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RESEARCH ARTICLE / ARAŞTIRMA MAKALESİ

Relationship Between Bilirubin Levels and Heart Rate Recovery in Polycystic Ovary Syndrome Patients

Polikistik Over Sendromlu Hastalarda Bilirubin Düzeyleri ile Kalp Hızı Toparlanması Arasındaki İlişki

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Abstract

Aim: Bilirubin is a final product of heme metabolism and also has antioxidant properties. But there is no information on the effect of bilirubin in the pathogenesis of in Polycystic Ovary Syndrome (PCOS). The goal of the present study was to

JOURNAL OF HUMAN RHYTHM

investigate the relationship between bilirubin levels, PCOS and heart rate recovery (HRR).

Material and Methods: Subjects were included in this study from our center's Department of Gynecology & Obstetrics between March 2012 and May 2013. Serum bilirubin levels and other blood parameters in at least 12-hour fasting states were determined. Exercise stress test was performed on all patients and control participants.

Results: Thirty four patients with a diagnosis of PCOS aged younger than 40 year and twenty seven healthy women matched by age, BMI, heart rate and blood pressure were included in the study. Total bilirubin levels in PCOS group were significantly lower than the control group. The HRR was significantly decreased in women with PCOS compared the control group. In the Pearson correlation test, HRR was significantly correlated with homeostasis model assessment insulin resistance (HOMA-IR) index and bilirubin levels.

Conclusions: The bilirubin levels may affect HRR in PCOS patients. Therefore, in the treatment of PCOS patients, oxidative stress and its harmful effects should be considered.

Key words : Bilirubin, heart rate recovery, polycystic ovary syndrome, exercise test

Özet

Amaç: Bilirubin antioksidan özelliklere sahip Hem metabolizmasının son ürünüdür. Bilirubin Polikistik Over Sendromu (PCOS) patojenezi üzerindeki etkisi bilinmemektedir. Çalışmamızın amacı bilirubin, PCOS ve kalp toparlanma hızı (HRR) arasındaki ilişkiyi araştırmaktır.

Materyal ve Metod: Jinekoloji ve Obstetrik Bölümünde Mart 2012 ve Mayıs 2013 arasındaki hastalar çalışmaya dahil edildi. Serum bilirubin düzeyleri ve diğer kan parametreleri en az 12 saat açlık sonrası ölçüldü. Egzersiz testi tüm hasta ve kontrol grubuna uygulandı.

Bulgular: PCOS tanılı 40 yaşından genç Otuz Dört hasta ve yas, vücut kitle indeksi, kalp hızı ve kan basıncı açısından eşit olan Yirmi Yedi sağlıklı bayan çalışmaya dahil edildi. Toplam bilirubin düzeyleri PCOS grubunda anlamlı şekilde daha düşüktü. Kalp toparlanma hızı PCOS grubundaki hastalarda kontrol grubuna göre anlamlı şekilde daha düşüktü. Pearson korelasyon testinde, Kalp toparlanma hızı bilirubin düzeyleri ve homeostaz modeli değerlendirme insülin direnci (HOMA-IR) endeksi ile anlamlı şekilde korele idi.

Sonuç: Bilirubin düzeyleri PCOS hastalarında kalp toparlanma hızını etkileyebilmektedir. Bu etkiden

JOURNAL OF HUMAN RHYTHM

dolayı, PCOS hastalarının tedavisinde oksidatif stres ve zarar verici etkileri dikkate alınmalıdır.

Anahtar kelimeler: Bilirubin, kalp toparlanma hızı, polikistik over sendromu, egzersiz test

Introduction

Polycystic Ovary Syndrome (PCOS) is a common endocrine disorder in women. The estimated prevalence is between 4-14%.[1] Metabolic and endocrine factors are dominant in the pathogenesis of PCOS. Although there is no clear relationship, these factors are responsible for the high prevalence of pathologies in PCOS patients such as diabetes mellitus[2], hypertension[3], dyslipidemia[4], diastolic dysfunction.[5] Increased oxidative stress may be one of the possible mechanisms. There are studies showing that decreased antioxidant defenses and increased oxidative stress in patients with PCOS.[6-8] Increased oxidative stress is associated with increased production of reactive oxygen species (ROS). ROS leading to oxidative damage to cellular lipids, proteins and DNA that alter cell and tissue functions. Autonomic nervous system is also damaged tissue is one of these. Hoeldtke and colleagues found that sympathetic dysfunction associated with increased oxidative stress.[9] In another study showed that the severity of diabetic neuropathy is associated with oxidative stress.[10]

For the first time in 1994, Imai and colleagues found that vagally mediated heart rate recovery (HRR) after exercise is accelerated in well trained athletes but blunted in patients with chronic heart failure.[11] In another study showed that decreased vagal activity is a strong predictor of mortality independent of the extent of myocardial ischemia.[12] This negative result explained by an increased risk of arrhythmia depending reduced vagal activity as a result of impaired

autonomic balance.[13] There are studies indicating that impaired HRR observed in PCOS patients.[14-15] However, the underlying mechanisms are not known.

Bilirubin is a final product of heme metabolism and also has antioxidant properties.[16-18] Therefore bilirubin is located in the pathogenesis of many diseases such as coronary artery disease.[19-20] diabetes mellitus[21,22], metabolic syndrome. [23] But there is no information on the effect of bilirubin in the pathogenesis of PCOS. The goal of the present study was to investigate the relationship between bilirubin levels, PCOS and HRR.

Methods

Subjects

All patients were referred to our Department of Gynecology & Obstetrics between March 2012 and May 2013. Thirty four patients with a diagnosis of PCOS aged younger than 40 year and twenty seven healthy women matched by age, BMI, heart rate and blood pressure were included in the study. All patients underwent physical examination, 12-lead electrocardiography (ECG) and transthoracic echocardiographic evaluation. Patients with any cardiovascular disorder including hypertension, diabetes mellitus, thyroid and renal diseases, hyper-cortisolism, use of oral contraceptives or other hormonal therapy within the prior 3 months, pregnancy or breast-feeding, hyper-prolactinemia, congenital adrenal hyperplasia, androgen-secreting tumors, signs or symptoms of other endocrinopathies, smoking, chronic alcohol consumption, or history of use of any medications in the last 3 months were excluded. PCOS was diagnosed according to the Rotterdam criteria in the presence of at least two of the following three features: oligo or

anovulation, hyperandrogegism, and polycystic ovaries. The study protocol was approved by the local Medical Research Ethics Committee and consistent with the Declaration of Helsinki.

Measurements

Blood glucose, high-density lipoprotein (HDL), low-density lipoprotein (LDL) cholesterol, TG, insulin and transaminase enzyme levels in at least 12-hour fasting states were determined. Serum bilirubin concentrations were determined by the enzymatic colorimetric method by a clinical chemistry auto analyzer. Insulin resistance was calculated by using the homeostasis model assessment insulin resistance index (HOMA-IR) according to the formula described by Matthews et al. [24]

Exercise Stress Test Protocol

Subjects underwent a maximal graded exercise test on an electronic treadmill with Bruce protocol. Predicted peak heart rate was calculated as (220 - age) and the aim was to reach at least 85% of the age-predicted heart rates. Blood pressure, heart rate, and symptoms were recorded every minute. HRR was calculated as the difference between heart rate at peak exercise and heart rate at first minute of the cool-down period. Estimated workload, expressed in metabolic equivalents (METs; 1 MET equals 3.5 mL of oxygen uptake per kilogram of body weight per minute) was recorded into the database.

Statistics

Statistical analyses were performed using SPSS version 20.0 (SPSS, Inc.,Chicago, IL). After certification of normal distribution, data were reported as mean ± SD; categorical variables were defined as percentage. Two group comparisons were performed using independent Student's t tests for continuous variables and the chi-square test for categorical variables. Correlation

analyses were performed using the Pearson coefficient of correlation. $P < 0.05$ was considered significant.

Results

Clinical characteristics and laboratory findings of patients included in the study are shown in Table 1. Compared the control group, PCOS group was had significantly higher BMI, waist-to-hip ratio, fasting glucose, insulin and HOMA-IR levels. On the other hand, the total bilirubin levels in this group were significantly lower than the control group. The HRR was significantly decreased in women with PCOS compared the control group (Table 2).

In the Pearson correlation test (Table 3), HRR was significantly and negatively correlated with HOMA-IR (r: -0.359, p: 0.005) and bilirubin levels were positively and significantly correlated with HRR (r: 0.556, p: 0.001). No correlation was found for the other parameters.

Discussion

To the best of our knowledge, this is the first study to examine the relationship between total bilirubin levels and polycystic ovary syndrome. In addition, serum bilirubin levels were associated with heart rate recovery. Pearson correlation analysis showed that HRR was significantly correlated with bilirubin and HOMA-IR.

Oxidative mechanisms are important in energy production, but also they provide a potentially harmful reactive oxygen species.[25] If the amount of ROS reaches levels that the body's defense mechanism cannot overcome, a number of disorders occur in cells and tissues. Oxidative stress may be one of the reasons underlying the pathogenesis of PCOS. There are many studies about this relationship. Sabuncu and colleagues found that increased oxidative stress and insufficient

antioxidative status are in PCOS patients. [26] A similar result was also observed in another study.[27] Antioxidant enzyme PON-1 activity was found to decrease in PCOS patients.[7] A recent meta-analysis found that markers of the oxidative stress are abnormal in PCOS patients regardless of weight [28]. Increased oxidative stress is likely to be associated with insulin resistance[29], obesity[30] and hormonal changes.[6] However, PCOS and a molecule antioxidant bilirubin relationship has not been studied previously. In our study, PCOS group compared to the control group had lower levels of bilirubin. Lin et al found that the low serum bilirubin levels are strongly associated with increased HOMA-IR and insulin levels.[31] In accordance with these results, PCOS group had low bilirubin levels and high insulin resistance status compared with control group in our study. Similar results were observed in a study in patients with metabolic syndrome. [23] Thus, decreased bilirubin levels may be responsible from some negative effects caused oxidative stress in PCOS patients.

HRR is an indirect indicator of autonomic nervous system effect on the heart and it has been associated with many disorders.[12,32-34] PCOS is one of them. Giallauri and colleagues found in their study, PCOS women showed a significantly reduced HRR compared to healthy controls.[15] Another study found similar results and researchers suggested increased catecholaminergic stimulation might be responsible from these adverse effects.[14] There are studies that ovarian sympathetic tone or general sympathetic tone could be responsible from this adverse effect.[35] Our hypothesis was that impaired HRR in PCOS patients may relationship with damaged autonomic nervous system caused by oxidative stress. Sears and colleagues found that in their study nitric oxide synthase inhibition affect heart rate recovery by cholinergic system.[36] In an animal study, improvement in autonomic function was monitored by reduction of oxidative stress.[37] A study

conducted in patients with impaired fasting glucose showed that cardiac autonomic function associated with oxidative stress and insulin resistance.[38] Our study had similar results. Bilirubin levels were lower in the PCOS group and in this group bilirubin level had a positive correlation between the HRR. These findings may indicate indirectly that oxidative stress responsible from impaired HRR in PCOS patients.

The present investigation has several limitations. Firstly, there were a small number of patients. The second limitation of our study was the lack of other markers of oxidative stress such as malondialdehyde, superoxide dismutase. However our study leads way in furthering the efforts to understand the connecting links between PCOS and oxidative stress. Large control studies are needed to understand and validate our results.

As a result of our study, we found bilirubin levels associated HRR in polycystic ovary syndrome patients. Therefore, adverse effects of oxidative stress were shown on cardiac autonomic function. In the treatment of PCOS patients, this point should be considered.

Conflict of interest

The authors declare that they have no conflict of interest.

All persons gave their informed consent prior to their inclusion in the study.

Table 1 : Baseline clinical and biochemical PCOS patients and control subjects.

Variables	**PCOS (n=34)**	**Controls(n =27)**	**P**
Age	24.7 ± 6.7	24.7 ± 5.3	0.998
BMI (kg/m2)	26.3 ± 4.9	23 ± 4.1	**0.003**
Metabolic Syndrome %(n)	55(19)	66(18)	0.39
Waist circumference (cm)	84.2 ±13.3	79.9 ± 10.1	0.168
Waist to hip ratio	0.80 ± 0.7	0.75 ± 0.8	**0.004**
SBP (mmHg)	106 ± 8.7	111 ± 11.6	0.490
DBP (mmHg)	64.4 ± 5.8	68.8 ± 8.8	0.563
Heart rate (beat/min)	81.6 ±13.3	86.7 ± 2.2	0.455
Fasting glucose (mg/dl)	95.6 ±13.8	83.1 ± 9.1	**0.001**
Low-density lipoprotein (mg/dl)	104.1±176	98.1 ± 11.6	0.135
Triglycerides (mg/dl)	111.3 ±40.2	109.5 ±27.7	0.840
Fasting insulin (mIU/l)	15 ± 5.9	10.4 ± 2.2	**0.001**
HOMA-IR	2.9 ± 0.9	2 ± 0.5	**0.001**
Bilirubin(mg/dl)	0.38 ±0.08	0.45 ± 0.08	**0.001**

JOURNAL OF HUMAN RHYTHM

AST(U/L)	18.3 ± 8.6	18.4 ± 7.8	0.991
ALT(U/L)	24.3 ± 7.5	22.6 ± 6.2	0.355

HOMA-IR - homeostasis model assessment - insulin resistance, PCOS - polycystic ovary syndrome

Table 2: Comparison of treadmill exercise test parameters

Variables	**PCOS (n=34)**	**Controls(n=27)**	**P**
Maximal METs	11 ± 1.3	10.8 ± 2.1	0,105
Exercise duration (minutes)	9.9 ± 1.3	9.3 ± 1.2	0,648
Maximal heart rate (beat/min)	178.6 ± 9.4	176.3 ± 12.1	0,421
Heart rate recovery at 1 min (beat/min)	27.8 ± 11.2	34.2 ± 10.8	**0.027**

METs = metabolic equivalent

Table 3: Correlation between heart recovery rate (HRR) and risk factors

	HRR	
	r value	**p**
BMI	- 0,167	0,347
Waist circumference	- 0,178	0,315
Waist to hip ratio	0,211	0,232

LDL	- 0,153	0,386
TG	0,051	0,774
Bilirubin	0,556	**0.001**
Fasting insulin	- 0,018	0,918
HOMA-IR	- 0,359	**0.005**
Fasting glucose	- 0,204	0,247

LDL: Low-density lipoprotein TG: Triglycerides
HOMA-IR: Homeostasis model assessment - insulin resistance

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