar letters refer to non-significant differences.

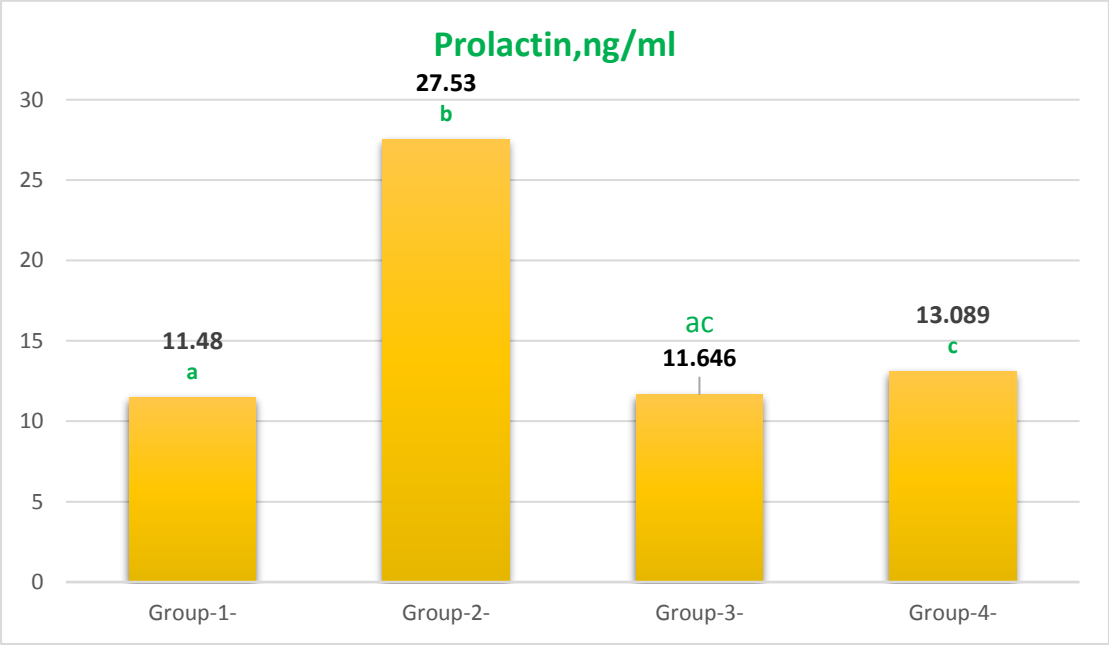
Figure 4.5. LH: FSH ratio associated with PCOS in type 2 diabetic women.

4.1.6. Prolactin

The results revealed that the prolactin concentration in the second group (27.530 ±2.582) ng/ml increased significantly ($p \leq 0.05$) in comparison with the first group (11.480 ±0.953) ng/ml.

No significant differences were observed between the third group **(**11.646 ±1.077**)** ng/ml and the control group, but there was a significant ($p \leq 0.05$) decrease in comparison with the second group. Also, no significant differences were observed between the third group and the fourth groups.

In fourth group (13.089 ±1.29) ng/ml a significant ($p \leq 0.05$) increase was observed in comparison with the first group, but a significant decreased were observed in comparison with second group. No significant differences were observed between the fourth group and third group. Figure (4.6) and table (4.1).



- Values represent mean ± SD.
- Different letters refer to a significant difference at ($p \leq 0.05$).
- Similar letters refer to non-significant differences.

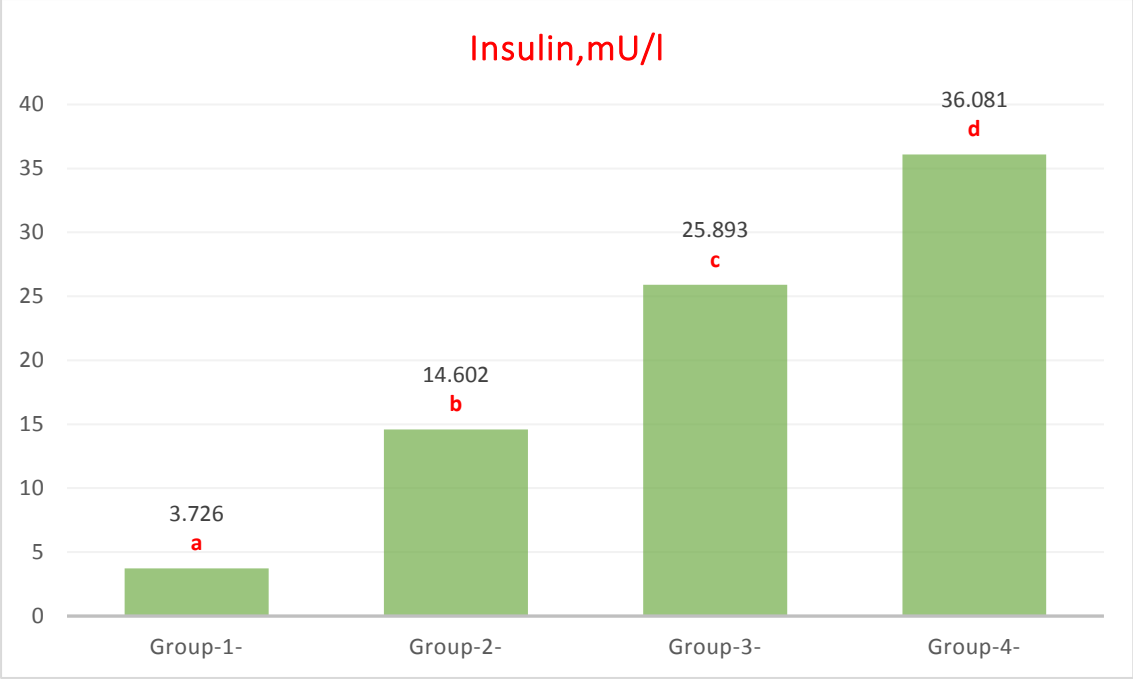
Figure 4.6. Prolactin hormone concentration associated with PCOS in type2 diabetic women.

4.1. 7. Fasting Insulin

The results revealed that the value of insulin concentration in the second group (14.602 ±1.944) mU/l was increased significantly ($p \leq 0.05$) in comparison with the control group (3.726 ±1.192) mU/l.

In third group (25.893 ±1.323) mU/l a significant increase ($p \leq 0.05$) was observed in comparison with the control and second groups.

In fourth group (36.081±1.726) mU/l a significant increase ($p \leq 0.05$) was observed in comparison with the control, second and third groups, figure (4.7) and table (4-2).



- Values represent mean ± SD.
- Different letters refer to a significant difference at ($p \leq 0.05$).
- Similar letters refer to non-significant differences.

Figure 4.7. The insulin concentration associated with PCOS in type2 diabetic women.

Table (4.1) Hormonal parameters (DHT, T, FSH, LH, LH: FSH ratio and PRL) associated with PCOS in type2 diabetic women.

Parameters	Group-1-	Group-2-	Group-3-	Group-4-
DHT, pg/ml	575.315±0.482 a	1009.182±0.369 b	1444.677±0.663 c	1984.238±0.556 d
Testosterone, ng/d	18.056±1.519 a	34.626±3.021 b	26.163±2.547 c	46.983±2.393 d
FSH, mIU/ml	4.338±1.066 a	3.730±0.848 b	4.382±1.029 a	3.707±1.101 bc
LH, mIU/ml	2.192±0.789 a	6.529±0.830 b	2.777±1.274 a	6.553±2.326 bc
LH: FSH ratio	0.545±0.274 a	1.811±0.332 b	0.636±0.249 a	1.970±0.966 bc
Prolactin, ng/ml	11.480±0.953 a	27.530±2.582 b	11.646±1.077 ac	13.089±1.289 c

- N=120
- Values represent mean ± SD.
- Different letters refer to a significant difference at ($p \leq 0.05$).
- Similar letters refer to non-significant differences.
- G1=healthy group (control).
- G2=Women with PCOS.
- G3=Women with T2DM.
- G4=Women with PCOS and T2DM.

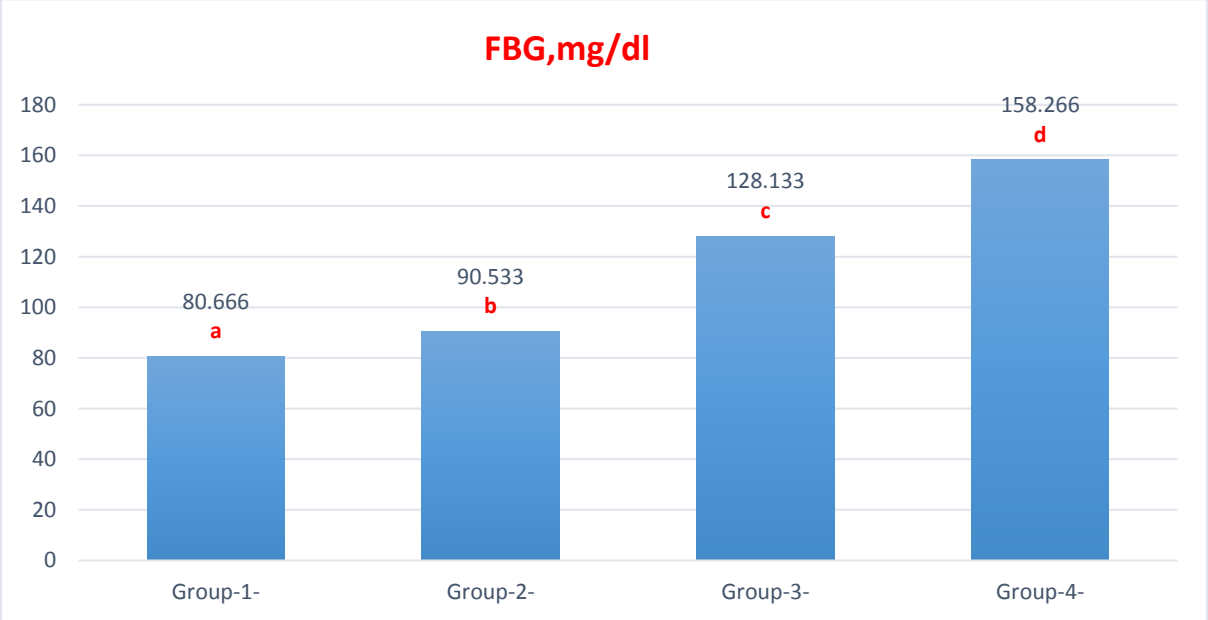
4.2. Biochemical Parameters:

4.2.1. Fasting Blood Glucose (FBG)

The results revealed that the value of fasting blood glucose concentration in the second group (90.533 ±3.257) mg/dl was increased significantly ($p \leq 0.05$) in comparison with the control group (80.666 ±3.447) mg/dl.

In third group (128.133 ±3.319) mg/dl a significant increase ($p \leq 0.05$) was observed in comparison with the control and second groups.

In fourth group (158.266 ±2.211) mg/dl a significant increase ($p \leq 0.05$) was observed in comparison with the control and second groups. Figure (4.8) and table (4-2).



- Values represent mean ± SD.
- Different letters refer to a significant difference at ($p \leq 0.05$).
- Similar letters refer to non-significant differences.

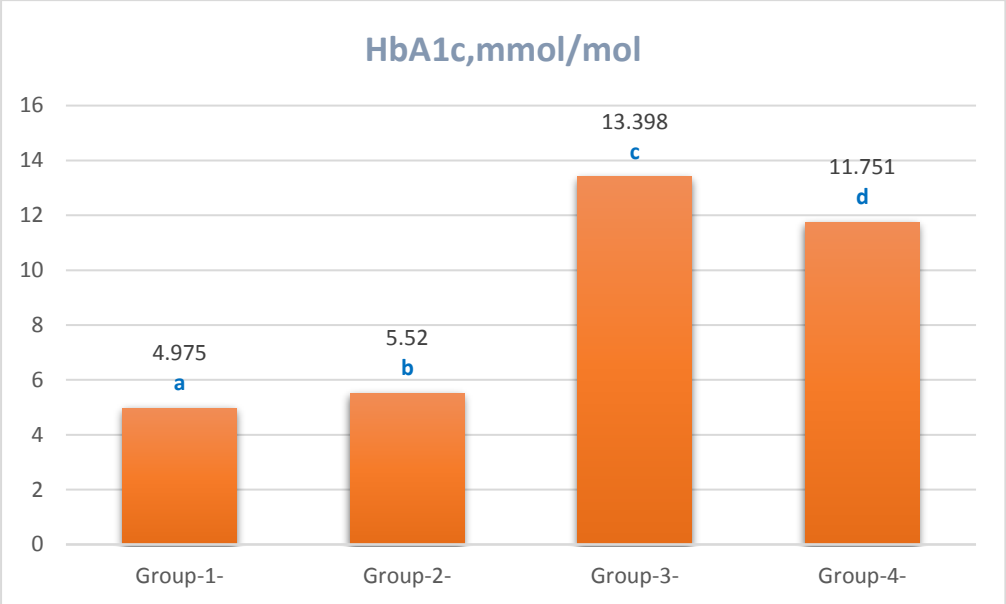
Figure 4.8. The Fasting blood glucose concentration associated with PCOS in type2 diabetic women.

4.2.2. Glycated Hemoglobin (HbA1c)

The results revealed that the value of HbA1c in the second group (5.520 ±0.614) mmol/mol was increased significantly ($p \leq 0.05$) compared with the control group (4.975 ±0.456) mmol/mol.

In third group (13.398 ±0.727) mmol/mol a significant increase ($p \leq 0.05$) was observed in comparison with the control and second groups.

In fourth group (11.751 ±0.765) mmol/mol a significant increase ($p \leq 0.05$) was observed in comparison with the control, second and third groups, but a significant decreased were observed in comparison with third group. Figure (4.9) and table (4.2).



- Values represent mean ± SD.
- Different letters refer to a significant difference at ($p \leq 0.05$).
- Similar letters refer to non-significant differences.

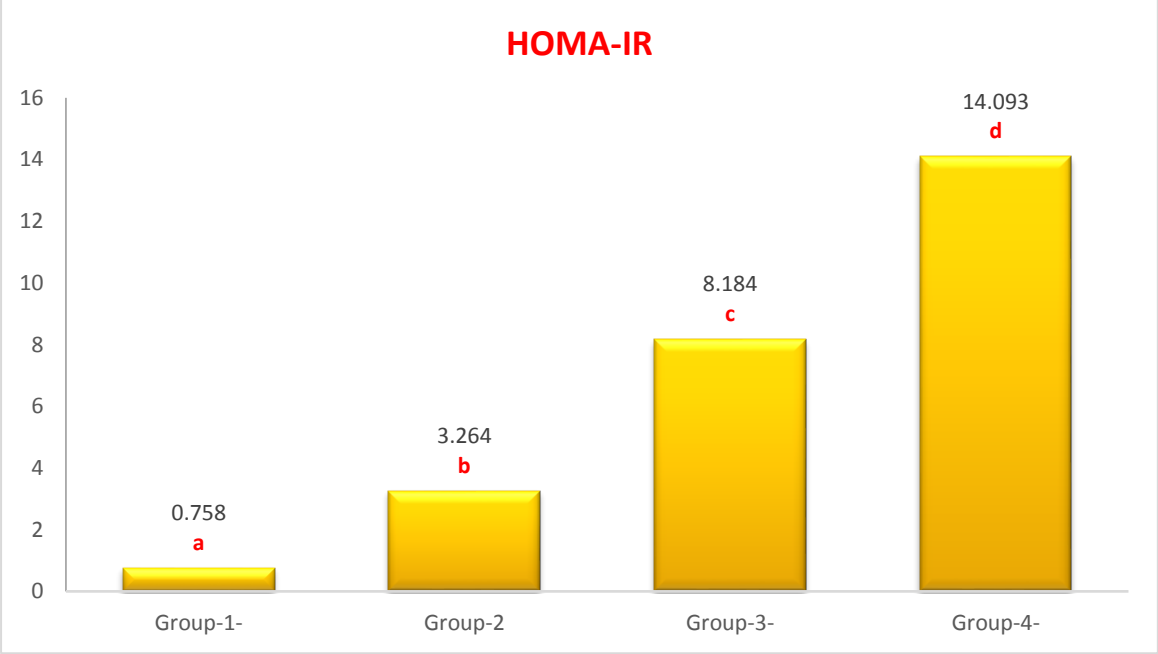
Figure 4.9. The HbA1c concentration associated with PCOS in type2 diabetic women.

4.2.3. Homeostasis Model Assessment of Insulin Resistance (HOMA-IR)

The results revealed that the value of HOMA-IR in the second group (3.264±0.457) was increased significantly ($p \leq 0.05$) in comparison with the control group (0.758±0.275).

In third group (8.184 ±0.308) a significant increase ($p \leq 0.05$) was observed in comparison with the control and second groups.

In fourth group (14.093±0.558) a significant increase ($p \leq 0.05$) in comparison with the control, second and third groups, figure (4.10) and table (4-2).



- Values represent mean ± SD.
- Different letters refer to a significant difference at ($p \leq 0.05$).
- Similar letters refer to non-significant differences.

Figure 4.10. HOMA-IR associated with PCOS in type 2 diabetic women.

Table 4.2. The FBG, HbA1c, Fs-insulin and HOMA-IR associated with PCOS in type2 diabetic women.

Groups	FBG, (mg/dl)	HbA1c, mmol/mol	Fs-insulin, (mU/l)	HOMA-IR
Group-1-	80.666±3.447 a	4.975±0.456 a	3.726±1.192 a	0.758±0.275 a
Group-2-	90.533±3.257 b	5.520±0.614 b	14.602±1.944 b	3.264±0.457 b
Group-3-	128.133±3.319 c	13.398±0.727 c	25.893±1.32 3 c	8.184±0.308 c
Group-4-	158.266±2.211 d	11.751±0.765 d	36.081±1.726 d	14.093±0.558 d

- N=120
- Values represent mean ± SD.
- Different letters refer to a significant difference at ($p \leq 0.05$).
- Similar letters refer to non-significant differences.
- G1=healthy group (control).
- G2=Women with PCOS.
- G3=Women with T2DM.
- G4=Women with PCOS and T2DM.

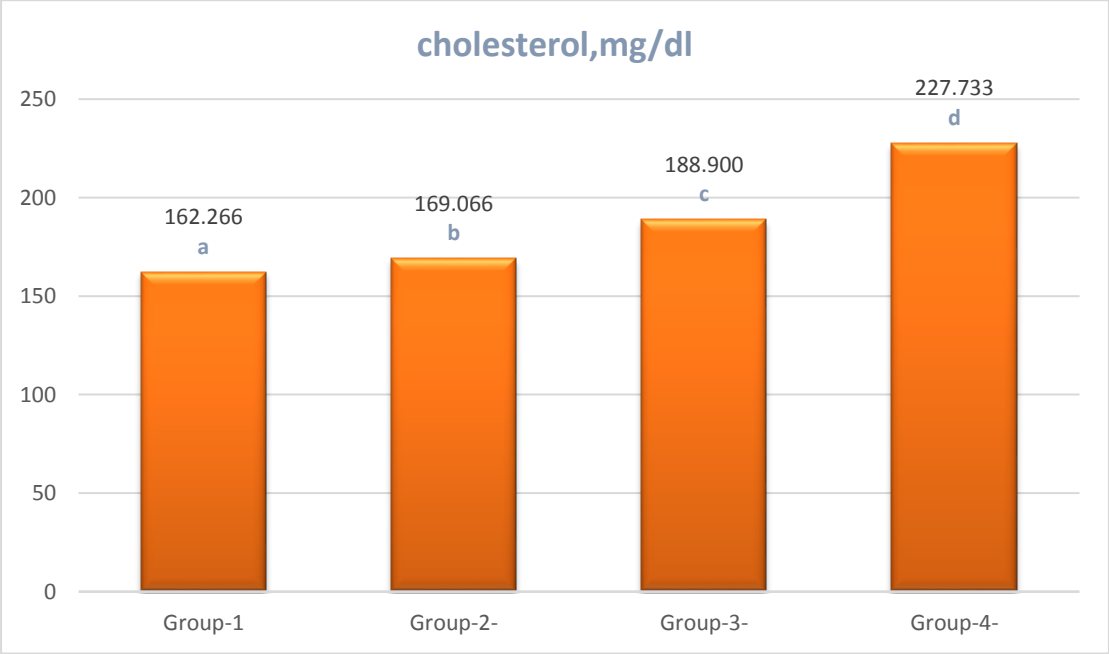
4.2.4. Lipid Profile

4.2.4.1. Total cholesterol

The results revealed that the value of cholesterol concentration in the second group (169.066±3.885) mg/dl was increased significantly ($p \leq 0.05$) in comparison with the control group (162.266 ±2.211) mg/dl.

In third group (188.900 ±3.565) mg/dl a significant increase ($p \leq 0.05$) was observed in comparison with the control and second groups.

In fourth group (227.733 ±4.126) mg/dl a significant increase ($p \leq 0.05$) was observed in comparison with the control, second and third groups, figure (4.11) and table (4.3).



- Values represent mean ± SD.
- Different letters refer to a significant difference at ($p \leq 0.05$).
- Similar letters refer to non-significant differences.

Figure 4.11. The total cholesterol concentration associated with PCOS in type2 diabetic women.

4.2.4. 2.Triglyceride

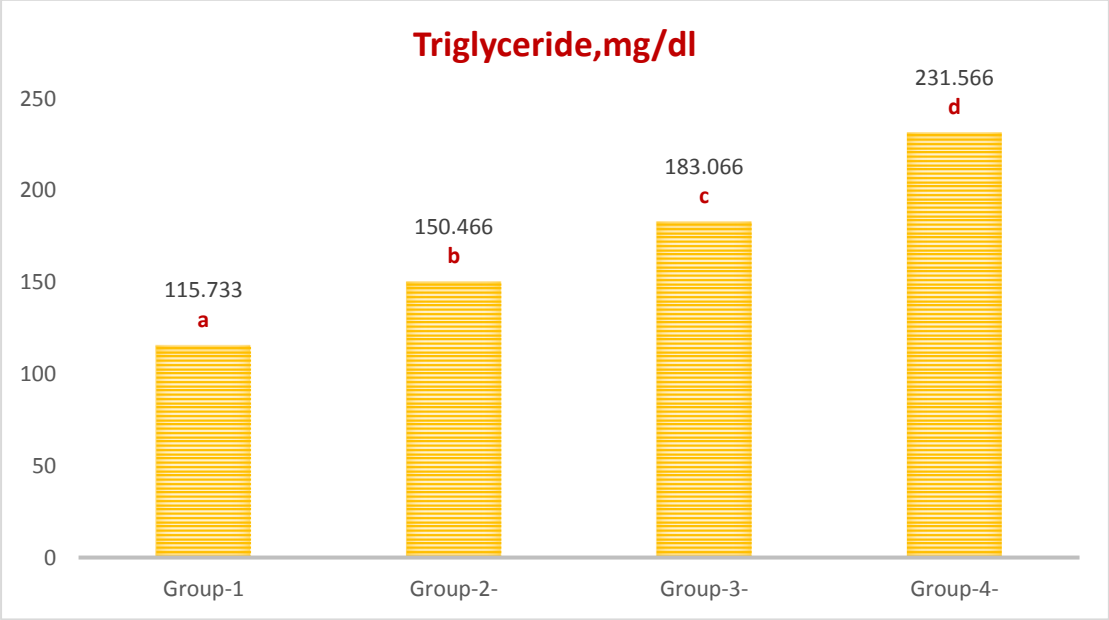
The results revealed that the value of triglyceride concentration in the second group (150.466 ±4.216) mg/dl increased significantly

($p \leq 0.05$) in comparison with the control group (115.733 ±3.433) mg/dl.

In third group (183.066±2.958) mg/dl a significant increase

($p \leq 0.05$) was observed in comparison with the control and second groups.

In fourth group (231.566 ±2.860) mg/dl a significant increase ($p \leq 0.05$) was observed in comparison with the control, second and third groups, figure (4.12) and table (4-3).

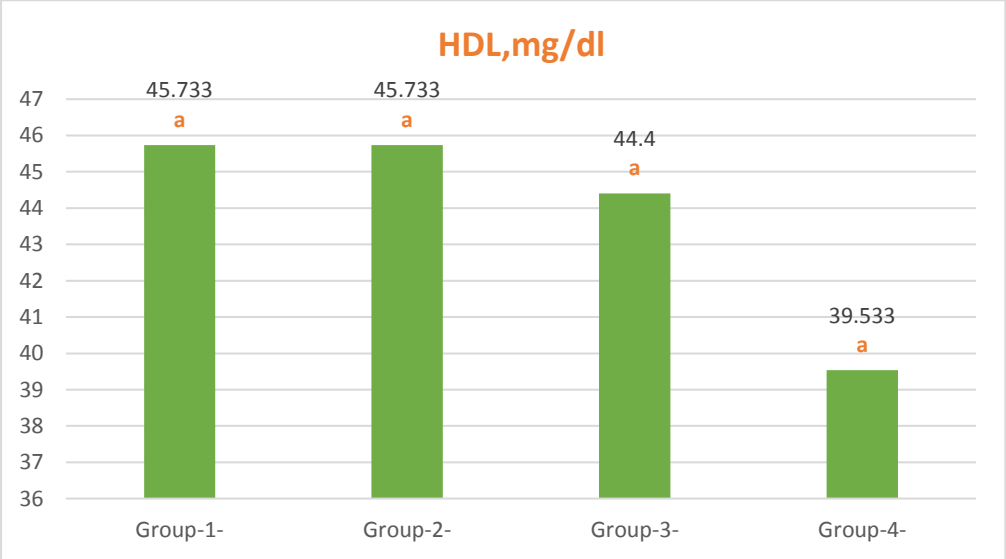


- Values represent mean ± SD.
- Different letters refer to a significant difference at ($p \leq 0.05$).
- Similar letters refer to non-significant differences.

Figure 4.12. The Triglyceride concentration associated with PCOS in type2 diabetic women.

4.2.4.3. High-Density Lipoprotein- Cholesterol (HDL-C)

The results of HDL revealed that a non-significant difference ($p \leq 0.05$) in the second (45.733 ±2.211) mg/dl, third (44.4 ±2.540) mg/dl and fourth groups (39.533 ±3.036) mg/dl in comparison with the control group (45.733 ±2.211) mg/dl, figure (4.13) and table (4.3).



- Values represent mean ± SD.
- Similar letters refer to non-significant differences.

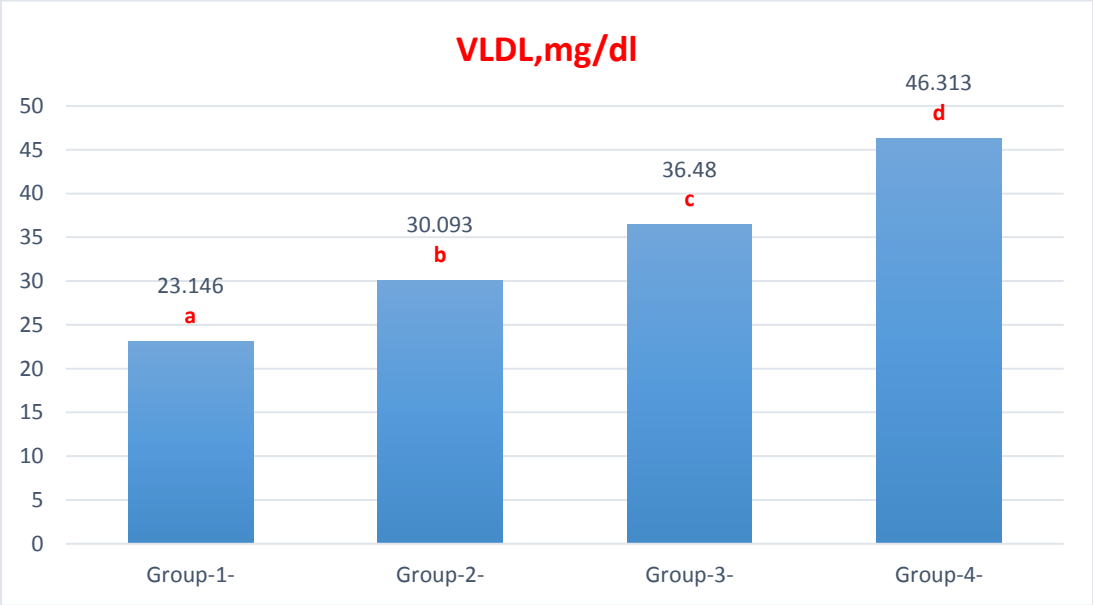
Figure 4.13. The HDL concentration associated with PCOS in type2 diabetic women.

4.2.4.4. Very Low–Density Lipoprotein- Cholesterol (VLDL-C)

The results revealed that the value of VLDL-C concentration in the second group (30.093 ±0.843) mg/dl increased significantly ($p \leq 0.05$) in comparison with the control group (23.146 ±0.686) mg/dl.

The third group (36.48 ±0.474) mg/dl increased significantly ($p \leq 0.05$) in comparison with the control and second groups.

In fourth group (46.313 ±0.572) mg/dl a significant increase ($p \leq 0.05$) was observed in comparison with the control, second and third groups, figure (4.14) and table (4.3).



- Values represent mean ± SD.
- Different letters refer to a significant difference at ($p \leq 0.05$).
- Similar letters refer to non-significant differences.

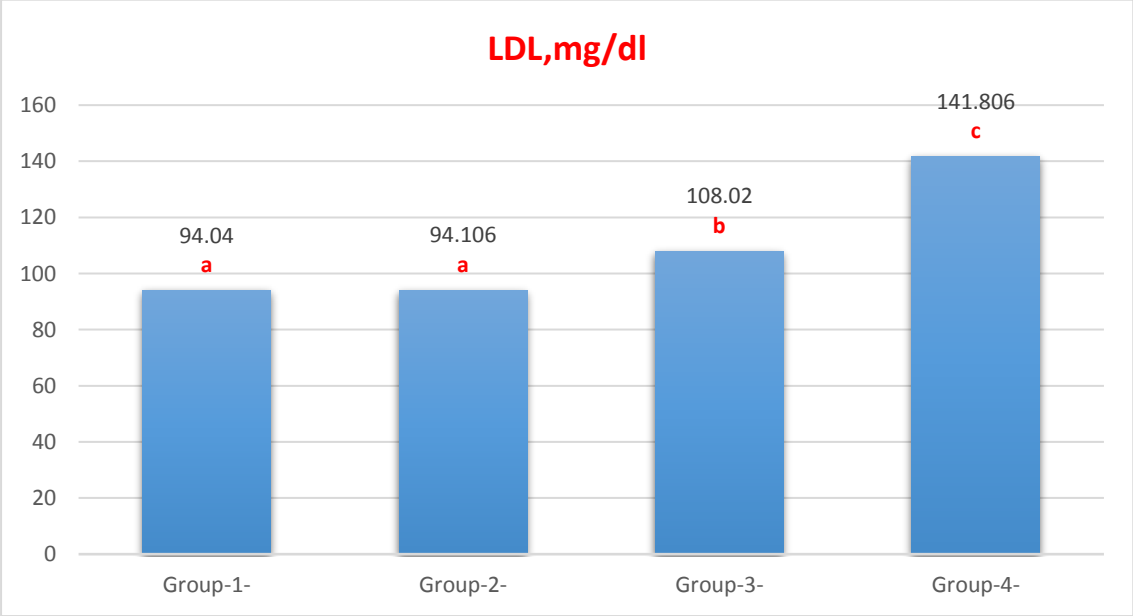
Figure 4.14. The VLDL concentration associated with PCOS in type2 diabetic women.

4.2.4.5. Low–Density Lipoprotein- Cholesterol (LDL-C)

The results revealed that the value of LDL-C concentration a non-significant difference ($p \leq 0.05$) in the second group (94.106 ±4.339) mg/dl in comparison with the control group (94.04 ±3.958) mg/dl.

The third group (108.02±4.296) mg/dl increased significantly ($p \leq 0.05$) in comparison with the control and second groups.

In fourth group (141.806±6.306) mg/dl a significant increase ($p \leq 0.05$) was observed in comparison with the control, second and third groups, figure (4.15) and table (4.3).



- Values represent mean ± SD.
- Different letters refer to a significant difference at ($p \leq 0.05$).
- Similar letters refer to non-significant differences.

Figure 4.15. The LDL concentration associated with PCOS in type2 diabetic women.

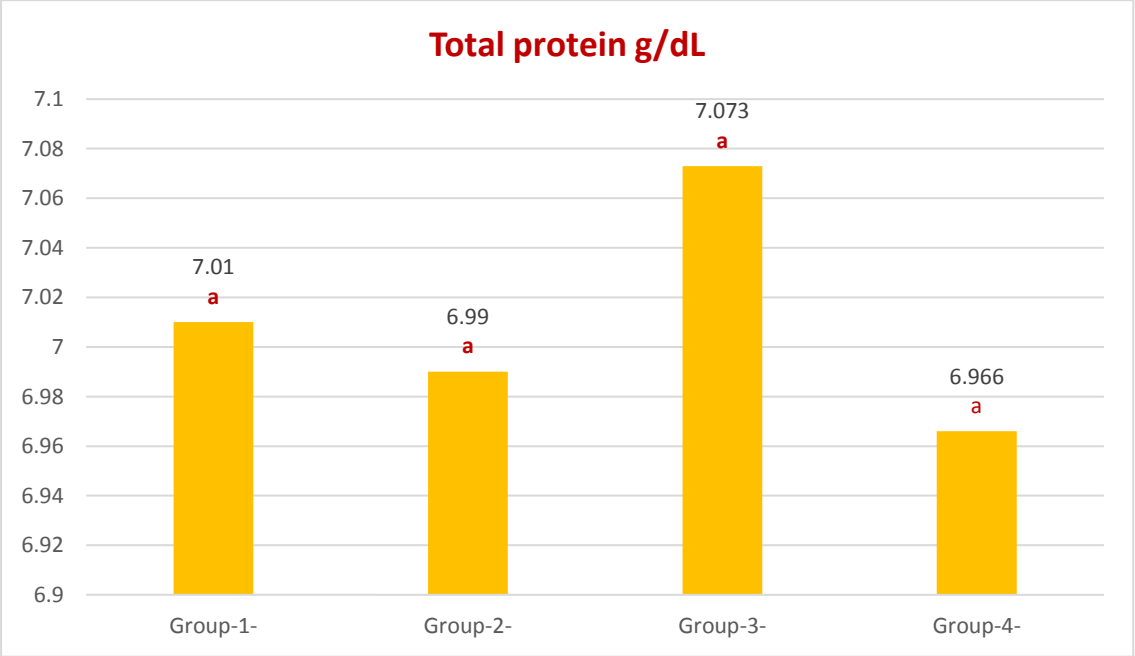
Table 4.3. The lipid profile concentration associated with PCOS in type2 diabetic women.

Groups	Cholesterol, (mg/dl)	Triglycerides, (mg/dl)	HDL-cholesterol, (mg/dl)	VLDL-cholesterol, (mg/dl)	LDL-cholesterol, (mg/dl)
Group-1-	162.266±2.211 a	115.733±3.433 a	45.733±2.211 a	23.146±0.686 a	94.04±3.958 a
Group-2-	169.066±3.885 b	150.466±4.216 b	45.733±2.211 a	30.093±0.843 b	94.106±4.339 a
Group-3-	188.9±3.565 c	183.066±2.958 c	44.4±2.540 a	36.48±0.474 c	108.02±4.296 b
Group-4-	227.733±4.126 d	231.566±2.860 d	39.533±3.036 a	46.313±0.572 d	141.806±6.306 c

- N=120
- Values represent mean ± SD.
- Different letters refer to a significant difference at ($p \leq 0.05$).
- Similar letters refer to non-significant differences.
- G1=healthy group (control).
- G2=Women with PCOS.
- G3=Women with T2DM.
- G4=Women with PCOS and T2DM.

4.2.5. Total Protein

The results revealed that the value of total protein concentration a non-significant differences ($p \leq 0.05$) in the second (6.990 ±0.690) g/dL, third (7.073 ±0.614) g/dL and fourth groups (6.966 ±0.482) g/dL in comparison with the control group (7.010 ±0.411) g/dL, figure (4.16) and table (4-4).



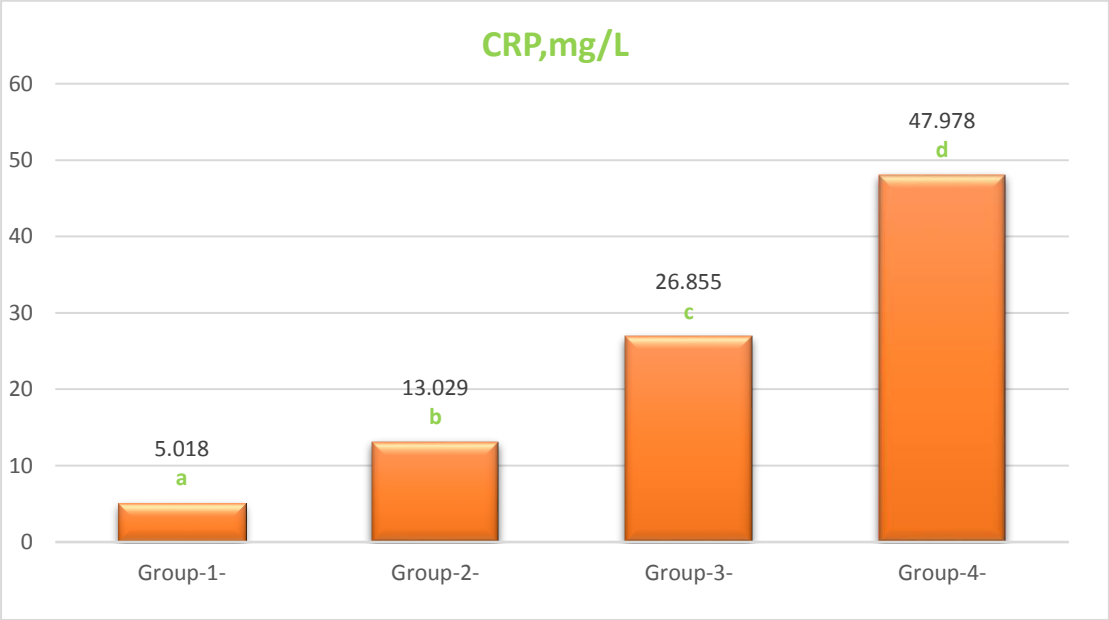
- Values represent mean ± SD.
- Similar letters refer to non-significant differences.

Figure 4.16. The total protein concentration associated with PCOS in type2 diabetic women.

4.2.6.C-Reactive Protein

The result revealed that the value of CRP concentration in the second group (13.029 ±0.645) mg/L increased significantly ($p \leq 0.05$) in comparison with the control group (5.018 ±0.695) mg/L.

The third group (26.855±0.544) mg/L increased significantly ($p \leq 0.05$) in comparison with the first and the second groups. In fourth group (47.978±1.086) mg/L a significant increase ($p \leq 0.05$) was observed in comparison with the control, second and third groups, figure (4.17) and table (4-4).



- Values represent mean ± SD.
- Different letters refer to a significant difference at ($p \leq 0.05$).
- Similar letters refer to non-significant differences.

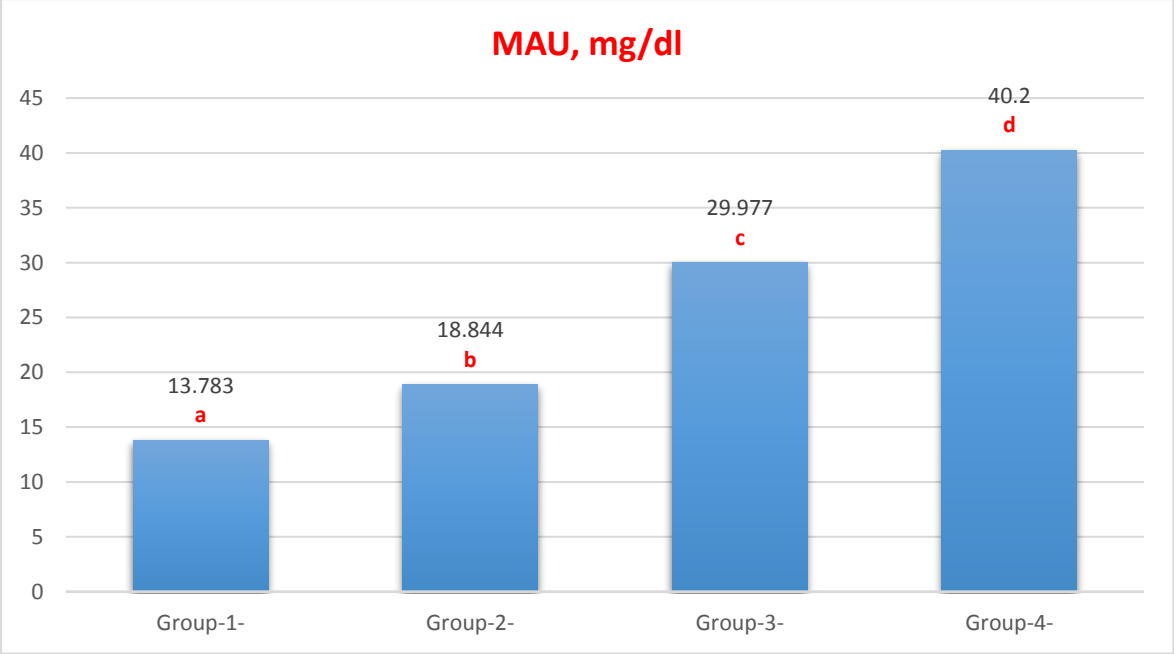
Figure 4.17. The C-Reactive protein concentration associated with PCOS in type2 diabetic women.

4.2.7. Microalbuminurea (MAU)

The results revealed that the value of MAU concentration in the second group (18.844 ±2.977) mg/dl increased significantly ($p \leq 0.05$) in comparison with the control group (13.78 3 ±1.532) mg/dl.

The third group (29.977 ±2.225) mg/dl increased significantly ($p \leq 0.05$) in comparison with the control and the second groups.

In fourth group (40.2 ±1.765) mg/dl a significant increase ($p \leq 0.05$) was observed in comparison with the control, second and third groups, figure (4.18) and table (4-4).



- Values represent mean ± SD.
- Different letters refer to a significant difference at ($p \leq 0.05$).

- Similar letters refer to non-significant differences.

Figure 4.18. The Microalbuminuria concentration associated with PCOS in type2 diabetic women.

Table 4.4. The Total Protein, CRP and MAU associated with PCOS in type2 diabetic women.

Groups	Total Protein, (g/dl)	CR-Protein, (mg/L)	MAU, (mg/dl)
Group -1-	7.010±0.411 a	5.018±0.695 a	13.783±1.532 a
Group -2-	6.990±0.690 a	13.029±0.645 b	18.844±2.977 b
Group -3-	7.073±0.614 a	26.855±0.544 c	29.977±2.225 c
Group -4-	6.966±0.482 a	47.978±1.0863 d	40.2±1.765 d

- N=120
- Values represent mean ± SD.
- Different letters refer to a significant difference at ($p \leq 0.05$).
- Similar letters refer to non-significant differences.
- G1=healthy group (control).
- G2=Women with PCOS.
- G3=Women with T2DM.
- G4=Women with PCOS and T2DM.

6.Conclusions and Recommendations

6.1. Conclusions

Results of the present study showed clearly the following conclusions:

1. Women with PCOS and T2DM are associated with infertility according to elevated level of androgen, prolactin, insulin, LH while decreased level of FSH hormone lead to inhibition of egg development, anovulation and cause infertility.
2. Neuroendocrine dysfunction occurs in women with PCOS due to high level of androgen production and a high ratio between the luteinizing hormone (LH) and the follicle-stimulating hormone (FSH).
3. Insulin works synergistically with LH in promoting the production of androgen from the theca cell by insulin receptors.
4. Women with PCOS and T2DM show a sign of severity of inflammation due to a high level of CRP in blood.
5. Increased lipid profile (TC, TG, LDL, VLDL) in a group of women with PCOS and T2DM leads to dyslipidemia.
6. A high level of MAU in women with PCOS and T2DM are an initial indicator of metabolic and kidney disorders.
7. The Insulin resistance plays a crucial role in pathophysiology of PCOS.

6.2. Recommendations

1. Future studies included more number of sample and other parameters like: oxidizing agents, related enzymes and other immunological factors in women with PCOS.
2. Studying the genetic expression of reproductive hormone and their receptors, especially androgen.
3. Future researches on the PCOS in women included the role of some hormones of adipokines (e.g. leptin, chemerin, respin……. etc.).
4. Studying the relationship between the PCOS and other metabolic disorders e.g. the obesity.

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الخلاصة

هدفت الدراسة الحالية إلى تقييم بعض المعايير الهرمونية والبايوكيميائية المرتبطة بمتلازمة تكيس المبايض في النساء المصابات بداء السكري من النوع الثاني في محافظة ميسان,حيث شملت الدراسة 120 امرأة تراوحت اعمارهن بين (35-45) سنة واللواتي يراجعن مستشفى الصدر التعليمي ومركز السكري والغدد الصم للفترة من 2017/12/3 - 2018 /6/20 .قسمت النساء الى أربع مجموعات ولكل مجموعة 30 امرأة كما يلي:
مجموعة (1) مجموعة السيطرة، مجموعة (2) نساء مصابات بل PCOS، مجموعة (3) نساء مصابات بداء السكري نوع 2 (T2DM) ومجموعة (4) نساء مصابات بل PCOS وT2DM.

أظهرت النتائج أن قيم الهرمون اللوتيني (LH)، نسبة الهرمون اللوتيني / الهرمون المنبه للجريبات (LH / FSH)، البرولاكتين (PRL)، التستوستيرون وديهدروتستوسترون (DHT) في المجموعتين الثانية والرابعة ارتفعت معنويا ($p \leq 0.05$) مقارنة مع المجموعة الأولى. في حين أن قيم الFSH في المجموعتين الثانية والرابعة قد انخفضت معنويا ($p \leq 0.05$) بالمقارنة مع المجموعة الأولى. لا توجد فروق ذات دلالة إحصائية في الFSH ، LH, نسبة LH / FSH والبرولاكتين عند مقارنة المجموعة الثالثة مع المجموعة الأولى. بينما زاد التستوستيرون وDHT في المجموعة الثالثة معنويا ($p \leq 0.05$) مقارنة بالمجموعة الأولى.

أظهرت النتائج أن مستوى سكر الدم (FBG)، السكر التراكمي (HBA1c)، الأنسولين ومقاومة الانسولين بال (HOMA-IR) في المجموعات الثانية والثالثة والرابعة زادت معنويا ($p \leq 0.05$) بالمقارنة مع المجموعة الأولى.

بينت نتائج الكولسترول والدهون الثلاثية (TG) والبروتين الدهني منخفض الكثافة جدا (VLDL) أن المجموعات الثانية والثالثة والرابعة زادت معنويا ($p \leq 0.05$) مقارنة بالمجموعة الأولى.

في حين أظهرت نتائج البروتين الدهني عالي الكثافة (HDL) والبروتين الكلي عدم وجود فروقات معنوية ($p \leq 0.05$) في المجموعات الثانية والثالثة والرابعة بالمقارنة مع المجموعة الأولى.

أوضحت نتائج البروتين الدهني منخفض الكثافة (LDL) أن الفروقات غير معنوية ($p \leq 0.05$) في المجموعة الثانية بالمقارنة مع المجموعة الأولى. في حين أن المجموعتين الثالثة والرابعة زادت معنويا ($p \leq 0.05$) بالمقارنة مع المجموعة الأولى والثانية.

أظهرت نتائج بروتين سي التفاعلي(CRP) والمايكرو البومين يوريا (MAU) أن المجموعات الثانية والثالثة والرابعة ارتفعت معنويا ($p \leq 0.05$) مقارنة بالمجموعة الأولى.

لقد تم مناقشة الابعاد الفسيولوجية لظاهرتي متلازمة تكيس المبايض وداء السكري من النوع الثاني للمتغيرات المدروسة ومن خلال ظاهرتي متلازمة تكيس المبايض وداء السكري من النوع الثاني استنادا الى ظاهرة hyperandrogenism (ارتفاع مستويات هرمونات الاندروجين) و hyperinsulinemia(ارتفاع مستوى الانسولين) للمجاميع المدروسة وتأثيراتها.

Appendix

Questionnaire

Name of patient	Age	Address	married		Family history of PCOS	
			Yes,	No,	Yes,	No,
Clinical assessment						
Clinical specimen	Blood		Urine			
Clinical features	Irregular menstrual cycle	Hirsutism	Obesity		Androgenic alopecia	Acne
Yes, or No,						