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The Effect of Vitamin D Administration on Androgen Levels in Addition to Metformin Treatment in Adolescent Girls with Hyperandrogenism

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Abstract

This retrospective study was planned to examine the relationship between glucose metabolism, androgen and lipid profiles after vitamin D and metformin treatments in adolescent girls with hyperandrogenism. Forty-five adolescent female patients diagnosed with hyperandrogenism were divided into three groups. The first group of patients was given metformin (M), the second group was given metformin and vitamin D drops (MdD), and the third group were those who received oral metformin and vitamin D ampoules (MsD). Biochemical and hormonal parameters at the end of 8 weeks were compared statistically. The vitamin D level was higher in the metformin-vitamin D stoss treated group than metformin-vitamin D drops. There was a positive correlation between vitamin D and SHBG in the metformin group ($r = 0.65$, $p<0.01$). A significant correlation was observed between triglyceride and insulin in both groups given vitamin D, and there was a decrease in these two values ($p<0.05$). There was a positive correlation between total testosterone and Alanine transaminase (ALT) in the MsD group ($p<0.05$). Additionally, a positive correlation was observed between SHBG and HDL-cholesterol in three groups. There was a significant correlation between androgen and lipid parameters in the 8-week metformin and vitamin D treated groups. Long-term studies using high-dose vitamin D are needed to support our results.

Keywords: Hyperandrogenism, adolescent, vitamin D, metformin, testosterone

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Introduction

Hyperandrogenism causes chronic anovulation in girls and causes many clinical findings such as polycystic ovary syndrome (PCOS), obesity, systemic arterial hypertension, dyslipidemia, and insulin resistance.[1] Hirsutism is the term used for androgen-sensitive male-pattern hair growth in girls.[2] The most common cause of hirsutism in adolescent girls is PCOS, and it is seen in 5%-10% of women of reproductive age.[3] Insulin resistance and increased insulin level have been shown to be the etiology of hyperandrogenism. The treatments used to date vary according to the source of hyperandrogenism and the clinical condition of the patient. Birth control pills, metformin, antiandrogens such as spironolactone and finasteride are some of these treatments.[4]

Highlights

- Hyperandrogenism causes chronic anovulation in adolescent girls and causes the development of many systemic diseases.
- Vitamin D supplementation is effective in curing some diseases. There are studies showing that androgen levels decrease with the addition of vitamin D to metformin.
- The current study shows that long-term and adequate dose of vitamin D is required to see this effect.

Obesity is the most common cause of insulin resistance and hyperandrogenism. Therefore, weight loss may be the first line of treatment for women with hyperandrogenism.[4] Metformin is the most commonly used drug for treating type 2 DM (Diabetes mellitus). This drug increases glucose uptake by peripheral tissues while reducing peripheral insulin levels and fatty acid oxidation. Additionally, various effects of this drug have reported such as, regulating ovulation, weight loss, lowering circulating androgen levels and reducing the risk of miscarriage in patients with PCOS.[5]

In recent studies, vitamin D has been one of the nutritional factors recommended for treating PCOS. Vitamin D, which belongs to the family of steroid hormones, is fat-soluble. Serum 25(OH)D is the best indicator of vitamin D level. Vitamin D (25(OH)D) level <20 ng/ml is defined as severe deficiency, 20-30 ng/ml as deficiency, 30-100 ng/ml level as adequacy, and more than 100 ng/ml level as toxicity.[6] It has been suggested that gene transcription is regulated through vitamin D receptors (VDRs) found in various tissues, including the skeletal system, parathyroid glands, heart, immune system, and ovaries.[3] Vitamin D deficiency appears to be associated with increased parathyroid hormone (PTH) level, PCOS, ovulatory infertility, and high testosterone.[3] Additionally, it has been reported that vitamin D supplementation significantly reduces the serum total testosterone level.[3] In our study, the files of adolescent female patients diagnosed with hyperandrogenism and given outpatient treatment were reviewed retrospectively. Our aim in this study is to show the relationship between vitamin D treatment and androgen levels in addition to metformin treatment. It is important because it is the first study conducted in adolescents in our country.

Material and Method

The study was carried out with the permission of Süleyman Demirel University Faculty of Medicine Clinical Research Ethics Committee (Date: 20.12.2017, Decision No: 226). Between December 2017 and September 2018 forty-five female patients were selected who applied to our endocrine outpatient clinic with complaints such as increased hair growth, menstrual irregularity, acne, male pattern hair loss and diagnosed with hyperandrogenism. File information was reviewed retrospectively.

Patients with systemic disease and using medication and vitamin therapy were excluded from the study. The height and weight of the patients were recorded according to the file information. Body mass index (BMI) was calculated with the formula body weight (kg) / height² (m²). Ferriman Gallwey scoring was used in patients with hirsutism, and patients with a score of 8 and above were considered hirsutism. Patients who did not want pelvic ultrasound were excluded from the study. Patients with normal ultrasound results were included. Glucose, vitamin D, insulin, Homa-IR (homeostatic model evaluation of insulin resistance), ALT (alanine aminotransferase), LDL (low-density lipoprotein), HDL (high-density lipoprotein), triglyceride, FSH (follicle stimulating hormone), LH (luteinizing hormone), estradiol, DHEAS (dehydroepiandrosterone sulfate), androstenedione, free testosterone, total testosterone and SHBG (sex hormone binding globulin) values were recorded. The total testosterone of the patients was >55 ng/dl. A Homa-IR above 4 was considered insulin resistance (Homa-IR: Fasting insulin (µu/ml) x fasting plasma glucose (mg/dl) /405).[7] The patients were divided into 3 groups according to the treatment they received (n=15). Group M was given 850 mg/day of metformin. Metformin-vitamin D 15 drops/day (2 000 IU) was given to the MdD group. Half of the metformin-vitamin D ampoule (150,000 IU/month) was given orally to the MsD group. After 8 weeks, biochemical and hormonal parameters were compared statistically.

Statistical Analysis

SPSS 23.0 program was used for statistical analysis in the study. Descriptive statistics were given as mean±standard deviation. The Kolmogorov-Smirnov test was used to examine the normal distribution of variables. Changes between the three groups were compared using the LSD post hoc pairwise comparison method using one-way analysis of variance. Significance level was set as $p<0.05$.

Results

File information about 270 female patients aged between 12 and 18 who were diagnosed with hyperandrogenism was analyzed. Among these patients, 90 (33%) patients due to thyroid hormone disorder, 62 (22%) patients due to diabetes, 20 (7.4%) patients due to non-classical congenital adrenal hyperplasia (non-classical CAH), 4 (1.4%) patients due to prolactinemia, 49 (18%) patients

who did not meet the study criteria and/or did not have sufficient file information was not included in the study (**Figure 1**). The study was conducted in 45 (16.6%) subjects who met the inclusion criteria.

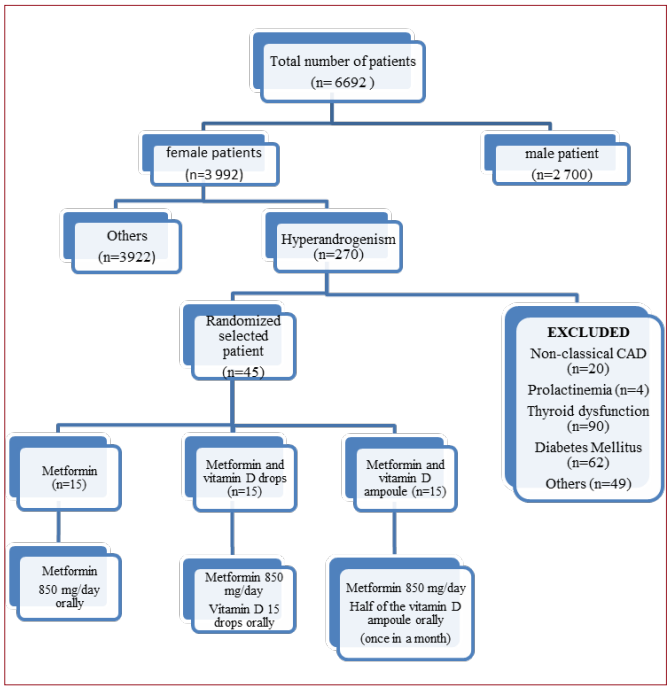

Figure 1. Summary of the patient follow-up chart

There was no significant difference between the mean age, weight and height of the groups before the study (p>0.05). The BMI of the M and MdD groups were significantly different from each other at the beginning of the study (p<0.01). However, after 8 weeks of treatment, there was no significant correlation between the BMIs of the groups (p>0.05) (**Table 1**).

Severe vitamin D deficiency (<20 ng/ml) was observed in 68.8% of all patients, vitamin D deficiency (20-30 ng/ml) in 26.6%, and vitamin D adequacy (>30 ng/ml) was observed in the remaining 4.4%. Severe vitamin D deficiency (<20 ng/ml) was observed in 68.8% of all patients, vitamin D deficiency (20-30 ng/ml) in 26.6%, and vitamin D adequacy (>30 ng/ml) was observed in the remaining 4.4%. While the mean total testosterone of those with severe vitamin D deficiency was 70.4 at the beginning of the study, it was 60.5 at the end of the study. SAI mean decreased from 10.8 to 10.2. The DHEAS average increased from 277 to 279. The mean total testosterone of those with vitamin D deficiency decreased from 70.7 to 57.1 with treatment. While the mean SAI was 9.8, it became 7.9 with treatment. The DHEAS average increased from 315 to 348. The mean total testosterone of those with adequate vitamin D levels decreased from 66.8 to 64.8. SAI average decreased from 4.2 to 4.9. The DHEAS average increased from 340 to 346. In the initial data of the study, there was no significant difference between the serum vitamin D levels of the groups (**Table 2**).

At the end of the 8-week treatment, a higher serum vitamin D level was observed in the MsD group compared to the MdD group. In our study, no significant correlation was found between vitamin D level and total testosterone, insulin, glucose, lipid profiles, FSH, LH, estradiol and DHEAS (**Table 3**).

Table 1
Physical characteristics of the patientsa

	M[b] (n=15)	MdD[d] (n=15)	MsD[c] (n=15)	p
Age (y)	14.8±1.86	15.7±153	16.2±1.29	0.67
Height (cm)	158.6±7.50	162.3±4.57	160.8±7.08	0.99
Weight (kg)	62.09±15.06	82.2±15.88	74.4±18.87	0.90
BMI (kg/m²)	24.50±5.05	31.07±5.40*	28.28±6.82	0.01
BMI (kg/m²) difference	-0.25±1.61	-0.10±1.23	-0.11±1.39	0.90

[a]Data mean±standard deviation, [b]Group receiving 850-mg metformin orally daily, [c]Group who took 850 mg metformin and half of the vitamin D ampoule (150 000 IU) once a month orally, [d]Group taking 850-mg metformin and 15 drops (2 000 IU) of vitamin D orally daily, *Statistical difference between M and MdD

Table 2
Effects of metformin and vitamin D supplements on glucose metabolism, lipid profile and androgen levels in adolescents with hyperandrogenisma (Primary data of the study)

	M[b] (n=15)	MsD[c] (n=15)	MdD[d] (n=15)	p
Vitamin D (ng/mL)	22.87±6.93	15.05±7.42	14.42±3.76	0.04
Glucose (mg/dL)	98.59±15.36	91.81±11.21	92.75±8.55	0.12
ALT (U/L)	19.78±11.45	19.62±13.94	19.94±9.28	0.95
Insulin (µU/mL)	33.48±7.30	35.28±14.73	32.23±11.35	0.25
HOMA-IR	8.16±2.34	8.25±4.41	7.35±2.69	0.09
HDL (mg/dL)	52.46±15.11	49.26±9.64	46.69±7.44	0.35
LDL (mg/dL)	86.60±23	94.23±22.98	97.95±40.54	0.33
Triglyceride (mg/dL)	116.23±54.80	105.35±53.32	123.25±63.26	0.45
FSH (mIU/mL)	6.18±1.87	6.47±2.33	4.98±1.52	0.16
LH (mIU/mL)	9.73±6.46	9.00±4.73	9.55±6.97	0.38
Estradiol (pg/mL)	78.13±47.78	75.07±61.70	69.87±41.15	0.68
DHEAS (ug/dL)	304.74±125	331.12±143	290±137	0.45
ANDR (ng/mL)	1.81±0.81	2.62±0.89	2.29±1.26	0.97
S.Testosterone (pg/mL)	2.12±1.11	2.52±0.68	2.25±0.74	0.15
T.Testosterone (ng/dL)	70.63±11.90	69.87±14.81	71.22±12.85	0.84
SHBG (nmol/L)	37.69±29.49	28.48±15.69	32.67±27.41	0.32

[a]Data mean±standard deviation, [b]Group receiving 850-mg metformin orally daily, [c]Group who took 850 mg metformin and half of the vitamin D ampoule (150 000 IU) once a month orally, [d]Group taking 850-mg metformin and 15 (2 000 IU) of vitamin D orally daily, ANDR: Androstenedione (ng/mL), ST: Free testosterone (pg/mL), TRG: Triglyceride (mg/dL), ESTR: Estradiol (pg/mL)

Table 3
The effects of metformin and vitamin D supplements on glucose metabolism, lipid profile and androgen levels in adolescents with hyperandrogenisma

	Latest data of the study				Differences between first and last data			
	M[b] (n=15)	MdD[c] (n=15)	MsD[d] (n=15)	p	M[b] (n=15)	MdD[d] (n=15)	MsD[c] (n=15)	p
Vitamin D (ng/mL)	23.58±7.95	20.34±6.32	25.04±10.90	0.31	0.70±8.76	5.91±4.67*	9.99±13.34	0.03
Glucose (mg/dL)	91.77±7.33	92.42±7.51	87.88±6.83	0.19	-6.83±15.79	-0.33±8.02	-3.93±12.23	0.36
ALT (U/L)	18.83±12.81	18.45±7.09	21.57±14.70	0.74	-9.95±9.13	-1.49±8.19	1.94±9.53	0.53
Insulin (µU/mL)	22.11±10.34	23.78±7.08	22.31±9.06	0.85	-11.38±9.66	-8.45±11.98	-12.98±15.74	0.61
HOMA-IR	5.06±2.46	5.48±1.82	4.89±2.20	0.75	-3.11±2.81	-1.88±2.80	-3.37±4.54	0.46
HDL (mg/dL)	51.36±9.93	49.89±8.19	52.43±12.61	0.79	-1.10±10.77	3.20±7.10	3.16±5.96	0.26
LDL (mg/dL)	89.08±14.82	98.75±41.38	96.44±26.32	0.64	2.48±25.90	0.79±20.76	2.20±13.58	0.97
Triglyceride (mg/dL)	118.45±60.55	116.35±73.98	113.69±50.28	0.97	2.22±47.60	-6.91±34.17	8.34±54.95	0.66
FSH (mIU/mL)	6.02±2.70	4.80±2.10	5.63±2.55	0.38	-0.16±2.68	-0.19±1.91	-0.84±3.58	0.75
LH (mIU/mL)	7.78±5.30	7.94±5,58	6.09±3,55	0.52	-1.96±5.95	-1.62±5.18	-2.91±5.69	0.80
Estradiol (pg/mL)	72.93±62.03	78.73±45.83	73.87±73.14	0.96	-5.20±52.44	8.87±67.64	-1.20±54.81	0.79
DHEAS (ug/dL)	335.77±149.32	275.64±112.89	341.25±133.63	0.33	31.03±79.25	-14.45±106.10	10.13±88.85	0.40
ANDR (ng/mL)	1.51±0.77	2.20±1.34	2.37±0.97	0.73	-0.30±0.80	-0.09±1.98	-0.26±1.44	0.92
S.Testosterone (pg/mL)	2.07±0.87	2.15±0.76	2.20±1.22	0.93	-0.05±0.73	-0.11±0.52	-0.33±1.36	0.69
T.Testosterone (ng/dL)	62.75±17.45	59.62±17.57	55.76±27.87	0.67	-7.89±21.40	-11.61±13.79	-14.12±16.17	0.61
SHBG (nmol/L)	41.78±31.34	36.04±33.23	59.62±70.36	0.38	4.08±30.07	3.37±28.96	31.14±63.68	0.15

[a]Data mean±standard deviation, [b]Group receiving 850-mg metformin orally daily, [c]Group who took 850 mg metformin and half of the vitamin D ampoule (150 000 IU) once a month orally, [d]Group taking 850-mg metformin and 15 drops (2 000 IU) of vitamin D orally daily

Although there was no statistically significant relationship between vitamin D and total testosterone levels in our MsD and MdD groups, a slight decrease in serum total testosterone levels was observed in the groups. Additionally, there was a positive correlation between total testosterone and ALT (r=0.58, p=0.02) in our group-given metformin-vitamin D drops. There was a positive correlation between SHBG and HDL levels in all three groups (p<0.05). There was a positive correlation between vitamin D and SHBG levels in the metformin group (r=0.65, p=0.009) and a negative correlation between SHBG level and androstenedione (r=-0.56, p=0.02) (**Table 4**). This consistent result is a desirable finding for treating hyperanrogenism, reaffirming the importance of high SHBG and low androstenedione levels.

A negative correlation was observed between insulin and LDL (r=-0.68, p=0.005) and BMI (r=-0.58, p=0.02) in the metformin group. A positive correlation was observed between insulin and triglycerides in the MsD and MdD groups (p<0.05) (**Table 4**) (**Figure 2**). There was a slight decrease in fasting insulin level and BMI in all 3 groups, but this decrease was not statistically significant.

Table 4
Statistically significant Pearson correlations between hormone and metabolic parameters in M, MsD, MdD groupsa

	M[a] (n=15)		MdD[b] (n=15)		MsD[c] (n=15)	
	r	p	r	p	r	p
Vitamin D						
SHBG	0.65	0.00	0.14	0.60	0.17	0.53
T. testosterone						
ALT	-0.22	0.43	-0.05	0.85	0.58	0.02
Estradiol	0.66	0.00	0.63	0.01	0.05	0.84
SHBG						
Vitamin D	0.65	0.009	0.14	0.60	0.17	0.53
Triglyceride	0.61	0.01	0.58	0.02	0.180	0.51
HDL	0.52	0.04	0.66	0.00	0.54	0.03
Androstenedion	-0.56	0.02	-0.43	0.10	0.05	0.85
Insulin						
Triglyceride	-0.36	0.18	0.64	0.01	0.54	0.03
Glucose	0.36	0.18	0.56	0.02	-0.05	0.83
LDL	-0.68	0.005	-0.33	0.22	-0.11	0.69
BMI	-0.58	0.02	0.22	0.42	-0.14	0.60

[a]Data mean±standard deviation, [b]Group receiving 850-mg metformin orally daily, [c]Group who took 850 mg metformin and half of the vitamin D ampoule (150 000 IU) once a month orally, [d]Group taking 850-mg metformin and 15 drops (2 000 IU) of vitamin D orally daily

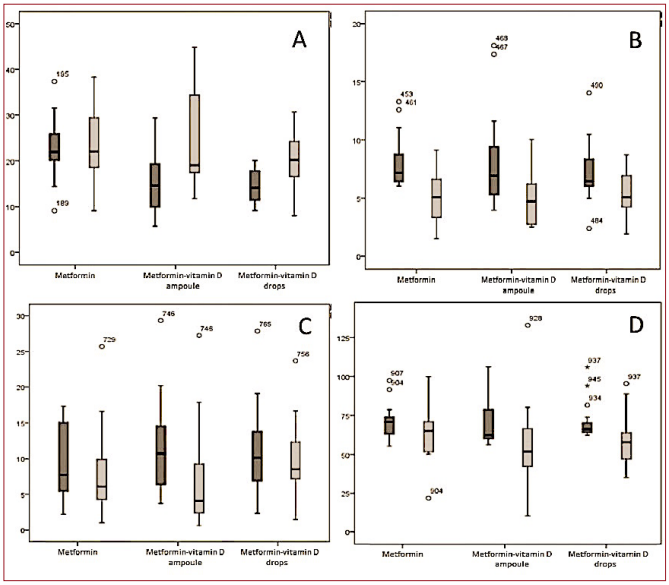

Figure 2. Comparisons of the groups

Discussion

Recent studies suggest that low vitamin D levels may be the primary factor in the initiation and progression of PCOS.[8] Therefore, dietary intake of vitamin D is thought to help restore the menstrual cycle in women with PCOS.[8] This is the first study in our country to examine vitamin D levels and androgen parameters in adolescents to the best of our knowledge.

Previous studies have addressed the effects of vitamin D and calcium supplementation on endocrine, inflammation and oxidative stress markers in women with PCOS who are vitamin D deficient.[9,10] Additionally, Rashidi et al. showed that the combined use of calcium-vitamin D and metformin in women with PCOS was more effective in

regulating the menstrual cycle and follicle maturation than the use of either drug alone.[8] Razavi et al. found that 8 weeks of vitamin D, vitamin K, and calcium supplementation in women diagnosed with PCOS in 2016 significantly reduced serum free testosterone and DHEAS compared to placebo, while not affecting other hormonal profiles.[10] In this study, we could no observe a significant decrease in total testosterone, free testosterone and DHEAS following metformin-vitamin D treatment for 8 weeks in the M, MsD, and MdD groups. However, total testosterone was positively correlated with ALT (r=0.58, p=0.02) in the MsD group. We can explain this situation by the fact that the high ALT level seen in women with PCOS is related to the effect of increased androgen level independent of obesity, insulin resistance and dyslipidemia.[11]

Vitamin D appears to play an important role in the secretion of insulin from pancreatic β cells and the stimulation of insulin receptors.[12] In some studies in patients with PCOS an inverse correlation was found between vitamin D levels and insulin resistance.[13] Mishra et al. showed in 2016 that low vitamin D is a risk factor for impaired glucose tolerance, insulin resistance, and type 2 DM.[14] However, Selimoğlu et al. showed that HOMA-IR decreased with a single dose of 300,000 IU vitamin D supplementation for 3 weeks in individuals with PCOS, but in this case, fasting insulin and glucose concentrations were not affected.[15] Garg et al. found that vitamin D supplementation given 4000 IU/day for 6 months did not have a significant effect on insulin levels in women with PCOS.[16] Although our study showed similar results, we found that vitamin D supplementation did not affect fasting insulin level, glucose concentration and HOMA-IR. In our study, adequate vitamin D level (>30 ng/dl) was achieved in only 4% of our patients after 8 weeks of vitamin D treatment. We predict that a more significant effect on insulin and androgen levels will occur with long-term vitamin D supplementation. We attributed this hypothesis to the study by Menichini et al. in which they found positive effects of high-dose vitamin D (4000 IU/day) administered for at least 12 weeks on fasting blood glucose, insulin sensitivity, hyperlipidemia, and fertilization in patients with PCOS.[17]

Vitamin D stimulates aromatase activity, which is effective in the conversion of testosterone to estrogens in granulosa cells, which causes a balance in androgen and estrogen levels in patients with PCOS.[18] In a study by Azadi-Yazdi et al. in 2017 on women with PCOS, it was revealed that vitamin D supplementation significantly reduced serum total testosterone level and did not affect SHBG and free testosterone levels.[3] In our study, there was a positive correlation between vitamin D and SHBG in the M group (p=0.009). This significant result we obtained in the group not given vitamin D can be explained by the fact that the vitamin D level of the M group was higher than the other groups at the beginning of the study. In support of this situation, in a study by Zhao et al. in 2017, low vitamin D concentration was associated with low SHBG and high free testosterone levels in men and women; showed that it was associated with low estradiol and high DHEA levels in women.[19]

In some studies examining the relationship between vitamin D deficiency and metabolic syndrome, it has been shown that vitamin D supplementation contributes to the improvement of metabolic syndrome in patients.[20] Hahn et al. found a positive correlation between vitamin D and HDL cholesterol (r=0.312, p<0.05).[21] Grunwald et al., on the other hand, did not find any relationship between vitamin D deficiency and metabolic parameters in obese children.[22] In a study by Foroozanfard et al. in 2017, they stated that in patients with PCOS, serum triglycerides, VLDL, LDL and total cholesterol ratios decreased significantly with 4000 IU/day vitamin D supplementation compared to 1000 IU/day vitamin D supplement given for 12 weeks. However, no significant effect was shown on HDL-cholesterol levels.[23] In our study, there was a significant relationship between insulin and triglycerides in the group-given metformin-vitamin D stoss treatment (r=0.54, p=0.03). There was a positive correlation between SHBG and HDL levels in all three groups (p>0.05). In a study by Temizsiz et al. in 2017, it was stated that the decrease in SHBG level is associated with obesity, hyperinsulinemia and dyslipidemia. They also found a positive relationship between SHBG and HDL.[24]

In adolescents with PCOS, increased total-LDL cholesterol and triglyceride levels may increase the risk of cardiovascular disease.[25] Therefore, the treatment of associated cardiovascular risk factors, including dyslipidemia, should be included in the routine healthcare program of patients with PCOS. Because vitamin D can reduce lipid profiles by increasing insulin sensitivity.[26] We think that this will lead to positive results in terms of reducing cardiovascular diseases accompanied by high triglyceride levels.

Conclusion

Significant correlations were found in androgen and lipid parameters with the addition of vitamin D to 8 weeks of metformin treatment. We think that the positive correlation between vitamin D and SHBG will have a positive effect on ensuring ovulation and keeping the androgen level within normal limits. Depending on the positive correlation between insulin and triglycerides, the decrease in insulin level may contribute to the improvement of dyslipidemia by reducing the triglyceride level. Additionally, the significant relationship between total testosterone and ALT may have a protective effect on liver functions by decreasing the androgen level and decreasing the ALT level. However, long-term studies are needed to support our results.

Author Contributions: Esranur Cig: design of the study, retrospective collection of data, statistical analysis, writing, methodology; Muge Atar: investigation; M.Ozgur Pırgon: design of the study, reviewing, editing. All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

Conflict of Interest: There are no conflicts of interest in connection with this paper, and the material described is not under publication or consideration for publication elsewhere.

Ethics Committee Approval: The study was carried out with the permission of Süleyman Demirel University Faculty of Medicine Clinical Research Ethics Committee (Date: 20.12.2017, Decision No: 226).

Financial Disclosure: The authors have no conflicts of interest to declare.

Informed Consent: Informed consent was obtained from the parents of the patients.

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