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Investigation the Relation Between Transforming Growth Factor-β1 Bioavailability with Vitamin D and Calcium in PCOS Patients

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Abstract

Objective: This study seeks to explore the association between TGF-β1 bioavailability and vitamin D/calcium levels in women with polycystic ovary syndrome (PCOS), aiming to uncover potential links that could contribute to understanding the pathogenesis of PCOS and its management.

Methods: Case-control study comprises of 60 women with PCOS and 60 healthy control women. Fasting serum TGF-β1, sEND, vitamin D and calcium were measured. Additionally, Anthropometric parameters: age, body mass index, blood pressure; diabetes parameters: fasting glucose, fasting insulin, homeostatic model assessment of insulin resistance index (HOMA-IR), homeostatic model assessment of beta–cell function (HOMA-B_2); Lipid profile: TC, TG, LDL, HDL, VLDL were assessed.

Results: There were significant increasing in serum TGF-β1 and its bioavailability in PCOS compared to control whereas there were significant decreasing in serum sENG in PCOS compared to control. Additionally, serum vitamin D was lower significantly in PCOS than in control while serum calcium was higher significantly in PCOS than in control. The correlation did not find between TGF-β1, sENG, TGF-β1/sENG with vitamin D and calcium and with other metabolic parameters. However, positive correlation was found between calcium and BMI and between TGF-β1 and it bioavailability (TGF-β1/sENG).

Conclusion: Elevated TGF-β1 bioavailability results from increase serum TGF-β1 combine with decrease serum sENG, suggesting a potential role of TGF-β1 bioavailability in arresting follicular development in PCOS patients. Moreover, significant decrease in vitamin D and significant increase of calcium in PCOS patients, indicating that vitamin D and calcium may be involve in PCOS pathogenesis.

Keywords: Calcium, polycystic ovary syndrome, transforming growth factor-β1, soluble endoglin, vitamin D

Introduction

Polycystic Ovary Syndrome (PCOS) is described as common endocrine disorder that achieved 5%–10% women in reproductive age. PCOS is characterized by anovulation, hyperandrogenism and infertility. PCOS is highly known as a metabolic syndrome of women who have the characteristic features of impaired glucose metabolism, insulin resistance, hypertension, dyslipidemia, obesity, depression, anxiety and endometrial carcinoma.[1-3] Even thought the pathogenesis of PCOS is still unclear, many studies propose that some angiogenesis factors and inflammatory cytokines,[4] such as Transforming Growth Factor- beta (TGF-β), may be involved in follicular development. TGF-β is considered to have many biological functions; angiogenesis, multifunctional cytokines, tissue fibrosis and fibroblast proliferation. Three human's isoforms of TGF-β (TGF-β1, TGF-β2 and TGF-β3) have been known to express by ovarian cells.[5] The TGF-β super family proteins are, activins, antimullerian hormone and inhibins which have an important role in pathophysiology of PCOS.[6-8] TGF-β is regulated by fibrillin genes (matrix components of extracellular microfibrils).[9] TGF-β dysregulation is linked with the allele 8 variant of fibrillin-3 gene, which can contributed to the metabolic disturbances in PCOS women.[10,11] It is shown that increase TGF-β1 level could have a potential role in elevated deposition of collagen in ovarian stroma and theca layers of PCOS ovary women[12] and increased vascularity.[13] Previous studies were shown that serum TGF-β1 level in PCOS patients is higher than in control.[14]

Endoglin (ENG), also called (CD105), is an important fraction of TGF-β1 and TGF-β3 receptor complexes. The proteolytic production of endoglin is soluble endoglin which is considered as circulating receptor that binds TGF-β1 and forms its bioavailability (TGF-β1/sEND). This binding leads to decrease TGF-β1.[15] It was shown that TGF-β1 bioavailability (TGF-β1/sENG) increase in PCOS patient since the level of TGF-β1 increase with decrease in the levels of sENG.[16,17] Tal et al, 2013 suggested that increase TGF-β1 combine with decrease sENG results of increase TGF-β1 bioavailability, which have a potential role in PCOS pathogenesis.[14]

Vitamin D active form, 1,25-dihydroxyvitamin D3 [1,25-(OH)2D3], has an important role in gonad glands function.[18] It has been suggested in many studies that vitamin D has a role in PCOS pathogenesis. It was found that vitamin D level decrease in PCOS patient in compared to healthy individual.[14,19] Furthermore, it has been reported that vitamin D and calcium homeostasis have a possible contribution in oocyte maturation and follicular devepolment.[20] Several studies showed that supplement of vitamin D and calcium improve the irregulator menstrual cycle and hyperandrgensim.[21,22] Interestingly, it was observed in heart and kidney fibrosis that administration of vitamin D decrease TGF-β1 level.[23,24] Furthermore, it was reported in PCOS patients that supplement of vitamin D decrease bioavailability of TGF-β1 (TGFβ1/sEND).[25] However,

there is no previous study clarify the relation of vitamin D and calcium with TGF-β1 bioavailability. Therefore, the main aim of the present study is to investigate the relationship between TGF-β1 bioavailability with vitamin D and calcium in PCOS patients.

Materials and Methods

Study Design

Case-control study was performed at Fertility Center, Al-Najef, Iraq, through the period from February to September 2020. A study consists of 120 women in reproductive age; 60 new diagnosed PCOS women and 60 healthy women. PCOS patients were diagnosed according to the Rotterdam criteria.[26] Patients who have congential adrenal hyperplasia, hyperparathyroidism, hyperprolactinaemia, androgen secret tumour and Cushing's disease[27] were excluded from the study.

Blood Collection

Fasting blood samples were obtained from vein during the follicular phase of the menstrual cycle (2rd–3thday). After 30 minutes, blood sample was centrifuged at 2,200 g for 10 minutes. Serum was stored at –20 ºC. Fasting blood glucose, calcium and lipid profile levels were measured by colorimetric methods. Serum insulin, dehydroepiandrosterone sulfate (DHEAS) and sex hormone binding globulin (SHBG) were measured by the electrochemiluminescence (ECL) technique (Cobas e411 analyzer, Roche Company, Germany). Transforming growth factor-β1 (TGF-β1), soluble endoglin (sENG) and free testosterone were determined via ELISA kits (Human, Germany). Serum vitamin D, luteinizing hormone (LH), follicle stimulating hormone (FSH), total testosterone were measured via immune fluorescence technique/ELFA (Minividas, Biomerieux, France). The body mass index (BMI) is expressed by the ratio of (weight/ height^2), with unit kg/m^2. Homeostatic model of assessment of insulin resistance index (HOMA-IR) is calculated by multiple value of fasting glucose (mmol/L) with fasting insulin (mU/L) and then divided by 22.5.[28] The homeostatic model of assessment of beta-cell function (HOMA-β_2) was calculated by formula: 20*fasting insulin (mU/ml)/(fasting glucose (mmol/L) –3.5).

Statistics Analysis

Normal distribution for each parameter in PCOS group and control group was tested by Kolmogorov-Smirnov test. Normal distribution values are expressed by mean± SEM and skewed distribution values are expressed as median. Unpaired student's t-test and mann-whiteny U test was used to test the significant differences in variables which normal and skewed distribution, respectively. The correlation between variables was tested by spearman test. The statistical analysis are performed by GraphPad prism "version 8.0.2" and SPSS "version 22, SPSS Inc, Chicago, IL,USA". Significant differences are setup when P value <0.05.

Results

The comparison in anthropometic, glucose, lipid and fertility parameters between PCOS patients and control was shown in Table 1.

There are no significant differences between the PCOS and control in terms of age, SBP, BMI and HOMA-β_2. Significant decreasing in HDL-C and SHBG was found in PCOS compare to control. On the other hand, significant increasing was observed in PCOS for each DBP, fasting glucose, fasting insulin, HOMA-IR, TC, TG, LDL, VLDL, FSH, LH, LH/FSH, free testosterone, total testosterone, and DHEAS.

As it was shown in Figure 1, serum TGF-β1, TGF-β1/sENG ratio and Ca^{+2} concentrations are significantly increase in women who have PCOS in comparison with control group (20.55 ± 1.54 vs 19.09 ± 1.3, P < 0.0008; 6.061 vs 4.26, P < 0.0001; and 9.501 vs 8.51, P < 0.0001) respectively. In contrast, serum sENG and vitamin D concentrations are significantly decrease in women who have PCOS in comparison with control (3.1 vs 4.5, P < 0.0001; 16.76 ± 4.56 vs 25 ± 7.07, P < 0.0001), respectively.

The relationships between TGF-β1, sEND, TGF-β1/sEND, vitamin D, calcium and anthropometric, diabetes, lipid profile, female sex hormone and with each other were investigated in PCOS patients (Table 2). Interestingly, there is significant negative association between sENG and HOMA-β_2. Also, significant positive association was shown between TGF-β1 and its bioavalibility, TGF-β1/sENG.

Discussion

PCOS is the major frequent cause of female infertility. The etiology of PCOS remains unknown. Substantial evidence confirmed that PCOS is a case of low grade inflammation due to, insulin resistance, type 2 diabetes mellitus, hyperandrogensim, obesity and cardiovascular disease.[29-32] Because TGF-β1 have role as inflammatory cytokines and angiogenesis factor, several studies suggest TGF-β1 bioavailability could have a potential role in pathogenesis of PCOS. Interestingly, supplement of vitamin D and calcium decrease the level of TGF-β1. Hence, the aim of this study is to investigate the relation between TGF-β1, sENG, TGF-β1 bioavailability with vitamin D and calcium.

In the present study, TGF-β1 level is significantly increase in PCOS women in compare to controls. This result is consistent with previous studies.[14,17,33] Elevation of TGF-β1 could be explained by its role in angiogenesis, fibrosis and inhibition of collagen and fibronectin.[34,35] Increase the fibroblast activity and vescularity are observed in ovarian stroma and theca layers in PCOS patients.[12,36] Additionally, TGF-β1 level has been increased in metabolic disease, such as diabetes,[37] obesity,[38] cardiovascular disease[39] and hypertension.[40] There is strong association between PCOS and metabolic disease.[41]

In addition to elevation of TGF-β1, it was revealed in this study that serum sENG is significantly lower in PCOS women in compared to controls, as a consequent, TGF-β1 bioavailability (TGF-β1/sENG) increase in PCOS. Interestingly, there is a significant positive correlation between TGF-β1 and its bioavailability (TGF-β1/sENG). This result is in agreement with previous studies.[14,17,33] It is hypothesized that sENG binds to TGF-β1 and decrease its bioavailability in the circulation under physiological condition. Furthermore, sENG protein is an anti-angiogenesis protein;[42] as a result, it can be suggested that sENG play a physiological protective mechanism to counterbalance angiogenic factor and

Table 1. **Clinical and biochemical variables of participated women**

Characteristic	Control (*N* = 60)	PCOS (*N* = 60)	*P*-value
Anthropometric parameters			
Age (years)	30.04 ± 6.18	28.05 ± 5.11	0.056
SBP (mm/Hg)	113(120–109)	112 (116–110)	0.418
DBP (mm/Hg)	71(80–69)	70 (80–65)	0.019
BMI (kg/m^2)	27.10 ± 2.92	30.68 ± 2.21	0.189
Diabetes parameters			
Fasting glucose (mmol/L)	4.26 (5.64–3.65)	4.89 (5.3–3.6)	0.011
Insulin (mU/ml)	5.71 ± 3.89	13.33 ± 2.22	0.000
HOMA-IR	1.030 (2.27–0.59)	2.615(4.05–1.78)	0.000
HOMA-β_2	108.3 (570.6–37.95)	202.2 (640–13.62)	0.217
Lipid profile			
TC (mg/dL)	118 (132–98)	177.5 (280–152)	0.000
TG (mg/dL)	116.03 ± 11.66	144.23 ± 18.87	0.000
HDL (mg/dL)	97.43 ± 17.09	42.90 ± 2.4	0.000
LDL (mg/dL)	45 (67.6–24.4)	106.4 (116–78.8)	0.000
VLDL (mg/dL)	19.9 ± 3.45	28.85 ± 3.77	0.000
Female sex hormones			
FSH (mIU/mL)	5.57 ± 1.46	6.76 ± 1.70	0.013
LH (mIU/mL)	5.44 ± 1.52	12.92 ± 1.9	0.000
LH/FSH	0.64 (1–0.18)	1.915 (3.43–1.34)	0.000
Free testosterone (pmol/L)	2.82 ± 1.26	11.95 ± 1.75	0.000
Total testosterone (ng/mL)	1.51± 0.33	2.86 ± 0.92	0.000
DHEAS (µg/dL)	129 (160.01–110.5)	143 (245– 109)	0.026
SHBG (nmol/L)	129.0 ± 13.60	24.82 ± 6.7	0.000

SBP; systolic blood pressure, DBP; diastolic blood pressure, BMI; body mass index, HOMA-IR; homeostatic model assessment of insulin resistance, HOMA-β_2; homeostatic model assessment of beta-cell function, TC; total cholesterol, TG; triglyceride, HDL; High density lipoprotein, LDL; low density lipoprotein, VLDL; very low density lipoprotein, FSH; follicular-stimulating hormone, LH; Luteinizing hormone, DHEAS; dehydroepiandrosterone sulfate, SHBG; sex-hormone binding globulin.

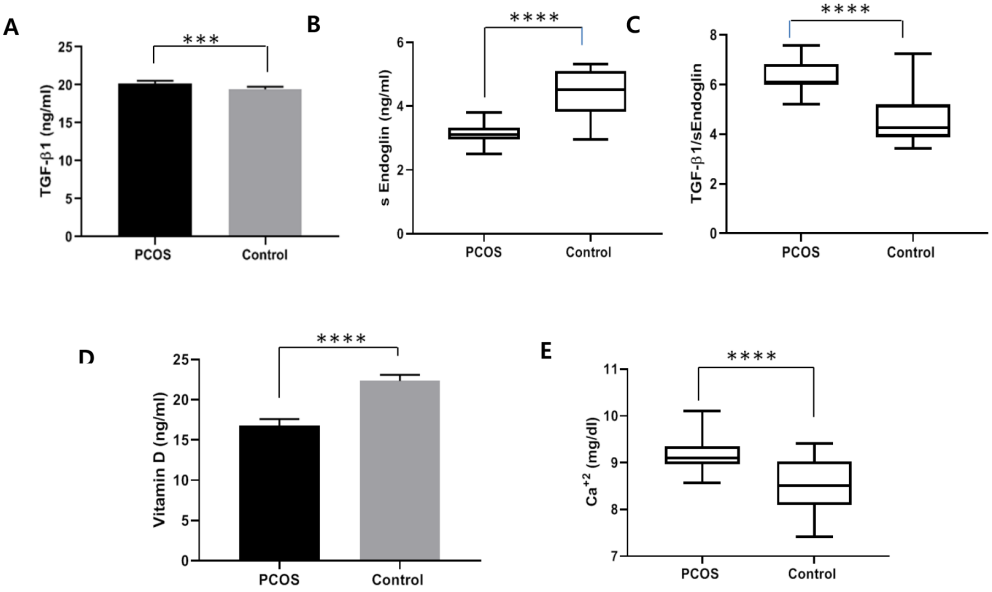

Fig. 1. **Comparison between PCOS patients and control serum of each TGF-β1 (A), sEndoglin (B), TGF-β1/sEndoglin (C), Vitamin D (D) and Ca^{+}2 (E). Normal distribution values were expressed by mean ± SEM and skewed distribution values were expressed by median. PCOS, polycystic ovary syndrome; TGF-β1, Transforming growth factor-beta and sEndoglin, soluble endoglin receptor, *** *P* < 0.005, **** *P* < 0.0001.**

Table 2. **Correlation of TGF-β1 values with clinical and biochemical parameters in PCOS patients**

Test	TGF-β1	sENG (ng/ml)	TGF-β1/sENG ratio	Vitamin D (mg/ml)	Calcium (mg/dl)
Anthropometric parameters					
Age (y)	0.005	–0.065	–0.038	0.053	0.267
SBP (mm/Hg)	0.176	0.037	0.031	0.157	–0.015
DBP (mm/Hg)	0.244	–0.240	0.212	–0.252	0.081
BMI (kg/m^2)	–0.052	–0.222	0.187	–0.291	0.404
Diabetes parameters					
Fasting glucose (mmol/L)	–0.179	0.056	0.140	0.340	–0.062
Insulin (mU/ml)	–0.007	0.056	0.177	0.359	–0.028
HOMA-IR	–0.132	–0.101	0.081	–0.092	0.313
HOMA-β_2	–0.025	–0.384	–0.032	–0.325	0.313
Lipid profile					
TC (mg/dL)	–0.256	0.034	0.102	0.077	0.141
TG (mg/dL)	–0.285	–0.122	0.078	–0.028	–0.097
HDL-C (mg/dL)	0.0507	–0.090	0.051	0.120	0.150
LDL-C (mg/dL)	–0.191	0.083	0.026	0.069	0.116
VLDL	–0.285	–0.122	0.078	0.078	–0.097
Female sex hormones					
FSH (mIU/mL)	0.332	–0.066	0.099	–0.145	0.195
LH (mIU/mL)	0.152	–0.006	0.011	0.087	–0.001
LH-FSH ratio	–0.222	0.076	–0.117	0.290	–0.186
Free Testosterone (pmol/L)	–0.308	0.333	–0.212	0.569	–0.257
Total Testosterone (ng/mL)	–0.148	–0.251	–0.004	0.303	0.113
DHEAS (µg/dL)	–0.042	–0.118	0.031	–0.072	–0.104
SHBG (nmol/L)	0.106	0.023	–0.012	–0.143	0.107
Interesting Parameter					
Calcium(mg/dL)	–0.069	–0.345	–0.036	–0.164	–
Vitamin D (mg/ml)	–0.213	0.063	–0.175	–	–0.153
TGF-β1	–	0.013	0.211	–0.161	–0.069
sENG	–0.013	–	–0.338	–0.002	–0.345
TGF-β1/sENG	0.275	–0.338	–	–0.226	–0.036

SBP; systolic blood pressure, DBP; diastolic blood pressure, BMI; body mass index, HOMA-IR; homeostatic model assessment of insulin resistance, HOMA-β_2; homeostatic model assessment of beta-cell function, TC; total cholesterol, TG; triglyceride, HDL; High density lipoprotein, LDL; low density lipoprotein, VLDL; very low density lipoprotein, FSH; follicular-stimulating hormone, LH; Luteinizing hormone, DHEAS; dehydroepiandrosterone sulfate, SHBG; sex-hormone binding globulin. *P*-Value < 0.05; significant, r; Spearman correlation.

reducing the risk of ovarian hyperstimulation syndrome. In PCOS patient, reduce sENG leads to absence of this protective mechanism which in turn increased risk of ovarian hyper stimulation syndrome.

In consistent with previous studies,[19,43-45] the level of vitamin D is lower in PCOS patients than in control subjects. Other studies reported that there were no significant differences in vitamin D level between PCOS and control.[46,47] However, our result disagrees with one study which was reported that the vitamin D level is higher in PCOS than in control.[48] In addition, calcium level is higher in PCOS than in control. These results confirm previous study which suggest the vitamin D and calcium abnormality have an important role in follicular development.[20] Interestingly, there is significant positive correlation between calcium and BMI in PCOS patients. However, in meta-analysis study in children and adults, it was suggested that increase calcium intake leads to decrease weight in both male and female.[49] Further investigation is needed to confirm the relation between calcium and BMI in PCOS.

There are no significant correlations between TGF-β1, sENG, TGF-β1/sENG, vitamin D and calcium with anthropometric (except calcium with BMI), diabetes parameters, lipid profile, female sex hormone, vitamin D, calcium, TGF-β1, sENG and TGF-β1/sENG (except TGF-β with TGF-β1/sENG). This result is not consistent with previous study.[33] The reason of absence of significant correlations might be because PCOS patients has BMI close to each other and this is one of

the limitation in this study. Another limitation factor is small size of sample.

Conclusion

Together, increase TGF-β1 with decrease sENG causes increasing of TGF-β1 bioavailability which could have a potential role in follicular development in PCOS patients. In addition, significant decrease in vitamin D and significant increase of calcium in PCOS patients in comparison with control, suggesting that vitamin D and calcium may be involve in PCOS pathogenesis. However, no correlation was observed between TGF-β1, sENG and TGF-β1 bioavailability with vitamin D and calcium. Thus, further investigations are needed to understand the influence of TGF-β1 bioavailability in PCOS pathogenesis.

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Conflict of Interest

No conflicts of interest regarding the publication of this article.

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