Study the extent of the relationship among hormonal disturbance, vitamin D deficiency and age in women with polycystic ovary Thi-qar city, southern Iraq

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Abstract

This study was design to investigate the relationship between the disturbance of LH, FSH, testosterone, estradiol, progesterone and metabolic parameters with AMH, and vitamin D in polycystic ovarian syndrome in woman.

The results showed that in respective to age, PCOS patients showed significant elevation of serum level of LH ($P< 0.01$), FSH ($P< 0.05$), LH/FSH ratio ($P< 0.05$), testosterone ($P< 0.01$), progesterone ($P< 0.05$), antimullerian ($P< 0.001$), glucose ($P< 0.01$), total cholesterol ($P< 0.01$), triglycerides ($P< 0.001$), LDL ($P< 0.01$), VLDL ($P< 0.05$) and Vitamin D ($P< 0.01$), while there were significant decline in the serum level of estradiol ($P< 0.01$), and HDL ($P< 0.01$).

It was clearly appeared that AMH was positively correlated with the elevated LH, FSH, LH/FSH ratio, testosterone, progesterone, glucose, total cholesterol, triglycerides, LDL, VLDL levels and negatively correlated with estradiol and HDL. While Vitamin D was negatively correlated with AMH. Low serum Vitamin D was associated with elevation of testosterone, LH, FSH, LH/FSH ratio, progesterone, total cholesterol, triglycerides, LDL and glucose. We can concluded that, AMH can potentially applicated as a diagnostic marker for PCOS, the results also showed a strong correlation between vitamin D deficiency and the pathophysiology of PCOS. Vitamin D serum levels showed an inverse correlation with hyperglycemia, hyperlipidemia, and hormonal disturbances in PCOS.

Key words: Hormonal disorders, metabolic disorders, Pcos, Vitamin D, AMH

Introduction

Polycystic ovarian syndrome (PCOS) is multifactorial endocrine disorder affecting women in child bearing age and is considered the main cause of infertility as a result of anovulation [1-2]. It was described as a syndrome of oligoamenorrhea and polycystic ovaries accompanied by hirsutism, acne, and obesity[3]. Excess secretion of androgen associated with changes in the ovarian morphology represented the main pathological factor in PCOS. Hyperlipidemia, insulin resistance, and psychosocial dysfunction are part of the symptoms and signs of PCOS[4]. PCOS is diagnosed by the presence of oligo amenorrhoea, polycystic ovaries morphology and hyperandrogenemia [5].

Increasing of anti-mullerian hormone (AMH), the glycoprotein secreted by the granulosa cells of ovarian early developing follicles was also used as marker in diagnosis of PCOS [6]. PCOS characterized by excessive amount of small antral follicles in the ovaries and excessive serum AMH levels [7].

Many studies have demonstrated that vitamin D deficiency is common among women with PCOS [8]. Menstrual irregularities, ovulation problems, and infertility were occurred during the periods of low vitamin D status [9-10]. Vitamin D deficiency was also associated with

high insulin resistance and elevated levels of total testosterone and dehydroepiandroster-one [10-11]. Vitamin D receptors were distributed in various human tissues, including ovary and endometrium, suggesting the active role of vitamin D in female reproductive tissues [12-13]. This study was design to investigate the relationship between serum vitamin D, with hormonal disturbances and age in PCOS women.

Materials and Methods

Fasting blood samples were collected from 60 married infertile women (20-40 years) with PCO attending Al-Shatra hospital, Bint AL-Hoda and Al-Hussein teaching hospital in Thi-Qar governorate- Iraq, who diagnosed by gynecologists, through the period from 1 September 2020 until the end of November 2020. The patients with suspicion of androgen-secreting tumor, hyperprolactinemia, Cushing syndrome, congenital adrenal hyperplasia, women with contraceptives hypoglycemic drugs, lipid lowering drugs and hormonal medications were excluded. Fasting blood samples were also collected from 30 healthy non pregnant, aged matched women, to serve as control. The study was approved by the ethical committee of health directorate of Thi-Qar governorate and the ethical board of the South technical university-Iraq. An informed consent was obtained from all participants. Glucose and lipid profile were determine by using Cobas C311 photometric assays and ion-selective electrode measurements (Kits and instrument Roche- Germany), while, LH, FSH, estradiol, progesterone, testosterone, AMH and vitamin D were determined by using Cobas 411 immunoassay analysis (Kits and instrument Roche- Germany) [14-15]. All results are reported as the mean ±SE. Differences in the mean values for individual hormone measurements were assessed by student t-test.

Results

In the group aged 20-25 years, PCO patients showed significantly elevated serum levels of LH ($P< 0.001$), FSH ($P< 0.05$), LH/FSH ratio ($P< 0.05$), total testosterone ($P< 0.05$), progesterone ($P< 0.05$), AMH ($P< 0.001$), glucose ($P< 0.05$), total cholesterol ($P< 0.05$), triglycerides ($P< 0.01$), and VLDL cholesterol ($P< 0.05$). While, the serum level of estradiol, HDL cholesterol and vitamin D were significantly declined ($P< 0.05$). Vitamin D level was positively correlated with estradiol and HDL cholesterol and negatively correlated with LH, FSH, LH/FSH ratio, total testosterone, progesterone, AMH, glucose, total cholesterol, triglycerides and VLDL cholesterol (table 3-1).

Table 1: Vitamin D, hormonal and metabolic alterations in PCOS patients aged 20-25 years in comparison with aged matched control.

Parameter studied		Control (15)	PCOS patients (37)	Significancy
LH (IU/L)		4.99±o.62	15.86±0.96	P< 0.001
FSH (IU/L)		3.42±0.32	4.68±0.45	P< 0.05
LH/FSH		1.45±0.08	3.38±0.12	P< 0.05
Total testosterone (ng/ml)		0.46±0.08	0.58±0.06	P< 0.05
Estradiol (pmol/l)		116.64±8.62	106.33±9.32	P< 0.05
Progesterone (nmol/ml)		0.67±0.06	1.44±0.09	P< 0.05
AMH(ng/ml)		2.40±0.12	10.00±0.23	P< 0.001
Glucose (mg/dl)		94.60±3.23	109.02±4.32	P< 0.05
Lipid profile	**Total cholesterol (mg/dl)**	170.00±6.16	176.00±7.22	P< 0. 05
	Triglycerides (mg/dl)	150.66±5.56	175.81±6.32	P< 0.01
	LDL (mg/dl)	98.81±3.51	97.17±4.12	NS
	VLDL (mg/dl)	30.76±1.45	35.18±1.94	P< 0.05
	HDL (mg/dl)	48.93±1.66	45.91±1.57	P< 0.05
Vitamin D (ng/ml)		16.95±0.96	14.26±0.84	P< 0.05

In the group aged 26-30 years, serum biochemical analysis of PCO patients revealed significant elevatation of serum levels of LH (P< 0.01), FSH (P< 0.05), LH/FSH ratio (P< 0.05), total testosterone (P< 0.05), AMH (P< 0.01), glucose (P< 0.05), total cholesterol (P< 0.05), triglycerides (P< 0.01), LDL cholesterol (P< 0.05), and VLDL cholesterol (P< 0.05). While, the serum level of estradiol (P< 0.05), HDL cholesterol (P< 0.01), and vitamin D (P< 0.05) were significantly declined in comparison with control. Vitamin D level was positively correlated with estradiol testosterone and HDL cholesterol and negatively correlated with LH, FSH, LH/FSH, total testosterone, AMH, glucose, total cholesterol, triglycerides, LDL cholesterol, and VLDL cholesterol(table3-2).

Table 2: Vitamin D, hormonal and metaolic alterations in PCOS patients aged 26-30 years in comparison with aged matched control.

Parameter studied		Control (6)	PCOS patients (11)	Significancy
LH (IU/L)		5.67±	9.58±	P< 0.01
FSH (IU/L)		3.79±	5.37	P< 0.05
LH/FSH		1.49±	1.78±	P< 0.05
Total testosterone (ng/ml)		0.42±	0.48±	P< 0.05
Estradiol (pmol/l)		107.90±	74.33±	P< 0.05
Progesterone (nmol/ml)		0.84±	0.76±	NS
AMH(ng/ml)		5.35±	8.96±	P< 0.01
Glucose (mg/dl)		97.80±	101.00±	P< 0.05
Lipid profile	**Total cholesterol (mg/dl)**	190.00±	196.00±	P< 0. 05
	Triglycerides (mg/dl)	184.00±	211.50±	P< 0.01
	LDL (mg/dl)	91.00±	99.70±	P< 0.05
	VLDL (mg/dl)	41.20±	45.60±	P< 0.05
	HDL (mg/dl)	53.80±	40.72±	P< 0.01
Vitamin D (ng/ml)		21.96±	17.28±	P< 0.05

PCO patients aged 31-35 years, showed significant elevation of serum levels of LH (P< 0.001), FSH (P< 0.05), LH/FSH ratio (P< 0.01), total testosterone (P< 0.05), AMH (P< 0.001), glucose (P< 0.05), total cholesterol (P< 0.05), triglycerides (P< 0.001), LDL cholesterol (P< 0.05), and VLDL cholesterol (P< 0.05). However, the serum level of estradiol (P< 0.05), HDL cholesterol (P< 0.01), and vitamin D (P< 0.01) were significantly declined in comparison with control. Vitamin D level was positively correlated with estradiol and HDL cholesterol and negatively correlated with LH, FSH, LH/FSH, total testosterone, progesterone, AMH, glucose, total cholesterol, triglycerides, LDL cholesterol, and VLDL cholesterol (table 3-3).

Table3: Vitamin D, hormonal and metabolic alterations in PCOS patients aged 31-35 years in comparison with aged matched control.

Parameter studied		Control (5)	PCOS patients (7)	Significancy
LH (IU/L)		4.13±0.31	11.17±0.73	P< 0.001
FSH (IU /L)		3.44±0.05	4.71±0.08	P< 0.05
LH/FSH		1.20±0.05	2.37±0.06	P< 0.01
Total testosterone (ng/ml)		0.47±0.02	0.55±0.03	P< 0.05
Estradiol (pmol/l)		119.32±4.62	90.39±3.78	P< 0.05
Progesterone (nmol/ml)		0.37±0.03	0.54±0.04	P< 0.05
AMH(ng/ml)		2.41±0.07	11.27±0.26	P< 0.001
Glucose (mg/dl)		82.00±3.54	113.857±6.23	P< 0.05
Lipid profile	**Total cholesterol (mg/dl)**	198.00±7.82	205.00±9.63	P< 0. 05
	Triglycerides (mg/dl)	200.00±8.82	257.85±10.64	P< 0.001
	LDL (mg/dl)	101.25±3.62	105.83±3.82	P< 0.05
	VLDL (mg/dl)	44.50±1.28	56.83±1.64	P< 0.05
	HDL (mg/dl)	58.00±2.68	42.00±1.98	P< 0.01
Vitamin D (ng/ml)		20.51±2.82	14.96±1.64	P< 0.01

In comparison with control group, PCO patients aged 31-35 years, showed significant elevation of serum levels of LH (P< 0.01), FSH (P< 0.05), LH/FSH ratio (P< 0.01), total testosterone (P< 0.05), AMH (P< 0.01), glucose (P< 0.001), total cholesterol (P< 0.001), triglycerides (P< 0.001) and LDL cholesterol (P< 0.01). On the other hand, the serum levelof estradiol (P< 0.01), HDL cholesterol (P< 0.05), and vitamin D (P< 0.01) were significantly declined. Vitamin D level was positively correlated with estradiol and HDL cholesterol and negatively correlated with LH, FSH, LH/FSH, total testosterone, AMH, glucose, total cholesterol, triglycerides, and LDL cholesterol (table 3.4).

Table 4: Vitamin D, hormonal and metabolic alterations in PCOS patients aged 36-40 years in comparison with aged matched control

Parameter studied		Control (4)	PCOS patients (5)	Significancy
LH (IU/L)		6.54±0.42	10.32±0.85	P< 0.01
FSH (IU/L)		4.40±0.12	5.28±0.24	P< 0.05
LH/FSH		1.48±0.12	2.03±0.15	P< 0.05
Total testosterone (ng/ml)		0.52±0.06	0.68±0.08	P< 0.05
Estradiol (pmol/l)		92.00±4.62	60.00±3.32	P< 0.01
Progesterone (nmol/ml)		0.82±0.06	0.84±0.04	NS
AMH(ng/ml)		1.47±0.09	3.90±0.13	P< 0.01
Glucose (mg/dl)		104.66± 4.58	165.40±6.51	P< 0.001
Lipid profile	**Total cholesterol (mg/dl)**	180.00±8.72	227.00± 12.64	P< 0. 001
	Triglycerides (mg/dl)	156.66±8.62	210.00±10.28	P< 0.001
	LDL (mg/dl)	127.25±6.23	142.20±7.52	P< 0.01
	VLDL (mg/dl)	45.33±6.43	47.25±6.67	NS
	HDL (mg/dl)	46.00±2.12	42.80±1.23	P< 0.05
Vitamin D (ng/ml)		29.11±1.34	14.84±0.98	P< 0.01

Discussion

All tables showed that the results of all age subgroups of the control group were similar, furthermore the results of all age subgroups in PCO patient were also identical. The results was in agreement with many previous studies [16], [17], [18], mentioned that there no variations in the level of pathological markers among age groups in PCO women.

The hormonal disturbances recorded in PCOS in this study could be attributed to hypothalamic dysfunction. Hypothalamic dysfunction may be a primary or secondary cause to abnormal steroid feedback. In either case serum LH level, rises and increased levels are observed clinically in approximately 50% of PCOS women [19]. As a result of this derangement the ratio between FSH and LH levels which is normally around 2 to 1, become reversed and sometimes even more (2 or 3 to 1) in approximately 60% of the patients with PCOS [20], as that recorded in the current study. The elevation in LH hormone in turn lead

to increased androgen production and secretion by ovarian theca cells, increased level of testosterone also stimulated by insulin resistance associated with this syndrome [21].

The current study showed that AMH was positively correlated with the elevated LH, FSH, LH/FSH ratio, testosterone, progesterone, glucose and lipid profile and negatively correlated with estradiol, and HDL. While vitamin D was negatively correlated with AMH. These results were in agreement with many studies [22], [23], [24]. In *vitro* studies showed that the human AMH gene promoter contains a functional vitamin D responsive element, and treating human cumulus granulosa cells with vitamin D led to a down regulation in AMH receptor-II. In addition, treating PCOS women with vitamin D supplements normalized their serum AMH levels [23], [24].

Vitamin D deficiency is also associated with high insulin resistance and elevated levels of total testosterone and dehydroepiandrosterone sulfate patients with PCOS [25], [26]. The active form of vitamin D, 1,25-dihydroxyvitamin decline of Vitamin D was associated with elevation of LH, FSH, LH/FSH ratio, testosterone, progesterone, glucose, total cholesterol, triglycerides, LDL and VLDL levels.

Vitamin D receptors (VDRs) are distributed across various human tissues, including ovary and endometrium, which suggested an active role of vitamin D in female re-productive tissues [26], [27], [28]. PCOS is associated with abnormal calcium and phosphate metabolism, and low vitamin D levels [26] [29].

Many studies have reported low levels of 25(OH) D with a range between 11 and 31 ng/ml, with the majority having values <20 ng/ml (67–85%) in women with PCOS. The relationship between vitamin D level and high LH and testosterone in addition to PCOS symptoms (infertility, hirsutism and insulin resistance) was confirmed by many studies [30], [31].

Vitamin D plays a role in enhancing certain key steroidogenic enzymes such as 3b-HSD. During the normal menstrual cycle, luteinized human granulose cells usually form the corpus luteum which produces large amounts of progesterone (and some estrogens) and induces endometrial changes such as decidualization to support a pregnancy. 1,25- dihydroxyvitamin D3 potentiates granulosa cell luteinization as reflected by increased progesterone production, thus providing a better endometrial environment [24].

In other hand, vitamin D deficiency is a contributing factor to insulin resistance, obesity, and metabolic syndrome, all of which are commonly observed in PCOS and associated with ovulatory dysfunction [31], [32]. An improvement in insulin sensitivity was recorded after supplementation of PCOS patients with vitamin D. Vitamin D activates the transcription of human insulin receptor gene, as the promoter of this gene showed vitamin D responsive element. Furthermore, secretion of insulin from β cells is a calcium-dependent process. Supplementation with vitamin D in PCOS patients improved insulin sensitivity, reduced total testosterone and reducing AMH level [33], [34].

Conclusion:

The results strongly correlated between vitamin D deficiency and the pathophysiology of PCOS. Vitamin D could participated in the occurrence of these hormonal and metabolic changes in patients with PCOS. As well as, the impact of vitamin D in the ovary might potentially encourage the use of vitamin D as an adjunct therapy in treatment of PCOS.

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Ethical considerations

The research was approved by the ethical committee of health directorate of Thi-Qar governorate andthe ethical board of the South technical university-Iraq. Furthermore, a written informed consent was obtained from all participants.

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