Clinical research

Assessment of the relationship between serum vitamin (A, B_{12}, C, D, folate) and zinc levels and polycystic ovary syndrome

Mehmet Kulhan, Nur Gozde Kulhan, Umit Arslan Nayki, Cenk Nayki, Nahit Ata, Pasa Ulug, Cuma Mertoglu

Department of Obstetrics and Gynecology, School of Medicine, Erzincan University, Erzincan, Turkey

Submitted: 6 February 2017
Accepted: 14 March 2017

Arch Med Sci Civil Dis 2017; 2: e62–e69
DOI: https://doi.org/10.5114/amscd.2017.66840

Corresponding author:
Mehmet Kulhan
Department of Obstetrics and Gynecology
School of Medicine
Erzincan University
24030 Erzincan, Turkey
Phone: +90 5054918090
E-mail: Mehmet_kulhan@yahoo.com

Abstract

Introduction: There is increasing evidence that vitamin D affects insulin and glucose metabolism, and a low vitamin D status is suspected to be a risk factor for impaired glucose tolerance, insulin resistance and so polycystic ovary syndrome (PCOS), but there is no evidence to suggest that there is a relationship between vitamin A, vitamin B_{12}, vitamin C, folate, zinc (Zn) and PCOS in the literature. We aimed to investigate the levels of vitamins A, B_{12}, C and D and zinc and the association between vitamins A, B_{12}, C and D, folate and zinc level and hormonal-biochemical parameters in PCOS.
Material and methods: We recruited 65 women with PCOS and 67 healthy individuals. Correlations between clinical and metabolic parameters and vitamins A, B_{12}, C and D and zinc status were analyzed separately in patients and controls.
Results: Women with PCOS showed a decreased serum level of vitamin A compared with the control group ($p < 0.05$), but they showed no differences in the levels of vitamin D, vitamin B_{12}, vitamin C, folate or Zn ($p > 0.05$).
Conclusions: Our study found no differences in the absolute levels of serum vitamins B_{12}, C, D, folate or Zn between PCOS patients and matched controls, but the vitamin A level was lower in PCOS patients. Prevalence of vitamins A, B_{12}, C and D and Zn insufficiency was equally common among both patients and controls.

Key words: polycystic ovary syndrome, vitamin A, vitamin B_{12}, vitamin C, vitamin D, folate, zinc.

Introduction

Polycystic ovary syndrome (PCOS) is a syndrome in which the etiology is still unclear even today. This syndrome is considered to be primarily a functional disorder of the ovary and many theories and mechanisms have been put forward on its causes to date [1]. The most popular theory of the pathophysiology of PCOS is insulin resistance [2]. Great progress has been made in this regard by molecular and clinical studies, but some questions still need answers. If the underlying cause in the pathophysiology of PCOS is insulin resistance, what is the reason for this insulin resistance? Why can insulin resistance not be shown in every case? Is there a preventive treatment? Other important factors are thought to play a role in the pathophysiology of PCOS besides insulin resistance. These factors are closely related to insulin, because even though insulin resistance cannot be demonstrated in typical PCOS cases, the therapeutic use of insulin sensitizers (metformin, rosiglitazone) provides improvements in androgen levels and ovulation [3]. This is theoretically explained by a factor that causes insulin resistance but prevents compensatory hyperinsulinism. That is, the same reason has to play a role in both the synthesis phase and the effect phase of insulin. There is increasing evidence that vitamin D affects insulin and glucose metabolism, and a low vitamin D status is suspected to be a risk factor for impaired glucose tolerance and insulin resistance [4], but there is no evidence to suggest that there is a relationship between vitamin A, vitamin B_{12}, vitamin C, folate, zinc (Zn) and PCOS in the literature. In the current study, we examined whether a low vitamin A, vitamin B_{12}, vitamin C, vitamin D, folate and zinc status can be observed in Turkish women with PCOS. In addition, we investigated the relationship between serum vitamin A, vitamin B_{12}, vitamin C, vitamin D, folate and zinc concentration and metabolic or clinical profiles of PCOS.

Material and methods

This study was performed on 132 women, 65 patients with PCOS and 67 healthy individuals, during 2015–2016. The study was planned as prospective, controlled and single centered and was approved by the ethics committee of our university. All women in the 16–44 age group (reproductive age) were recruited from our clinic in Turkey. According to the revised Rotterdam diagnostic criteria, PCOS was defined by the presence of at least 2 out of the following 3 features: oligo-anovulation (menstrual cycle length > 45 days or less than 6 menstrual cycles a year), clinical or biochemical hyperandrogenism (Ferriman-Gallwey (FG) score > 8 or elevated serum testosterone levels), and polycystic ovaries on transvaginal ultrasonography (TVUSG) (enlarged ovaries with increased stromal volume and more than 10 follicles which measure 2–8 mm in diameter and localize along the periphery of the ovary in a way to form 'a pearl necklace' appearance). Thyroid functions, luteinizing hormone (LH), follicle-stimulating hormone (FSH), estradiol (E2), prolactin, and total and free testosterone were assessed in patients with PCOS and control subjects, and those with thyroid diseases, hyperprolactinemia, Cushing's disease, or congenital adrenal hyperplasia and those who were administered agents such as hormonal agents, ovulation-inducing agents, glucocorticoids, anti-androgens, or anti-hypertensives over the last 6 months prior to the study were excluded from the study. A healthy woman was defined as a woman in reproductive age with regular cycles. They came to our clinic for an annual check-up. Women with congenital adrenal hyperplasia, hyperprolactinemia, hyperparathyroidism, and androgen secretory tumors were excluded using specific laboratory tests. Furthermore, women who used medications suspected to affect carbohydrate metabolism or Zn/vitamin concentrations during 6 months prior to the study, who had a history of chronic disease or endocrinopathies, and who had a history of smoking or drug abuse were excluded.

Clinical assessment

The hirsutism score was calculated according to the FG score system. Nine anatomic regions were assessed on a 4-point scale where 0 meant no growth of terminal hair and 4 meant maximum hair growth. Scores below 8 were regarded as normal and scores between 8 and 36 were regarded as pathological. Scores rose in parallel to the severity of hirsutism [5]. In addition to family history of metabolic disorders, a detailed history including menstrual cycle pattern, temporal or situated in the temples of the head profile, severity of unwanted hair growth, and drug intake was taken at the time of enrollment. Clinical examination included measurement of body weight (kg), height (cm) as well as FG scoring. FG scoring was done by a single observer, and scores above 8 were taken as significant. A single observer performed transabdominal or transvaginal ultrasonography to reveal findings of polycystic ovarian morphology (presence of at least 10 follicles around the ovary that measure 2–8 mm in size, with increased ovarian volume and/or echogenic ovarian stroma) [6].

Laboratory tests

Blood samples were taken from the patients at the early follicular phase (between the third and the fifth days of the spontaneous or gestagen-induced menstrual cycle). Venous blood was taken from the forearm between 8.00 and 10.00 am after 8 h of fasting. Blood samples were immediately centrifuged, and sera were kept at –80°C until laboratory testing.

Vitamin B_{12}, vitamin D, folate, follicle-stimulating hormone (FSH), luteinizing hormone (LH), estradiol 2 (E_2), prolactin, total testosterone, and insulin levels were determined by electrochemiluminescence immunoassay using Acridinium Ester technology (SIEMENS CENTAUR XP, USA). Free testosterone levels were determined by the ELISA method (DiaMetra, Milano Italy). Fasting glucose and Zn levels were determined spectrophotometrically (OLYMPUS AU 2700, USA). C-reactive protein (CRP) was measured by the nephelometric method (SIEMENS BN II, USA). Vitamin A, vitamin C and HbA_{1c} levels were measured by high performance liquid chromatography (HPLC) (ADAMS ARKRAY A 10). The homeostasis model assessment of insulin resistance (HOMA-IR) was used to evaluate insulin resistance, using the following formula: HOMA-IR = (fasting plasma glucose (mg/dl) × insulin (U/ml)/405) [7]. Patients with HOMA-IR levels above 2.5 were considered to have insulin resistance (IR) [8].

Statistical analysis

Statistical Package for the Social Sciences (SPSS 22.0) was used for data analyses. Data are expressed as mean and standard deviation. *T* test and nonparametric Mann-Whitney *U* test were used for comparison of clinical and biochemical data between the groups. Whether intra-group variables demonstrated a normal distribution or not was determined by the Kolmogorov-Smirnov test. Spearman's correlation and Pearson test analysis was used for investigation of correlations between the values. *P*-value < 0.05 was accepted as significant.

Table I. Clinical and biochemical features of PCOS patients and matched controls

Parameter	PCOS (*n* = 65)	Controls (*n* = 67)
Clinical:		
Age [years]	24.06 ±6.12	25.34 ±5.82
BMI [kg/m^2]	26.00 ±4.52	25.27 ±2.68
Hirsutism score (median [range])	8.32 ±3.67	1.28 ±1.73
Biochemical:		
Total T [ng/ml]	0.65 ±0.15	0.40 ±0.29
Free T [pg/ml]	1.34 ±0.43	0.59 ±0.58
FSH [IU/l]	5.85 ±2.08	6.67 ±3.23
LH [IU/l]	9.84 ±6.18	8.00 ±7.14
E_2 [pg/ml]	66.02 ±44.07	83.12 ±0.85
Glucose [mg/dl]	87 ±21.84	85 ±11.70
Insulin [μU/ml]	10.71 ±5.47	6.48 ±2.34
HOMA-IR	2.37 ±0.77	1.92 ±0.58
HbA_{1c} (%)	5.64 ±1.05	5.09 ±0.76
CRP [mg/dl]	2.65 ±3.08	2.56 ±2.76
Sedimentation [mg/dl]	9.21 ±8.34	12.49 ±2.95
Zn [μg/dl]	95.45 ±10.94	89.22 ±9.83
Vitamin A (retinol) [μg/dl]	41.70 ±13.54	43.5 ±9.59
Vitamin C [mg/ml]	2.09 ±3.21	6.23 ±1.92
25-(OH) vitamin D_3 [ng/ml]	18.36 ±17.69	21.84 ±19.34
Vitamin B_{12} [pg/ml]	314.61 ±92.95	300.23 ±87.06
Folate [ng/ml]	19.01 ±53.22	10.79 ±5.75
Prevalence of Zn insufficiency (< 70 μg/dl)	1 (1.54%)	–
Prevalence of vit. A insufficiency (< 30 μg/dl)	12 (18.46%)	2 (2.98%)
Prevalence of vit. C insufficiency (< 0.5 mg/ml)	6 (9.23%)	10 (14.92%)
Prevalence of vit. D insufficiency (< 30 ng/ml)	49 (75.38%)	52 (77.61%)
Prevalence of vit. B_{12} insufficiency (< 211 pg/ml)	7 (10.76%)	9 (13.43%)
Prevalence of folate insufficiency (< 5 ng/ml)	5 (7.69%)	3 (4.48%)

Table II. Correlation of Zn, vit. A, vit. B_{12}, vit. C, 25-(OH) vitamin D_3, folate levels with clinical and biochemical parameters

Parameter	PCOS		Controls	
	r	*p*	*r*	*p*
Zinc (Zn)				
Clinical parameters:				
Age [years]	0.048	0.705	0.059	0.633
BMI [kg/m^2]	0.022	0.859	–0.088	0.479
Hirsutism score (median [range])	0.088	0.486	–0.116	0.351
Biochemical parameters:				
Total T [ng/ml]	–0.118	0.348	–0.252	0.040
Free T [pg/ml]	0.138	0.273	–0.235	0.056
FSH [IU/l])	–0.029	0.821	0.078	0.528
LH [IU/l]	0.090	0.475	0.110	0.377
E_2 [pg/ml]	–0.042	0.739	0.032	0.799
Glucose [mg/dl]	0.241	0.054	–0.049	0.692
Insulin [μU/ml]	0.036	0.776	–0.253	0.039
HOMA-IR	0.129	0.304	–0.022	0.860
HbA_{1c} (%)	–0.042	0.740	–0.135	0.277
CRP [mg/dl]	0.154	0.220	–0.087	0.481
Sedimentation [mg/dl]	0.007	0.954	0.035	0.777
Vitamin A (retinol) [μg/dl]	–0.095	0.451	0.327	0.007
Vitamin C [mg/ml])	0.020	0.877	0.133	0.285
25-(OH) vitamin D_3 [ng/ml]	0.125	0.323	–0.111	0.372
Vitamin B_{12} [pg/ml]	–0.074	0.560	–0.102	0.411
Folate [ng/ml]	0.124	0.326	0.080	0.522
Vitamin A				
Clinical parameters:				
Age [years]	–0.081	0.520	–0.155	0.211
BMI [kg/m^2]	–0.095	0.451	–0.148	0.231
Hirsutism score (median [range])	0.104	0.410	–0.101	0.417
Biochemical parameters:				
Total T [ng/ml]	0.065	0.609	0.000	0.997
Free T [pg/ml]	–0.216	0.084	–0.132	0.286
FSH [IU/l]	0.002	0.988	–0.062	0.620
LH [IU/l]	–0.106	0.400	0.012	0.921
E_2 [pg/ml]	0.007	0.957	–0.050	0.687
Glucose [mg/dl]	–0.243	0.051	–0.012	0.922
Insulin [μU/ml]	–0.188	0.134	0.072	0.561
HOMA-IR	–0.169	0.179	0.149	0.229
HbA_{1c} (%)	–0.119	0.344	0.038	0.758
CRP [mg/dl]	0.078	0.536	0.038	0.759
Sedimentation [mg/dl]	–0.012	0.927	–0.134	0.280
Zn [μg/dl]	–0.172	0.171	0.358	0.003
Vitamin C [mg/ml]	0.084	0.506	0.425	< 0.001
25-(OH) vitamin D_3 [ng/ml]	0.060	0.636	–0.163	0.189
Vitamin B_{12} [pg/ml]	0.085	0.503	0.002	0.986
Folate [ng/ml]	–0.065	0.607	0.153	0.217

Table II. Cont.

Parameter	PCOS		Controls	
	r	*p*	*r*	*p*
Vitamin B_{12}				
Clinical parameters:				
Age [years]	0.111	0.378	–0.001	0.997
BMI [kg/m²]	–0.147	0.243	–0.021	0.864
Hirsutism score (median [range])	–0.145	0.250	–0.017	0.894
Biochemical parameters:				
Total T [ng/ml]	0.013	0.916	–0.033	0.788
Free T [pg/ml]	–0.045	0.720	0.044	0.723
FSH [IU/l]	0.047	0.709	–0.097	0.436
LH [IU/l]	0.077	0.543	0.008	0.949
E_2 [pg/ml]	0.049	0.696	0.090	0.470
Glucose [mg/dl]	0.104	0.409	0.143	0.249
Insulin [µU/ml]	–0.180	0.151	0.132	0.286
HOMA-IR	0.050	0.694	0.108	0.385
HbA_{1c} (%)	–0.002	0.985	0.136	0.274
CRP [mg/dl]	–0.129	0.307	–0.067	0.587
Sedimentation [mg/dl]	–0.130	0.304	–0.066	0.598
Vitamin A (retinol) [µg/dl]	0.085	0.503	0.002	0.986
Vitamin C [mg/ml]	–0.139	0.269	–0.185	0.134
25-(OH) vitamin D_3 [ng/ml]	–0.120	0.342	–0.157	0.204
Zn [µg/dl]	0.092	0.466	–0.019	0.881
Folate [ng/ml]	0.176	0.161	0.046	0.709
Vitamin C				
Clinical parameters:				
Age [years]	–0.165	0.189	–0.225	0.068
BMI [kg/m²]	0.012	0.924	–0.267	0.029
Hirsutism score (median [range])	0.190	0.130	–0.187	0.130
Biochemical parameters:				
Total T [ng/ml]	0.066	0.604	0.081	0.513
Free T [pg/ml]	0.156	0.216	0.009	0.941
FSH [IU/l]	–0.033	0.795	0.020	0.872
LH [IU/l]	0.078	0.536	0.029	0.817
E_2 [pg/ml]	–0.082	0.517	–0.132	0.288
Glucose [mg/dl]	0.126	0.318	–0.099	0.426
Insulin [µU/ml]	0.398	0.001	–0.017	0.890
HOMA-IR	0.314	0.011	–0.143	0.250
HbA_{1c} (%)	0.206	0.099	0.043	0.727
CRP [mg/dl]	0.013	0.920	–0.088	0.481
Sedimentation [mg/dl]	0.083	0.510	–0.129	0.299
Vitamin A (retinol) [µg/dl]	–0.132	0.296	0.252	0.040
Zn [µg/dl]	0.020	0.877	0.133	0.285
25-(OH) vitamin D_3 [ng/ml]	0.052	0.682	–0.013	0.917
Vitamin B_{12} [pg/ml]	–0.038	0.765	–0.192	0.120
Folate [ng/ml]	–0.260	0.036	0.049	0.691

Table II. Cont.

Parameter	PCOS		Controls	
	r	*p*	*r*	*p*
25-(OH) vitamin D_3				
Clinical parameters:				
Age [years]	0.030	0.812	–0.446	< 0.001
BMI [kg/m²]	0.093	0.460	–0.197	0.110
Hirsutism score (median [range])	0.103	0.413	0.009	0.939
Biochemical parameters:				
Total T [ng/ml]	0.167	0.184	–0.019	0.879
Free T [pg/ml]	0.164	0.190	0.026	0.836
FSH [IU/l]	–0.250	0.044	–0.125	0.315
LH [IU/l]	–0.125	0.319	–0.158	0.201
E_2 [pg/ml]	0.091	0.472	0.093	0.456
Glucose [mg/dl]	–0.059	0.641	–0.070	0.574
Insulin [µU/ml]	0.107	0.394	–0.069	0.577
HOMA-IR	0.119	0.344	–0.176	0.154
HbA_{1c} (%)	0.060	0.636	0.026	0.838
CRP [mg/dl]	0.045	0.719	–0.098	0.429
Sedimentation [mg/dl]	0.039	0.759	–0.038	0.758
Vitamin A (retinol) [µg/dl]	0.100	0.426	–0.168	0.173
Vitamin C [mg/ml]	0.052	0.682	–0.013	0.917
Zn [µg/dl]	0.125	0.323	–0.111	0.372
Vitamin B_{12} [pg/ml]	–0.071	0.573	–0.186	0.132
Folate [ng/ml]	–0.098	0.436	–0.009	0.939
Folate				
Clinical parameters:				
Age [years]	0.177	0.159	0.147	0.237
BMI [kg/m²]	–0.084	0.506	0.079	0.526
Hirsutism score (median [range])	–0.265	0.033	0.027	0.830
Biochemical parameters:				
Total T [ng/ml]	–0.031	0.806	0.124	0.319
Free T [pg/ml]	–0.083	0.510	0.044	0.721
FSH [IU/l]	–0.169	0.177	–0.096	0.439
LH [IU/l]	0.033	0.791	–0.144	0.246
E_2 [pg/ml]	–0.120	0.341	0.000	0.997
Glucose [mg/dl]	–0.114	0.364	–0.054	0.666
Insulin [µU/ml]	–0.387	0.001	0.142	0.251
HOMA-IR	–0.108	0.391	0.127	0.304
HbA_{1c} (%)	–0.289	0.020	0.001	0.992
CRP [mg/dl]	–0.078	0.536	0.196	0.112
Sedimentation [mg/dl]	–0.098	0.439	0.056	0.656
Vitamin A (retinol) [µg/dl]	0.028	0.822	0.250	0.041
Vitamin C [mg/ml]	–0.260	0.036	0.049	0.691
25-(OH) vitamin D_3 [ng/ml]	–0.098	0.436	–0.009	0.939
Vitamin B_{12} [pg/ml]	–0.070	0.580	–0.009	0.940
Zn [µg/dl]	0.124	0.326	0.080	0.522

Results

A total of 132 cases were evaluated: 65 in the patient group and 67 in the control group.

The metabolic and hormonal characteristics of women with PCOS and the control group are presented in Table I. The PCOS patients had higher hirsutism scores, and there were significant differences in the levels of fasting insulin and HOMA-IR between women with PCOS and the control group.

Women with PCOS showed decreased serum levels of vitamin A compared with the control group, but they showed no differences in the levels of 25-(OH)D_3, vitamin B_{12}, vitamin C, folate or Zn. The correlations between serum vitamin A, vitamin B_{12}, vitamin C, vitamin D, folate, Zn and clinical or metabolic profiles in both PCOS patients and the control group are presented in Table II.

Discussion

The PCOS is characterized by ovarian androgen excess and infertility. Recent experiments have suggested that several genes involved in retinoic acid synthesis may be differentially expressed in PCOS theca cells and may contribute to excessive theca-derived androgen production. For this reason, it is very important to investigate expression levels of genes encoding proteins involved in this complex system. In a study by Wood *et al.* the expression of genes involved in retinoic acid biosynthesis was found to be increased in theca cells from women with PCOS [9]. All-trans retinoic acid (atRA) is a lipid that is essential for the normal function of somatic cells in male and female gonads. Studies have shown that the PCOS cases have more atRA conversion in the theca cells. The overexpression of atRA synthase causes CYP11A1 and CYP17 gene expression in the theca cells. atRA also regulates cell division and differentiation. For this reason, increased levels of atRA are also associated with arresting follicle growth [10]. In another study, it was reported that differential responses to retinol and retinoids in normal and PCOS theca suggest that altered retinoic acid synthesis and action may be involved in augmented CYP17 gene expression and androgen production in PCOS [11]. Therefore we investigated whether vitamin A (retinol) levels are related to PCOS. Our study found differences in the absolute level of serum vitamin A and prevalence of vitamin A insufficiency between women with PCOS and matched controls. Vitamin A insufficiency was observed in 18.46% of the PCOS group and 2.98% of the control group. On the other hand, we did not find any correlations between serum vitamin A and hormonal or metabolic profiles in either PCOS patients or controls (Table II).

It has been previously demonstrated that folate and vitamin B_{12} treatment improved insulin resistance in patients with metabolic syndrome [12]. Kaya *et al.* been reported that insulin resistance, obesity, and elevated homocysteine were associated with lower serum vitamin B_{12} concentrations in PCOS patients [13]. Our study found no differences in the absolute level of serum vitamin B_{12} and folate between women with PCOS and matched controls, but we found negative correlations between serum folate and fasting insulin, HbA_{1c}, and vitamin C levels (Table II). Our findings suggest that the role of vitamin B_{12} and folate in the pathogenesis of PCOS is not yet clear.

Oxidative stress may play a role in the pathophysiology of PCOS. Insulin resistance (IR) also can be found in young non-obese women with PCOS. Hyperglycemia may increase reactive oxygen species production and decrease antioxidant levels [14]. The association of vitamin C, a well-known anti-oxidant, with PCOS has been shown in several studies in the literature [14, 15]. Our study found no differences in the absolute level of serum vitamin C between women with PCOS and matched controls, but we found negative correlations between vitamin C levels and folate, fasting insulin and HOMA-IR (Table II).

Increasing evidence suggests that vitamin D might have a regulatory role in PCOS-associated symptoms, including ovulatory dysfunction, insulin resistance and hyperandrogenism [16]. Therefore, the search for an association between PCOS and vitamin D metabolism appears to be justified. Vitamin D level was found to be lower in PCOS patients than the control group in the majority of the studies [17, 18]. Our study found no difference in the absolute level of serum vitamin D between women with PCOS and matched controls (Table II).

Zn is an essential trace element and acts as a catalytic, structural and regulatory ion. At the same time, Zn has a very important role in the synthesis, storage and the potentialization of insulin, and it also plays a role as an antioxidant [19]. We assessed the association between zinc and PCOS in our study. There was no differences in the absolute level of serum zinc between women with PCOS and matched controls (Table II).

In conclusion, the serum levels of vitamins A, B_{12}, C, and D and Zn may change in patients with PCOS. The findings in this study should be investigated with further trials in order to obtain new insights into PCOS.

Conflict of interest

The authors declare no conflict of interest.

References

1. Lord J, Wilkin T. Metformin in polycystic ovary syndrome. Curr Opin Obstet Gynecol 2004; 16: 481-6.

2. Cebeci F, Onsun N, Mert M. Insulin resistance in women with hirsutism. Arch Med Sci 2012; 8: 342-6.
3. Dunaif A, Segal KR, Futterweit W, Dobrjansky A. Profound peripheral insulin resistance, independent of obesity, in polycystic ovary syndrome. Diabetes 1989; 38: 1165-74.
4. Liu E, Meigs JB, Pittas AG, et al. Plasma 25-hydroxyvitamin D is associated with markers of the insulin resistant phenotype in nondiabetic adults. J Nutr 2009; 139: 329-34.
5. Ferriman D, Gallwey JD. Clinical assessment of body hair growth in women. J Clin Endocrinol Metab 1961; 21: 1440-7.
6. Balen AH, Laven JS, Tan SL, Dewailly D. Ultrasound assessment of the poly-cystic ovary: international consensus definitions. Hum Reprod Update 2003; 9: 505-14.
7. Legro RS, Castracane VD, Kauffman RP. Detecting insulin resistance in polycystic ovary syndrome: purposes and pitfalls. Obstet Gynecol Surv 2004; 59: 141-54.
8. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 1985; 28: 412-9.
9. Wood JR, Ho CKM, Nelson-Degrave VL, McAllister JM, Strauss JF. The molecular signature of polycystic ovary syndrome (PCOS) theca cells defined by gene expression profiling. J Reprod Immunol 2004; 63: 51-60.
10. Wood JR, Nelson VL, Ho C, et al. The molecular phenotype of polycystic ovary syndrome (PCOS) theca cells and new candidate PCOS genes defined by microarray analysis. J Biol Chem 2003; 278: 26380-90.
11. Wickenheisser JK, Nelson-DeGrave VL, Hendricks KL, et al. Retinoids and retinol differentially regulate steroid biosynthesis in ovarian theca cells isolated from normal cycling women and women with polycystic ovary syndrome. J Clin Endocrinol Metab 2005; 90: 4858-65.
12. Kilicdag EB, Bagis T, Tarim E, et al. Administration of B-group vitamins reduces circulating homocysteine in polycystic ovarian syndrome patients treated with metformin: a randomized trial. Hum Reprod 2005; 20: 1521-8.
13. Kaya C, Cengiz SD, Satiroğlu H. Obesity and insulin resistance associated with lower plasma vitamin B12 in PCOS. Reprod Biomed Online 2009; 19: 721-6.
14. Kurdoglu Z, Ozkol H, Tuluce Y, Koyuncu I. Oxidative status and its relation with insulin resistance in young nonobese women with polycystic ovary syndrome. J Endocrinol Invest 2012; 35: 317-21.
15. Zhang D, Luo WY, Liao H, Wang CF, Sun Y. The effects of oxidative stress to PCOS. Sichuan Da Xue Xue Bao Yi Xue Ban 2008; 39: 421-3.
16. Li HWR, Brereton RE, Anderson RA, Wallace AM, Ho CKM. Vitamin D deficiency is common and associated with metabolic risk factors in patients with polycystic ovary syndrome. Metab Clin Exp 2011; 60: 1475-81.
17. Li HW, Brereton RE, Anderson RA, Wallace AM, Ho CK. Vitamin D deficiency is common and associated with metabolic risk factors in patients with polycystic ovary syndrome. Metabolism 2011; 60: 1475-81.
18. Mazloomi S, Sharifi F, Hajihosseini R, Kalantari S, Mazloomzadeh S. Association between hypoadiponectinemia and low serum concentrations of calcium and vitamin D in women with polycystic ovary syndrome. ISRN Endocrinol 2012; 2012: 949427.
19. Ebisch IM, Thomas CM, Peters WH, Braat DD, Steegers-Theunissen RP. The importance of folate, zinc and antioxidants in the pathogenesis and prevention of subfertility. Hum Reprod Update 2007; 13: 163-74.