

Study the markers of follicle stimulating hormone and transforming growth factor - β 1 in patients with polycystic ovarian syndrome in AL-Najaf Al-Ashraf province.

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Abstract

Polycystic ovarian syndrome (PCOS) is an endocrine disorder affecting 6-15% of women in age (12-45) year . It is characterized by disturbance in menstrual cycle ,androgenic features and presence of multiple small cyst in ovaries in ultrasound. It is the important cause of infertility. The etiology of the disease remain unclear and may be associated with insulin resistance, obesity , type 2 diabetes mellitus, hyperandrogenism , environmental and familial genetic factors . PCOS has a role is the appearance of hirsutism , acne , hypertension and dyslipidemia with increase risk factor of cardiovascular disease (CVD).

Objective : Aim of the study to investigate the pathogenicity, disease severity and early diagnosis roles of follicle stimulating hormone (FSH) and transforming growth factor-beta (TGF- β 1) in women with polycystic ovarian syndrome.

Conclusion : Polycystic ovarian syndrome was related to the decrease level of follicle stimulating hormone (FSH) in different age groups where comprised 18.75% in PCOS patients , while increase marker of transforming growth factor- β 1(TGF- β 1) where comprised 46.25% in PCOS patients , these two result cause failure of follicle growth to become mature .

Key words : PCOS , FSH , TGF- β 1 and infertility.

Polycystic ovarian syndrome

1. Introduction

Polycystic ovarian syndrome (PCOS) is the most well-known female endocrine and metabolic disorders influencing (6 - 15%) of women of reproductive age (12 – 45)year and is believed to be one of the leading sources of female [infertility](#)^[1].The disturbance resulting multiple abnormal cysts in amplified ovaries, so they do not create the typical number of eggs and do not ovulate regularly^[2].

The illness is available during childbirth yet does not cause any symptoms till puberty.The essential features are obesity, [anovulation](#) (bringing about sporadic or irregular [menstruation](#)), acne and excessive amounts of [androgenic](#) hormones,although the reasons of PCOS are obscure, yet there are solid correlation between's insulin resistance (IR), diabetes and [obesity](#) with PCOS^[3].Rotterdam consensus workshop demonstrated PCOS introduce if (2) out of the following(3) criteria are found^[4] :-

1. Polycystic ovaries are present by the ultrasound.
2. Increased level of male hormones(androgen).

3. Menstrual disorders.

The luteinizing hormone (LH) level is higher than follicle stimulating hormone (FSH) level in most cases of PCOS, this state is called a lifted LH to FSH proportion^[5]. Exactly when ladies having PCOS implies that their ovaries are not getting the benefit hormonal signals from their pituitary gland, without these signals there will be no ovulation and the menstrual cycle, may be irregular or lost. Frequently, ladies with PCOS have high testosterone level, develop to acne, hair growth (hirsutism) predominantly on the lower face, neck, chest and upper back^[6]. In previous years it has become recognized that women with PCOS may be more susceptible than other women to a wide range of psychological problems, especially anxiety and depression, as indicated by a portion of the scientists in PCOS, there is still a yet far way until the point that we set up authoritative causative links between the physiological and the psychological phenomena that happen in PCOS^[7].

Several metabolic features are related with PCOS, include a predisposition to develop insulin resistance, metabolic syndrome, diabetes and obesity other metabolic issues involve hypertension, and non-alcoholic fatty liver disease^[8]. The obesity is a major impact on the metabolic affiliations and complexities of the syndrome.

Among different variables, obesity is clearly identified with the fruitlessness of PCOS and expand the hazard for metabolic disorders and the grouping of cardiovascular hazard factors in these ladies^[9].

There is a critical increment in the rate of impaired glucose tolerance (IGT) and type 2 diabetes mellitus (T2DM) in ladies with PCOS, this study showed that on the underlying assessment with a 2-hours glucose tolerance test (GTT) 30-40% of PCOS already have IGT or T2DM^[10].

Patients of polycystic ovarian syndrome (PCOS) are at expanded danger of increase levels of low density lipoprotein (LDL), which if ignored, can prompt a heart attack or stroke. Alternately, women with PCOS have bring down levels of high density lipoprotein (HDL), which is another dangerous factor for cardiovascular disease (CVD)^[11], other than an assumed hereditary susceptibility, different features related with PCOS may influence future health of offspring, such as obesity and unfavorable metabolic dysfunction of the mother, placental abnormalities, and increased pregnancy complications^[12].

Follicle stimulating hormone (FSH) : -

Follicle stimulating hormone is a glycoprotein dimer consisting of two subunits: α (alpha), FSH α and β (beta), FSH β subunits. The α -subunit consists of (92) amino acids, The β -subunit consists of (118) amino acids, which allows the differential function of each hormone. FSH assumes a very important role in reproductive control of ladies as a the generation and maturation of gonadal development amid the reproduction and differentiation of its objective cells by join to its objective receptor exist on the membrane of granulosa cells^[13].

On the off chance that there is a disturbance in follicular development, the FSH action will be unworkable^[14]. FSH cooperation with its receptor is pivotal for the development and maturation of follicles, therefore any alteration in the genotype of the receptor cause defect stuck its capacity to connect to the FSH which prompts disorder in the signaling pathways^[15]. The reason for 75% of infertility is anovulation PCOS^[16]. The granulosa cells of this follicles are lower sensitive to LH and their size is little than different follicles with typical cycle and creates unusual value of estradiol because of their little sizes and suppresses FSH level and prohibit the normal follicular development^[17].

FSH regulate granulosa cells function that makes the change of the androgen to estrogens by aromatase enzyme^[18].

FSH begins to rise a few days preceding the beginning of menses and is in charge of the induction of an accomplice of ovarian follicles and in addition a chosen of the dominant follicle. FSH encourage granulosa cell growth and actuates aromatase activity^[13]. FSH level at that point begin to downhill owing to estrogen offspring by the growing follicular granulosa cells. In spite of this reduce in the FSH level, the dominant follicle keeps on developing as it ensure the most elevation in concentration of FSH receptors, making it increasingly impervious to the drop in FSH level also, the drop in FSH level causes a higher androgenic microenvironment in the non dominant follicles. FSH at that point decreases after ovulation of the dominant follicles^[19].

Transforming growth factor- β 1 (TGF- β 1) :-

Transforming growth factor- β 1 (TGF- β) is a multifunctional groups of cytokine exist in mammals in three isoforms (TGF- β 1, TGF- β 2, and TGF- β 3), encoded by different genes, the TGF- β 1 is at first synthesized as a 390 amino-acid precursor molecule called pre-pro-TGF- β 1, Pre-pro-TGF- β 1 then processed by many cleavage to yield mature TGF- β 1^[20]. TGF- β 1 is useful in regulating multiple reproductive functions, including folliculogenesis, steroidogenesis, cumulus expansion, oocyte maturation, and ovulation^[21]. They are also influence growth, differentiation, cell migration, formation and degradation of cell matrix components, as well as processes of chemotaxis and apoptosis, TGF- β role had been exhibit in progesterone and inhibin production by granulosa cells^[22].

TGF- β 1 is normally found in the plasma and serum (TGF- β 1 isoform), and bound to extra cellular matrix (ECM) proteins throughout the body, TGF- β 1 also is expressed in the oocytes, granulosa cells and theca cells of the growing follicles, it have seven receptors type 1 [activin-like kinases (ALKs) in human^[23]. These present in both theca and granulosa cells in normal follicles, of the ovary, the TGF- β is responsible for the differentiation and proliferation of granulosa and theca cells and also inhibition of androgen production^[24]. While in PCOS it expressed in the ovary and has been implicated in the pathogenesis of abnormal follicle development and hyperandrogenism, other factors, such as endocrine, environmental, and metabolic disorders, might also play an important role in dysregulated TGF- β signaling in PCOS patients^[25]. TGF- β 1 levels increase in various cardio metabolic complications, hypertension, obesity, insulin resistance, diabetes and coronary artery disease^[26]. These findings of increased TGF- β 1 reveal the bioavailability in PCOS is consistent with an important role for TGF- β 1 in the pathogenesis of the cardio-metabolic complications seen in Iraqi women with PCOS, TGF- β 1 regulates the expression of genes that promote inflammation, such as interleukin 6^[27]. Circulating and molecular markers of oxidative stress and inflammation are highly correlate with circulating androgens. These findings raise the possibility that in PCOS, either hyperandrogenemia to account for the hyperglycemia induced inflammation or conversely that glucose-stimulated inflammation promotes ovarian androgen production in PCOS^[28]. TGF- β 1 level dysregulated in women with PCOS, which might play a role in the pathophysiology of this syndrome, also implicate that TGF- β 1 activation is associated with hypertension and endothelial dysfunction, there is dispersed guide for the relationship between TGF- β 1, metabolic syndrome, visceral adiposity, non-alcoholic fatty liver illness and T2DM^[28].

In PCOS ovaries show all the assay marks of TGF- β upregulation, including increased collagen deposition in ovarian theca cell, supported by angiogenesis and increased vascularity, TGF- β 1 has been shown to be increased in the PCOS^[29]. Genetic study exhibited strong indicator that TGF- β 1 dysregulation in PCOS was associated with insulin resistance in women with PCOS. A recent association study in a group of Korean women showed that single nucleotide polymorphisms in

the TGF- β 1 gene were associated with the development and characteristics of PCOS, suggesting a potential role for TGF- β 1 in the pathogenesis of this chronic disease^[30]. Other recent study using human ovarian tissues showed that (TGF- β), supposed TGF- β receptors, and its corresponding signal molecules are expressed by the various cell types (oocytes, granulosa and theca cells) of ovarian follicles in a different stage during follicular growth, these study occur by using cellular and molecular advanced technologies have implicated intraovarian TGF- β 1 as crucial modulators of all sides of ovarian functions, from germ cell formation to follicular development, steroidogenesis, cell-cell connection, oocyte maturation, ovulation and luteal function^[31]. It is established that a TGF beta protein must be collapsed accurately in order to fulfill its biological function, on account of mutations, this factor might be misfolded, which can cause numerous fertility disorders, for example, primary ovarian deficiency or PCOS^[32]. In PCOS, if defect or change in TGF- β signaling within the ovary reason for structural changes (eg, increased ovarian stroma and tunica features of PCOS) that encourage functional changes (eg, elevated secretion of androgens by theca cell that surrounds the antral follicle and failure of a follicle to mature and develop into a dominant follicle because the increase stroma and enlarge of the ovary), resulting in the PCOS reproductive phenotype of chronic hyperandrogenic anovulation^[33].

2. Materials and methods

2.1 Subject

The present study comprised of 120 women (80 patients with PCOS and 40 apparently healthy control) recruited from Al-Furat teaching hospital and fertility center of Al-Sadder medical city in Al-Najaf province. They were seen from (June/ 2018 – October/ 2018). All these patients and healthy control were examined for PCOS diagnosis by ultrasound, measuring parameters including follicle stimulating hormone (FSH) and transforming growth factor (TGF- β 1). The FSH measured by enzyme linked fluorescence assay (ELFA) technique by using minividas instrument (Biomérieux, France) and TGF- β 1 measured by ELISA technique (Human, Germany).

2.2. Samples

Venous blood samples (5 mL) were collected from all patients and healthy controls by using disposable syringe in the sitting position, the specimens were taken during 2nd – 7th days of the menstrual cycle (follicular phase) from those of patients and healthy control. The samples were collected by venous puncture and pushed slowly into a dry clean gel tubes, then centrifuged directly after drawn, the serum from all blood samples were separated by centrifugation at (4000 rpm) for 10 minutes. Serum markers of FSH was measured immediately before storage and the remaining of the sera were stored at deep freeze in disposable plain plastic tubes, until the time of the analysis for measure serum TGF- β 1.

2.3. A. Inclusion criteria:

1. All patients must fulfill the international diagnostic criteria for PCOS.
2. All patients and control should be more than (15 and less than 40) year.
3. The PCOS is diagnosed for not than one year ago.

B. Exclusion criteria:

1. Patients that do not fulfill the international diagnostic criteria for PCOS.
2. Any diagnosed case of PCOS under current medication.

3. Control with a previous history of PCOS and recovered by (medication and/or surgery).
4. Patients of unexplained causes of infertility
5. Patients with other chronic disease as autoimmune, genetic, infectious and others.

2.4 . Statistical analysis : All the statistical analyses were done by using computer through the graph pad prism(version-8) and SPSS programme (version-22).

3.Result and discussion :

Table (1) : The levels of FSH and TGF- β 1among studied groups.

Parameters	Patients n=80	Control n=40	Statistics <i>t</i> test	<i>p</i> value	Sig.
	Mean $\pm$ SD	Mean $\pm$ SD			
FSH	4.37 $\pm$ 1.67	6.22 $\pm$ 1.98	<i>t</i> =5.34, df = 118	<0.001	HS
TGF-B1	68.48 $\pm$ 23.86	39.23 $\pm$ 14.55	<i>t</i> = 7.109, df = 118	<0.001	HS

The level of FSH in sera of patients and healthy control.

Table (1) and figure (1) showed a highly significant elevation($P<0.01$) in the mean concentration of FSH hormones in sera of patients in comparison with the mean serum concentration of healthy control [FSH (4.37 $\pm$ 1.67 mIU/mL) and (6.22 $\pm$ 1.98 mIU/mL) respectively, with a highly significant difference ($P<0.01$), appearance when PCOS patients are compared with the healthy control. At an optimum cut-off level of (2.2) mIU/mL, FSH showed the high sensitivity and specificity of 92% and 100% respectively ,with the AUR 0.238 (95% CI: 0.151- 0.325). as explain in table and figure (2) below .

Table (2): ROC for studied parameters.

Parameters	Cutoff	AUR	95%CI	Sensitivity	Specificity
FSH	2.2	0.238	0.151-0.325	92	100
TGF-B1	21.65	0.857	0.789-0.925	100	90

FSH level explained a highly significant difference ($P<0.01$) between PCOS patients and healthy control, this study showed a decrease in result of FSH hormone in PCOS patients in compared with healthy group, this result is an agreement with several recent studies done by (Shalal *et al.*2017)^[34] in Baghdad city of Al-Iraq and study done by (Haldun.2019) ^[35] in Turkey. Other study in Oman accomplished by(Gowri . *et al* .2013) ^[36] improved the decreases FSH levels in PCOS patients below than healthy control these results fits with the result in this study. While the Iraqi study in Baghdad done by (Shakir *et al.* 2014) ^[37] explained that the level of FSH hormone were in the normal range in PCOS patients , exhibited different result with this study.

Finally more recent study in Al-Najaf province by (Aljelawi *et al.*2019)^[38] appeared no significant difference were observed in serum FSH levels between PCOs and healthy group , the result disagreement with this study the reason may be due to the differences in the criteria followed by this study that included the result of FSH must be lower in PCOS patients than control group as an additional factor for improved the diagnosis of PCOS.

The result obtained from this study it is compatible with the criteria for the diagnosis of PCOS , where the levels of FSH hormone in PCOS patients decreased or normal level than healthy control. The reduction levels of FSH can be explained by the increase of the conversion of androstenedione in adipose tissue which additionally stimulates LH and inhibits FSH ^[39].

The reduction in the concentration of FSH may be due to the adrenal gland, which produces adrenaline, which in turn affects the concentration of FSH through the secretion of androgens and estrogens. We believe that this low level of hormone may be due to the mechanism of hormonal restraining of FSH by the mechanism of feedback negative, and this corresponds to the results of the study ^[40].

The negative feedback control of FSH is critical for development of the single mature oocyte that characterizes normal reproductive function in women .The reduction level of FSH lead to inhibition of aromatase activity thus causing accumulation of androgen, also leads to impaired follicular development and therefore, granulosa cell atresia and not a single follicle is permitted to mature enough for ovulation to occur, this is important feature of PCOS^[41].

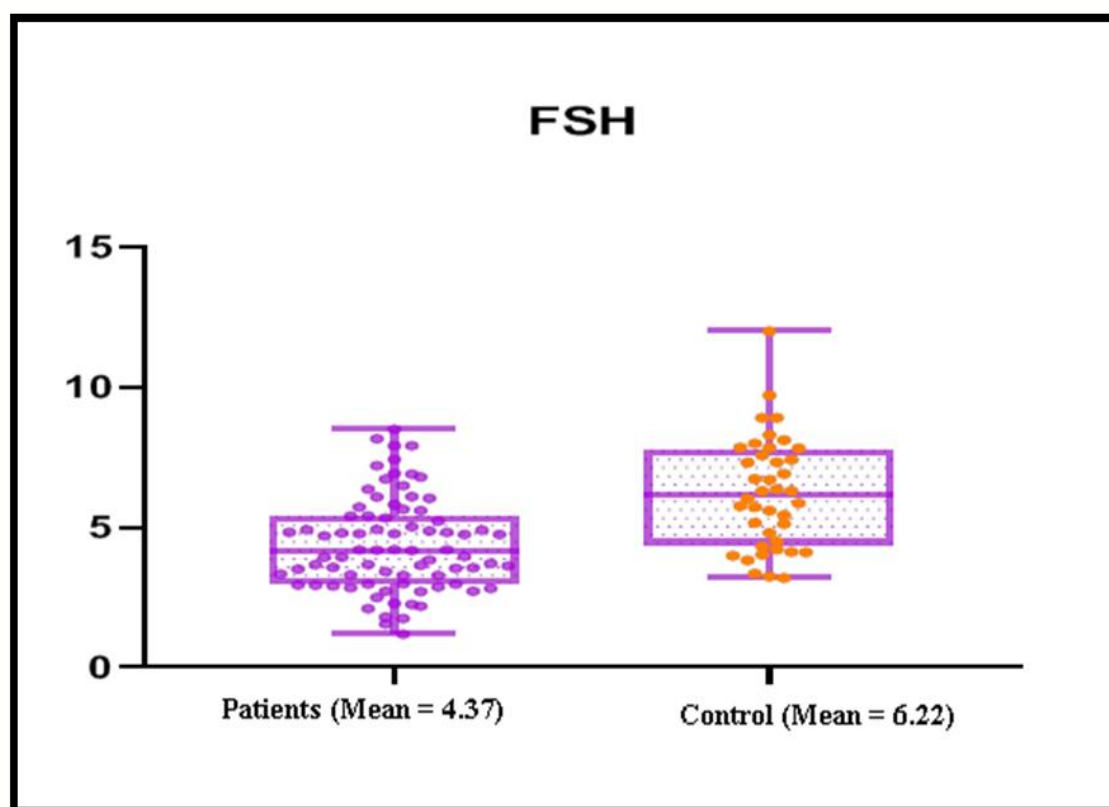


Figure (1): Boxplot of FSH levels in studied groups.

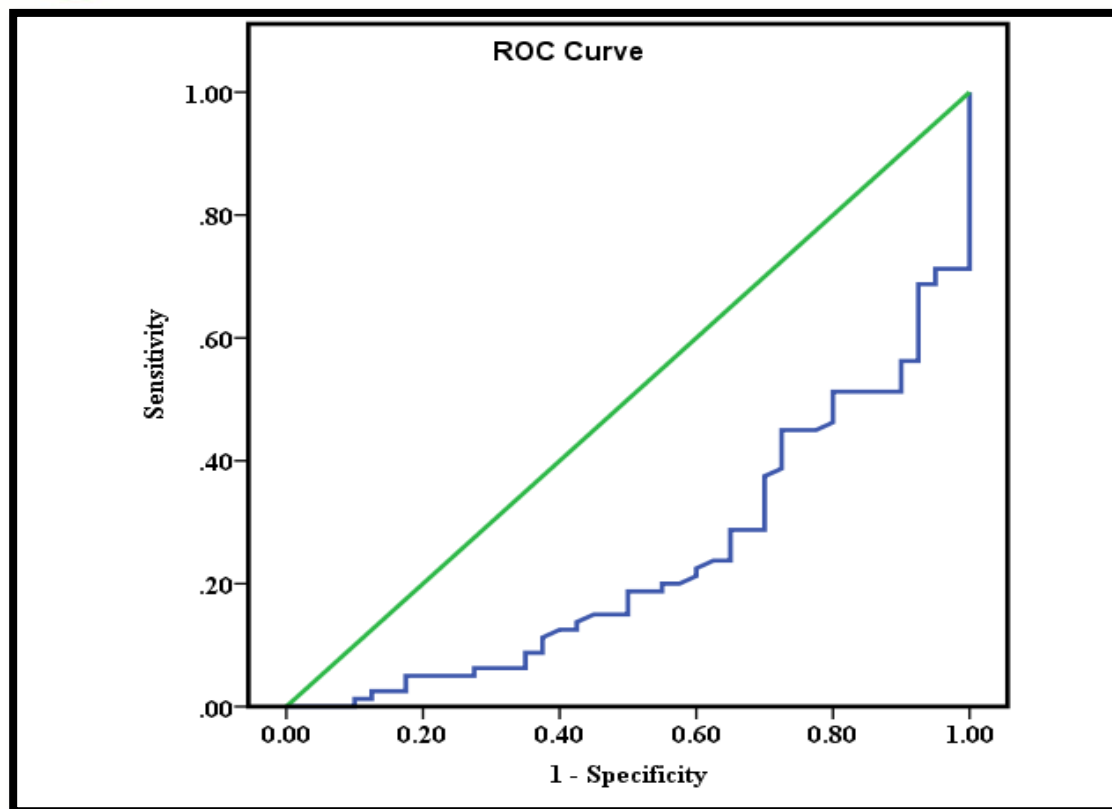


Figure (2): ROC curve analysis of FSH to discriminate the studied groups.

The level TGF- β 1 in sera of patients and healthy control.

The serum TGF- β 1 (ng/mL) in Table (1) and Figure (3) exhibited a highly significant elevation ($P < 0.01$) in the mean concentration of TGF- β 1 in sera of patients [serum TGF- β 1 (68.48 ± 23.86) ng/mL] in comparison with mean serum concentration of control group [serum TGF- β 1 (39.23 ± 14.55) ng/mL]. At an optimum cut-off value of (21.65) ng/mL, TGF- β 1 showed the high sensitivity and specificity as 100 % and 90 % respectively, with a AUR (0.857) (95% CI: 0.789-0.925), as showed in table (2) above and figure (4) below.

The result of this study agreed with many studies reported by (Mahdi *et al.* 2016)^[42] in Baghdad city, (Rashad *et al.* 2018)^[43] in Egypt and finally by (Tal *et al.* 2013)^[44] in America. These studies revealed statistically significant higher values of TGF- β 1 in PCOS compared to healthy control, higher values of TGF- β 1 appeared in overweight and obese PCOS compared to lean PCOS group.

The causes of increased TGF- β 1 in PCOS may associated with increased number of follicles in the ovaries of women with PCOS, because it produce from granulosa, theca cell and oocyte of these follicles^[45]. Also suggested the higher AMH levels, may still lead to greater ovarian TGF- β 1 production, which could have significant local effects in the ovary. The increased TGF- β 1 bioavailability in patients with PCOS may explain several aspects of this syndrome, which TGF- β 1 is a potent growth factor involved in angiogenesis, fibroblast activation and tissue fibrosis^[46]. Thus, it may play an important role in the increased fibrosis of the ovarian stroma and theca as well as in the increased angiogenesis and vascularity seen in the ovaries of women with PCOS^[47]. TGF- β 1 is responsible for the differentiation and proliferation of granulosa and theca cells, as well as inhibition of androgen production^[48]. The receptors of TGF- β 1 present in both

theca and granulosa cells ,when defect occur can effect on developing follicle in different stage of folliculogenesis, it can act as an inhibitor or stimulator of granulosa and theca cells proliferation. Also, TGF- β role had been demonstrated in progesterone and inhibin secretion by granulosa cells^[49].TGF- β 1 has a regulatory effect on primordial follicle growth and is involved in maintaining the primordial follicle pool . It was observed that there was a decrease in the amount of primordial and growing follicles when ovaries were cultured with TGF- β 1. Therefore, there is a possibility that this protein's relatively low level enables the activation of primordial follicles^[50].

Conditional of surgical removal of TGF- β 1 receptor in mice results in the altered functionality of the female reproductive tract, such as defective oviduct and uterus development, and prevents embryo implantation, thus causes problems with fertility^[51]. A low chronic inflammatory state, characterized by elevation levels of proinflammatory molecules and acute phase proteins, can be present in obesity and insulin resistance^[52]. The TGF- β 1 of overweight and obese groups showed a higher significant positive correlation with insulin resistance, insulin. In other studies, increased circulating TGF- β 1 level in various cardio metabolic complications, hypertension, obesity, insulin resistance, diabetes and coronary artery disease were found^[53].These findings of increased TGF- β 1 reveal the bioavailability in PCOS is consistent with an important role for TGF- β 1 in the pathogenesis of the cardio-metabolic complications seen in Iraqi women with PCOS. Targeting TGF- β 1 dysregulation may be particularly important in PCOS as women at increased risk of impaired glucose tolerance and type 2 diabetes due to underlying insulin resistance. Excessive TGF- β 1 activity has been implicate in the pathogenesis of arterial disease in patients with altered glucose metabolism^[54].Dysregulated TGF- β 1 activity may contribute to atherosclerosis by stimulating smooth muscle cells in the vasculature to proliferate and synthesize collagen. TGF- β 1 regulates the expression of genes that promote inflammation, such as interleukin 6 which highly correlate with circulating androgens^[55].These findings raise the possibility that in PCOS, either hyperandrogenemia pre-activate mononuclear cells (MNC) to account for the hyperglycemia induced inflammation, or conversely that glucose-stimulated inflammation promotes ovarian androgen production in PCOS. There is data to support that both mechanisms may occur^[56].

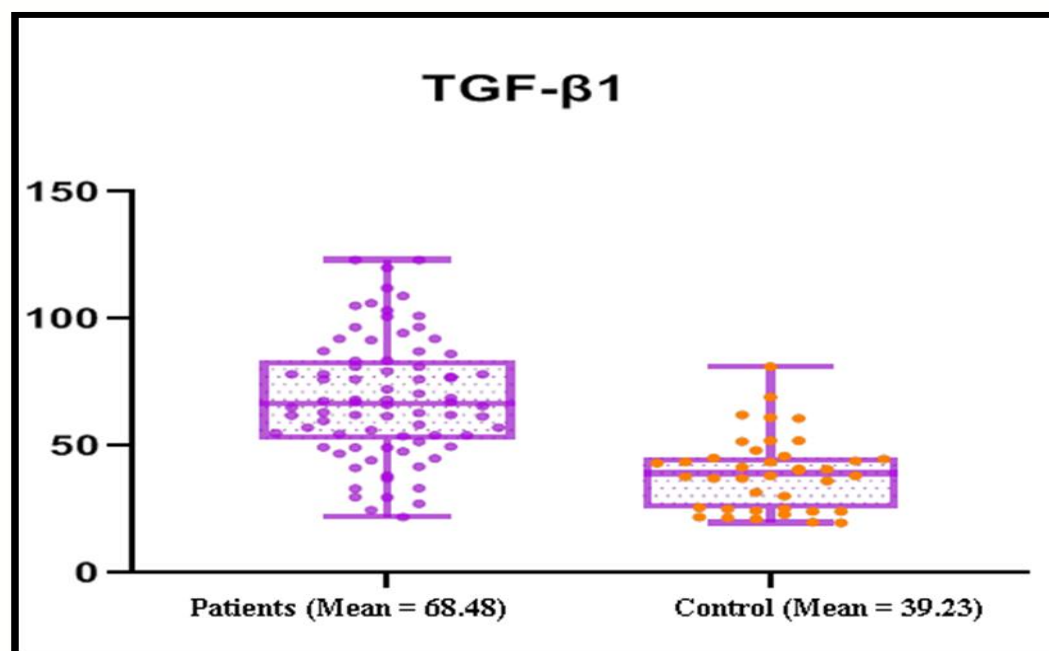


Figure (3): Boxplot of TGF- β 1 level in studied groups.

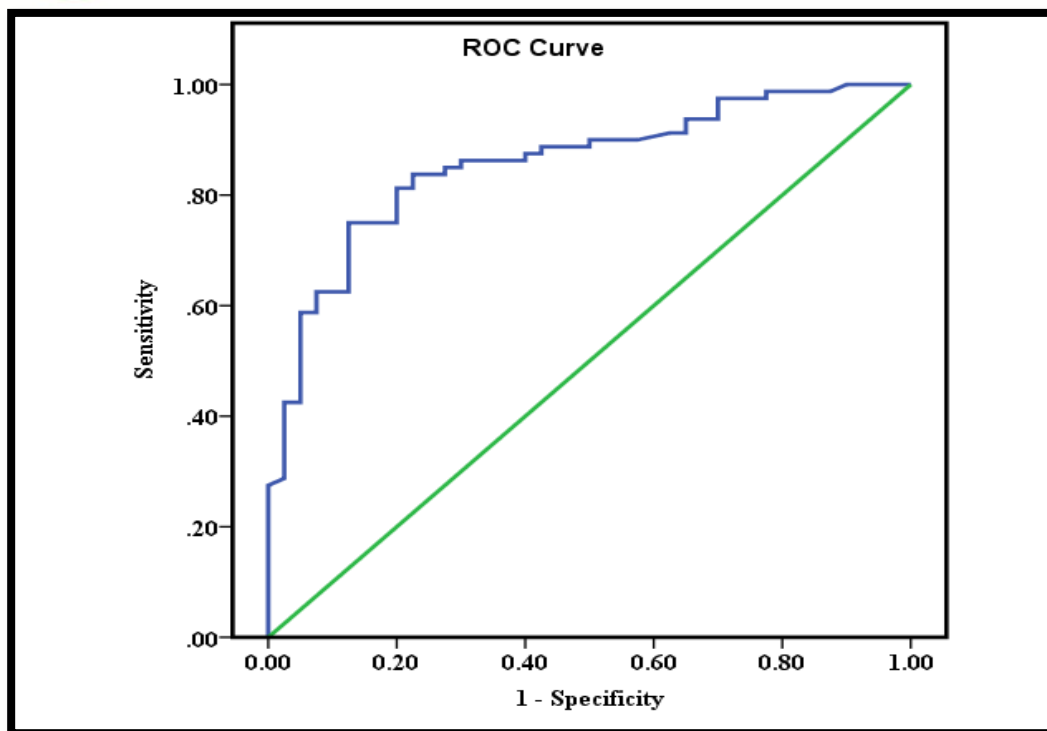


Figure (4): ROC curve analysis of TGF-B to discriminate the studied groups.

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