Doi:10.47572/muskutd.947753

Evaluation and Importance of Hypoxia Inducible Factor (HIF)-1-Alpha in Adolescents with Polycystic Ovary Syndrome

Polikistik Over Sendromu Olan Adölesanlarda Hipoksiyle İndüklenen Faktör (HIF) 1-Alfanın Değerlendirilmesi ve Önemi

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Öz

Adölesan polikistik over sendromu (PCOS) tanısında, serum hipoksiyle indüklenen faktör (HIF)-1 alfanın yerinin, klinik öneminin ve bir biyobelirteç olarak kullanılıp kullanılamayacağının araştırılması amaçlanmıştır. Üçüncü Basamak Kadın Sağlığı Eğitim ve Araştırma Hastanesinde, yaşları 15 ve 22 arasında değişen 30 PCOS'lu hasta, 30 sağlıklı adölesan ve genç yetişkin kadın çalışmaya dahil edildi. Her hastada genel fizik muayene, pelvik muayene ve ultrasonografik değerlendirme yapıldı. Serum açlık glukoz ve insulin seviyesi, lipid profili, HIF-1 alfa seviyesi hastaların esas parametreleri olarak kayıt edildi. Gruplar arasında serum HIF-1 alfa seviyeleri arasında istatistiksel olarak fark bulunmadı. PCOS hasta grubunda ortalama HIF-1 alfa seviyesi, sağlıklı popülasyona göre daha yüksek bulunmasına ragmen bu fark istatistiksel olarak anlamlı değildi. Bel/kalça çevresi oranı, serum LH seviyesi, LH/FSH oranı ve serum androjen seviyeleri PCOS'lu hastalarda kontrollere göre anlamlı olarak yüksek bulundu. Çalışmaya az sayıda hasta dahil edilmesi ve hasta grubunun PCOS semptom ve bulgularının henüz netleşmediği bir dönem olan adölesan yaş aralığını kapsaması, çalışmamızın limitasyonlarını oluşturmaktadır ve olasılıkla çalışma bulgularımızı etkilemiştir. PCOS tanısının konulmasında HIF-1 alfanın yerinin değerlendirilmesi için daha geniş hasta grupları içeren çalışmalara ihtiyaç vardır.

Anahtar Kelimeler: Adölesan, HIF-1 Alfa, Oksidatif Stress, PCOS

Abstract

It was aimed at investigating the place of serum hypoxia inducible factor (HIF)-1 alpha level in the diagnosis of adolescent polycystic ovary syndrome (PCOS), its clinical significance and whether or not it can be used as a biomarker. 30 patients with PCOS and 30 healthy adolescent and young adult women with ages ranging between 15 and 22 were recruited in a grade III Women's Health Education and Research Hospital. A general physical examination, pelvic examination and ultrasonographic evaluation was carried out on each patient. Serum fasting glucose and insulin levels, lipid profile, HIF-1 alpha levels were recorded as the main parameters of patients. No statistically significant differences were found in serum HIF-1 alpha levels between groups. While the mean HIF-1 alpha levels in PCOS patients was higher as compared to the healthy population, this difference was not statistically significant. Waist/hip circumference ratio, serum LH level, LH/FSH ratio and serum androgen levels in patients with PCOS were significantly higher as compared to control cases. The small number of patients included in the study and the age range covering the adolescence, which is the period that PCOS symptoms and findings have not become clear yet constitute the limitations of our study, and it is possible that they have affected out study results. Place of HIF-1 alpha level in the diagnosis of PCOS must be supported with studies including larger patient groups.

Keywords: Adolescent, HIF-1 Alpha, Oxidative Stress, PCOS

Introduction

Polycystic ovary syndrome (PCOS) is the most common endocrine disorder in women. While its prevalence is between 5% and 10% based on the National Health Institute (NIH) criteria, it reaches 15% when Rotterdam criteria are applied (1). It typically presents itself in early reproductive ages, and while clinical findings vary, generally involves oligo-anovulation, hyperandrogenism (biochemical and/or clinical) and the presence of polycystic ovaries. PCOS, beyond being a disease by itself, it is an integral body of endocrine disorders creating increased risk in the long term for diseases such as metabolic syndrome, type 2 diabetes, possible cardiovascular diseases and endometrium (2,3). Starting the diagnosis and treatment of PCOS in adolescence, during which PCOS picture and metabolic processes start to settle, has gained importance recently. Therefore, young females with progressive hirsutism, treatment-resistant acne, menstrual irregularity and obesity must be evaluated well for PCOS. Despite many studies, pathophysiology of PCOS has not been clarified fully. The most recent evidence-based studies have shown that PCOS is a disorder of ovarian steroidogenesis. PCOS is typically characterized with intraovarian androgen increase, which has been found responsible for not only cutaneous findings of the syndrome, but also with the anovulation and polycystic appearance of the ovaries.

The increased oxidative stress and secondary chronic inflammation in patients with PCOS are factors frequently discussed in the pathophysiology of PCOS in the recent years Macut D. and colleagues showed that PCOS is associated with oxidative stress, free oxygen radicals (super oxide, hydrogen peroxide, hydroxyl radical) increase and antioxidant

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Başvuru Tarihi / Received: 03.06.2021
Kabul Tarihi / Accepted : 04.10.2021

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levels and antioxidant enzyme decrease in PCOS (4). Based on another study, levels of homocysteine, malonaldehyde, dimethylarginine and superoxide dismutase freely circulating in the serum increase in PCOS patients independently from weight. It was stated based in the above that oxidative stress plays a role in PCOS pathophysiology (5). It has been found that intracellular reactive oxygen radical levels increase in the granulosa cells in PCOS patients (6).

Factor-I (HIF-I) that plays an important role in oxygen homeostasis and induced with hypoxia is the transcriptional regulator of metabolic events such as angiogenesis, erythropoiesis, iron and glucose metabolisms. In hypoxia, HIF-I alpha cannot be hydroxylated, and degradation slows down. Stabilized HIF-1 alpha is activated through co-activators including cAMP and protein/p300 and passes to the nucleus, and regulates the expression of genes providing response to hypoxia. Regulation of many physiologic events, angiogenesis, increased red blood cell production and metabolic changes like anaerobic glycolysis are regulated through hypoxia (7,8). Most of these events that are responsible for responses to changes in cellular oxygen amounts are regulated by HIF-1. It has been shown with immunohistochemical studies that HIF-1 increases in many human malignancies including colon, breast, lung, ovary, prostate, skin and gastric cancers. It has been stated that HIF-1 alpha protein levels can be used when planning the diagnostic and therapeutic strategies.

It has been shown that increased HIF-1 alpha production in hypoxia contributes to the mitochondrial activity (9), and ROS production (10,11). ROS plays a role in processes including migration, apoptosis, angiogenesis regulation and decreases the activity of prolyl hydroxylase (PHD) in hypoxia. PHD affects the HIF pathway through changings its phosphorylation status or by directly binding to PHDs and obstructing the availability of oxygen.

Based on this information, we aimed at investigating the place of serum HIF-1 alpha level in the diagnosis of adolescent polycystic ovary syndrome, its clinical significance, and whether or not it can be used as a biomarker.

Material and Method

Ethical approval was obtained from T.C. Zekai Tahir Burak Woman's Health Training and Research Hospital Clinical Researches Ethics Committee (Date: 17.1.2017, Decision Number:10/2017) and the study was conducted in accordance with the Helsinki Declaration. 30 patients with PCOS and 30 healthy adolescent and young adult women with ages ranging between 15 and 22 were recruited through the Health Sciences University, Zekai Tahir Burak Women's Health Education and Research Hospital, Youth Center Outpatient Clinic between March 2017 and August 2017.

PCOS diagnosis was made based on the recommendations of the latest Amsterdam ESHRE/ASRM Conference. Individuals meeting all the Rotterdam criteria were included in the PCOS group. These criteria include the ovaries appearing polycystic in the ultrasonography, menstrual irregularities (chronic anovulation and oligomenorrhea) and clinically or biochemically shown hyperandrogenism. Patients included in the study had no infective diseases, did not use oral contraceptives, sex hormones or other drugs affecting carbohydrate or lipid metabolism, they were not smokers, they did not have endocrinologic disorders. Purpose and contents of the study were explained to the patient and control groups, and informed consents of patients and parents were obtained in written form.

A general physical examination, pelvic examination and ultrasonographic evaluation were carried out in each patient, and their detailed histories were taken and socio-demographic characteristics were recorded. Ages, body mass indices (BMI), waistlines, hip circumferences, waist/hip ratios, menarche ages, menstrual rhythms, hirsutism grades, basal hormone levels at early menstrual phase, fasting glucose and insulin levels, serum lipid profile, serum HIF-1 alpha levels were recorded as the main parameters of patients.

Blood samples collected for the measurement HIF-1 alpha level was centrifuged without keeping them, and after separating the serum, it was maintained at -80 ˚C till it was used. HIF-1 alpha levels were analyzed on serum samples at 37 C incubation temperature with enzyme-linked immunosorbent assay (ELISA) method using a commercial kit. Results were expressed as ng/mL

Statistical Package for the Social Sciences (SPSS) 22 program was used for the analysis of data. Compliance of data with normal distribution was examined based on Kolmogorov-Smirnov test and Shapiro-Wilk test; and parametric methods were used in the analysis of variables with normal distribution, while non-parametric methods were used for the analysis of variables without normal distribution. Descriptive analyses were presented using means and standard deviations for normally distributed variables and median (minimum-maximum) for non-normally distributed variables. Independent-Samples T test and Mann-Whitney U test were used for the comparison of two independent groups. Spearman and Pearson Correlation tests were used to examine the correlations of variable with each other. Data were analyzed with 95% confidentiality level, and results with $p < 0.05$ was accepted as significant.

Results

Anthropometric characteristics of cases are shown. Of the sixty cases in total, 30 were PCOS patients and 30 were healthy individuals who had applied to the outpatient clinic of the youth center. Mean age of PCOS patients and control group was 18±2. Age at menarche, body mass index, waistline, hip circumference and waist/hip ratio in PCOS patients were significantly higher as compared to the control group (Table 1).

Serum HIF-1 alpha level was measured as 0.926±0.75 ng/mL in patients with PCOS, and 0.701±0.70 ng/ml in the control group. Although serum HIF-1 alpha levels were found higher in patients PCOS as compared to the control group, the difference was not statistically significant (Table 2).

LH, DHEA-S and free T values were found lower in control cases as compared to the PCOS group to the level of statistical significance. LF/FSH ratio in PCOS patients was increased with 2.1. TSH was significantly lower in PCOS group as compared to the control cases ($p<0.05$). As regards FSH, Estradiol, PRL, 17 OH-P levels, no differences were found between the control cases and patients with PCOS. (Table 2).

Positive correlation was found between fasting insulin level, insulin resistance and triglyceride levels and the body mass index (Table 3).

Table 1. Anthropometric characteristics of PCOS and Control Groups

Variable	PCOS group (n=30)	Control group (n=30)	P value
Age (years)	18 (16-22)	18 (15-22)	0.600
Age at menarche (years)	13 (10-16)	13 (11-17)	0.580
BMI (kg/m^2)	22.70 (18.30-35.10)	20.85 (16.10-30.50)	0.016
Waistline (cm)	76.10±8.73	70.10±9.85	0.015
Hip circumference (cm)	100.73±8.10	95.20±7.55	0.008
Hip/waist ratio (cm)	0.75 (0.67-0.86)	0.72 (0.65-0.91)	0.046

PCOS, polycystic ovary syndrome; BMI, body mass index

Table 2. Comparison of HIF-I Alpha Levels and Hormonal Parameters in PCOS and Control Cases

Variable	PCOS group (n=30)	Control group (n=30)	P value
HIF-1 alpha (ng/ml)	0.926±0.75	0.701±0.70	0.237
FSH (IU/L)	4.93±1.10	5.02±1.50	0.801
LH (IU/L)	10.40±3.98	7.33±3.59	0.003
Estradiol (pg/dL)	34.49±17.99	39.59±22.01	0.330
PRL (IU/L)	21.33±9.06	18.36±8.00	0.184
TSH (mIU/L)	1.84±0.84	2.35±1.07	0.046
17 OH-P (ng/ml)	1.61±0.79	1.40±0.71	0.276
DHEA-S (µg/dl)	369.25±147.05	299.62±90.40	0.031
Free T (ng/dl)	1.87±0.65	1.32±0.27	<0.001
Total T (ng/dl)	0.42±0.17	0.31±0.12	0.006

PCOS, polycystic ovary syndrome; HIF-1 Alpha, Hypoxia Inducible Factor-1 Alpha; PCOS, polycystic ovary syndrome; FSH, follicle stimulating hormone; LH, luteinizing hormone; PRL, prolactin; TSH, Thyroid-stimulating hormone; 17 OH-P, 17-hydroxyprogesterone; DHEA-S, dehydroepiandrosterone-sulphate; Free/Total T, Free/Total Testosterone

Table 3. Correlation of other biochemical parameters in the PCOS and control groups and the entire cohort

BMI	PCOS		Control		Total	
Variables	**r**	**p**	**r**	**p**	**r**	**p**
HIF-1 Alpha	0.194	0.305	0.169	0.372	0.121	0.359
Insulin	0.278	0.136	0.577	0.001	0.440	<0.001
HOMA-IR	0.382	0.037	0.614	<0.001	0.505	<0.001
T. Cholesterol	-0.061	0.749	0.251	0.181	0.085	0.516
Free Testosterone	0.020	0.917	0.033	0.864	0.170	0.195
Total Testosterone	0.011	0.956	-0.223	0.235	0.043	0.744
DHEA-S	0.048	0.802	-0.156	0.410	0.069	0.600
LDL	-0.182	0.335	0.414	0.023	0.109	0.406
HDL	-0.047	0.805	-0.139	0.465	-0.120	0.362
TG	0.306	0.100	0.508	0.004	0.332	0.010

BMI, body mass index; PCOS, polycystic ovary syndrome; HIF-1 Alpha, Hypoxia Inducible Factor-1 Alpha; DHEA-S, dehydroepiandrosterone-sulphate; HOMA-IR, homeostatic model assessment; LDL, low-density lipoprotein; HDL, high-density lipoprotein; TG, triglyceride

Discussion

Based on the study carried out Chandel N.S. and colleagues, HIF-1 alpha, regulates the induction of some genes including erythropoietin and VEGF in hypoxia. At the same time, hypoxia increases mitochondrial reactive oxygen radicals and this in turn causes HIF-1 alpha stabilization (10). Likewise, in the studies carried out by Guzy R.D. and colleagues, mitochondrial electron transport chain (ETC) complex III is required for hypoxic stabilization of HIF-1 alpha and HIF-2 alpha and the increased reactive oxygen radicals (ROS) bind to these complexes to this complex and helps stabilization of HIF-1 alpha (11). Similar results were obtained in another study also (12).

Increased oxidative stress and chronic inflammation secondary to oxidative stress are among factors discussed frequently for PCOS pathophysiology recently. Macut D. and colleagues have shown that PCOS is associated with oxidative stress, free oxygen radicals increase in PCOS, and antioxidant levels and antioxidant enzyme levels decrease. A wide range of endocrine and metabolic conditions including obesity, hyperinsulinemia, dyslipidemia can be responsible for the oxidative stress associated with PCOS. It has been seen that omega 3 fatty acids, alpha-lipoic acid and N-acetyl cysteine supplementations have anti-inflammatory and antioxidant effects in women with PCOS (4). It is stated in a meta-analysis published in 2013 that reactive radicals circulating in free form in serum (superoxide, hydrogen peroxide and similar), homocysteine, malondialdehyde, asymmetric dimethylarginine and superoxide dismutase levels increase in PCOS patients. Markers as indicators of oxidative stress circulating freely were found in abnormally high levels in PCOS patients independently from body weight. In this meta-analysis, it was stated that oxidative stress plays a role in PCOS pathophysiology (5). In another study investigating poor oocyte quality and poor IVF-ET results in women with PCOS, increased intracellular reactive oxygen radical levels were found in granulosa cells. The increased ROS levels in granulosa cells induces apoptosis, and this in turn affects the oocyte quality and lowers the positive IVF-ET pregnancy outcomes in patients with PCOS (6).

In an experimental study on rats, it was reported that oxidative stress has a place in the etiology of alcoholic fatty liver. Also, HIF-1 alpha levels were found high in patients with alcoholic fatty liver. Forty-eight rats were divided into 3 groups as subjects with alcoholic fatty liver, subjects in this condition and resveratrol was administered to, and the control group. It was found that HIF-1 alpha protein expression and mitochondrial reactive oxygen radicals were decreased in the group treated with resveratrol as compared to the untreated group (13). Based on the study of Coimbra-Costa D. and colleagues, acute hypoxia increases the formation of reactive oxygen radicals in the brain. They found increases in HIF-1 alpha expression, lipid peroxidation, protein oxidation and nitric oxide levels in brain extracts following respiratory hypoxia, accompanied by significant decreases in antioxidant systems including superoxide dismutase (SOD), reduced glutathione (GSH) and glutathione peroxidase (GPx). However, it was shown in this study that oxidative stress parameters and antioxidant system returned to normal following 24-hour reoxygenation (14).

There are no other studies in the literature evaluating the HIF-1 alpha level in women with PCOS. Our study is a first in this area. In our study, the mean serum HIF-1 alpha level in adolescent and young adult females with PCOS was 0.926 ng/mL, while the same in the control group was 0.701 ng/ml. Although serum HIF-1 alpha levels in patients with PCOS were found higher as compared to the control group consistently with the literature, the difference was not statistically significant. One of the reasons for this can be that our study group included adolescents and young adults and the small number of our sample, because adolescence is a period that PCOS symptoms and findings have just started to settle and PCOS picture can vary.

Comparison of the anthropometric characteristics of the PCOS group and the control group in our study showed that the mean age of both groups was 18 and the mean age at menarche was 13. Frequency of polycystic ovary syndrome in ultrasound was 86.7% in PCOS cases and 43.3% in the control group; this finding of ours was also consistent with the literature (15). It was found in two separate study carried out in the United States America that 18% to 22% of adolescents with PCOS were overweight and patients with PCOS had greater tendency for adiposity in the upper part of the body when compared to healthy women in similar body weights. Based on another study, abdominal obesity is present in about 65% of patients with PCOS. Abdominal obesity was defined as the waistline exceeding the 75th percentile value or waist/hip ratio >0.8 (16). In our study, BMI', waistline and hip circumference measurements and waist/hip ratios of patients were significantly higher than in the control group. Thirty percent of adolescents we have included in our study were overweight (BMI>25). These findings were also consistent with the literature. In the study of Ates S. and colleagues, in which they examined the clinical and metabolic characteristics of 77 adolescent girls with polycystic ovary syndrome in Turkey in 2018, they found high BMI, waistline and LH level and LH/FSH ratios and increased free androgen levels. These findings were consistent with our study also. It has been stated in this study that the most important factor in the

development of metabolic disorder in adolescents with PCOS can be high BMI (17).

The best indicator of hyperandrogenism is persistently high levels of serum testosterone (18). Serum free testosterone concentrations are 50% more sensitive in determining hyperandrogenism as compared to total testosterone. It has been proven through studies that high levels of serum free testosterone are the single most sensitive test to determine the presence of hyperandrogenism (18,19). We also found serum total and free testosterone levels higher as compared to the control group in our study consistent with the literature data. Increased LH levels are the laboratory abnormality seen first in classical PCOS. We found significantly high levels of LH in the PCOS group in parallel with the information in the literature. In addition, we found that the LH/FSH was increased with 2:1. This finding was also consistent with the information in the literature. When we combined the findings of increased BMI and LH, it can be considered that a vicious cycle between excessive androgen and abdominal deposition of visceral fat results in PCOS, because excessive androgen may lead to abdominal deposition of visceral fat facilitating further androgen excess (17). It is known that increased BMI and LH levels were commonly seen in PCOS at reproductive age but we found these findings in adolescents which revealed this situation begins at younger ages in PCOS patients. According to these results, the management of PCOS should be initiated at earlier ages.

Lack of studies on adolescents, lack of specific criteria to define PCOS in the adolescent period, normal range of biochemical markers being unknown for the adolescent period, and our relatively small sample constitute the limitations of our study. HIF-1 alpha plays a critical role in human physiology and oxygen homeostasis through the gene expression of hypoxia. HIF-1 alpha can be a new hope for diagnosis and treatment in our times many diseases including cancer are discussed and explained with genetics

In conclusion, in our study that we have investigated the HIF-1 alpha levels in adolescents with PCOS, HIF-1 alpha level was higher in the PCOS group as compared to the control group; however, the difference was not statistically significant. There are no studies in the literature on PCOS patients in relation with HIF-1 alpha. In this sense, our study is a first. The small number of patients included in the study and the age range covering the adolescence, which is a period that symptoms and findings of PCOS has not clearly settled yet constitute the weak aspects of our study, and this had possible affected our study results. Place of HIF-1 alpha level in the diagnosis of PCOS must be supported with studies carried out on larger patient groups and young adults in reproductive period are included.

Ethics Committee Approval: Ethical approval was obtained from T.C. Zekai Tahir Burak Woman's Health Training and Research Hospital Clinical Researches Ethics Committee (Date: 17.1.2017, Decision Number:10/2017).

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