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The Relationship Between Premature Adrenarche and Platelet Aggregation

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What is already known on this topic?

Premature adrenarche (PA) has been associated with an increase in adrenal androgens. A hyperandrogenic hormonal environment is known to lead to increased platelet (PLT) aggregation.

What this study adds?

We have shown increased collagen-induced PLT aggregation in girls with PA. This is significant as PA may cause increased cardiovascular event risks later in life due to increased PLT aggregation.

Abstract

Objective: Premature adrenarche (PA) has been associated with an increase in adrenal androgens, and the hyperandrogenic hormonal environment is known to lead to increased platelet (PLT) aggregation. Here, we evaluated the effects of PA on PLT aggregation in PLT-rich plasma samples from female patients.

Methods: The study included 40 female patients diagnosed with PA between February, 2014 and June, 2018 and 30 healthy female individuals as a control group. Adenosine diphosphate (ADP) and collagen-induced PLT aggregation were studied via the photometric aggregometry method.

Results: There were no significant differences in the PLT count or volume values between those participants with PA and the control group. Additionally, the ADP-induced maximum aggregation time, value, and slope values did not significantly differ between the patient and control groups ($p > 0.05$). However, the collagen-induced maximum aggregation time, value, and slope values were significantly higher in the studygroup ($p < 0.001$).

Conclusion: Increased collagen-induced PLT aggregation was detected in female patients with PA. As PA is associated with a higher risk of cardiovascular events later in life, close follow-up of PA in this respect may be beneficial.

Keywords: Premature adrenarche, cardiovascular diseases, hyperandrogenism, platelet aggregation, ADP, collagen

Introduction

Premature adrenarche (PA) has been associated with an increase in adrenal androgens due to the premature maturation of the zona reticularis layer of the adrenal cortex before the age of 8 in girls and 9 in boys (1). The most secreted androgens from the adrenal gland are dehydroepiandrosterone (DHEA) and androstenedione, which are weak androgens. DHEA undergoes sulfation in the liver and becomes DHEA-sulfate (DHEAS), and it is considered a marker of adrenal androgenic activity (2).

Girls with PA are at higher risk of developing symptoms of metabolic syndrome, including obesity and type 2 diabetes, and cardiovascular disease later in life (3). The mechanisms

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underlying these relationships remain unclear but have been partially associated with excess adipose tissue in adulthood (4). Indeed, earlier puberty is predictive of a higher adult body mass index (BMI) and a greater risk of obesity in women (5).

Cardiovascular diseases cause significant morbidity and mortality worldwide. Risk scoring systems have been developed to identify people at high risk of developing an adverse cardiovascular event by evaluating known risk factors (6). However, many patients whose risk assessments for cardiovascular diseases are determined to be at low or moderate levels may experience a cardiovascular event. Platelet (PLT) activity, which is not routinely found in current risk score algorithms, may also be a factor increasing cardiovascular risk (7).

The relationship between PLT aggregation and cardiovascular events has been evaluated in several studies (8). Different measures of PLT separation and purification methods and PLT aggregation measurements with different agonists at varying concentrations have been used in most of these studies (8). Although the data on increased PLT aggregation leading to cardiovascular events are far from conclusive, some significant results have been reported (8). A moderate increase in spontaneous PLT aggregation was detected in vascular events (8,9). It was recently shown that having an increased PLT aggregation response is associated with future arterial thrombosis, and the incidence of coronary heart disease-related mortality may increase significantly in these individuals (10).

Although there are various studies in children and adolescents regarding PLT counts and coagulation factors, as well as various PLT aggregation studies regarding non-hematological diseases (11,12,13,14), there are no studies on PLT counts and PLT aggregation in girls with PA.

In this case-control study, we investigated how PLT counts and aggregation are affected by adenosine diphosphate (ADP) and collagen agonists in girls with PA.

Methods

The patient group of this case-control study included 40 female patients diagnosed with PA between February, 2012 and June, 2018 (Group 1) at the pediatric outpatient clinic of Gülhane Training and Research Hospital (Ankara, Turkey). After the cases were diagnosed with PA, the relevant laboratory studies were conducted prospectively, and the cases and controls were followed up over a 6-year period. Girls with PA who had at least one clinical sign of adrenal androgen action (i.e., adult-type body odor, greasiness of hair and skin, comedones/acne, and axillary or pubic hair) together with increased DHEAS secretion before the age of 8 years, and other sources of hyperandrogenism (including central puberty, congenital adrenal hyperplasia, and androgen-producing tumors) were excluded. DHEAS concentrations of >40 µg/dL were considered adrenarche (15). Thirty healthy female individuals were included as the control group (Group 2). All girls in the control group were healthy, did not use any medication, and did not have any premature signs of androgen action.

Written informed consent from the families of the patients and approval of the Gülhane Training and Research Hospital Local Ethics Committee (date: 30.06.2009, no: 135) were obtained.

The inclusion criteria for both groups of patients were as follows: no use of antiplatelet drugs within the last 30 days, and no hematological diseases, chronic heart, kidney, and/or liver diseases (excluding PA in the patient group).

Complete blood count results, DHEAS, DHEA, luteinizing hormone (LH), 17-hydroxyprogesterone (17-OH progesterone), 11-deoxycortisol, adrenocorticotropic hormone (ACTH) and cortisol hormone levels, ferritin levels, prothrombin time (PT), activated partial thromboplastin time (aPTT), fibrinogen levels, and PLT aggregation were analyzed in both groups. Hormone levels were measured with the chemiluminescence method using the Beckman Coulter DxI® 600 analyzer.

Erythrocyte indices, PLT counts, and mean PLT volume (MPV) values were obtained using an automated device (Technicon H-1 System, Technicon Co, Tournai, Belgium).

Venous blood samples were obtained from the antecubital vein citrate between 8:00 and 9:30 in the morning after 8-12 hours of night fasting and collected into plastic syringes containing 3.8% trisodium by 1/10 volume. PLT-rich and PLT-poor plasma were prepared by centrifugation (16). PLT aggregation was assessed by photometric aggregometry using a complete blood aggregometer (Model 560; Chrono-Log Corporation, Havertown, PA, USA).

Collagen (5 µg/mL, Chrono Par No: 385; Chrono-Log Corporation) and ADP (10 µmol, Chrono Par No: 384; Chrono-Log Corporation) were used as agonists. The maximum aggregation time (s), value (%), and slope (%/min) were determined from the aggregation curves. The effects of ADP and collagen on aggregation were evaluated in both the control and patient groups considering the effect of iron deficiency on aggregation (17,18).

Statistical Analysis

The statistical analysis was performed with Statistical Package for the Social Sciences (SPSS) software (version 22; SPSS Inc., Chicago, IL, USA). The data are presented as mean and standard deviation values. The normally distributed data were compared by independent samples t-test. We analyzed the correlations of DHEAS and DHEA levels with aggregation using the Pearson correlation test, and we used regression analysis for the aggregation values. Differences were considered statistically significant at $p < 0.05$.

Results

Forty female patients diagnosed with PA were included in the patient group (Group 1), and thirty healthy female individuals constituted the control group (Group 2). The demographic characteristics of Groups 1 and 2 are given in Table 1. There were no significant differences between the groups in terms of age, height, weight, or BMI ($p > 0.05$).

The complete blood count parameters of Groups 1 and 2 are given in Table 2. There were no significant differences between the groups in terms of their white blood cell count, red blood cell count, hemoglobin (HGB) level, hematocrit level, mean erythrocyte volume, mean erythrocyte HGB level, mean erythrocyte HGB concentration, red cell distribution width, PLT count, or MPV values ($p > 0.05$).

There was no significant difference between Groups 1 and 2 in terms of their plasma ferritin level, PT, aPTT, fibrinogen level, LH, 17-OH progesterone, 11-deoxycortisol, ACTH, or cortisol hormone level values ($p > 0.05$); however, in the patient group, DHEAS and DHEA levels were significantly higher than of those in the control group ($p < 0.05$; Table 3).

The mean maximum aggregation time, value, and slope induced by 10 µmol ADP and 5 µg/mL collagen in Groups 1 and 2 are shown in Table 4. In the patient group, at 10 µmol ADP, the mean maximum aggregation time, value, and slope did not significantly differ from the values in the control group ($p > 0.05$). However, in the patient group, at a collagen concentration of 5 µg/mL, the mean maximum aggregation time, value, and slope were significantly higher than of those in the control group ($p = 0.001$, 0.002, and 0.04, respectively). DHEAS was positively correlated with the maximum aggregation time ($r = 0.446$, $p = 0.013$), maximum aggregation value ($r = 0.397$, $p = 0.018$), and slope ($r = 0.263$, $p = 0.034$) values in collagen-induced PLT aggregation in the patients with PA. Similarly, DHEA was positively correlated with the maximum aggregation time ($r = 0.356$, $p = 0.024$), maximum aggregation value ($r = 0.308$, $p = 0.029$), and slope ($r = 0.217$, $p = 0.039$) values in collagen-induced PLT aggregation (Table 5). The results of the multivariate logistic regression analysis revealed that the maximum aggregation time [Odds ratio (OR); 95% confidence interval (CI) 1.42 (1.05–2.11);

Table 1. Demographic characteristics of the study and control groups

	Study group	Control group	
Patient characteristics	**n = 40**	**n = 30**	**p value**
Age (year)	7.38 ± 0.08	7.50 ± 0.09	0.321
Height SDS	0.79 ± 0.22	0.75 ± 0.20	0.944
Weight SDS	0.51 ± 0.12	0.56 ± 0.13	0.476
BMI SDS	0.22 ± 0.04	0.24 ± 0.03	0.216

Data are given as mean ± SD.
BMI: body mass index, SDS: standard deviation (SD) score

Table 2. Complete blood parameters in the study and control groups

	Study group	Control group	
Parameters	**n = 40**	**n = 30**	**p value**
WBC (/µL)	6,370 ± 188	6,433 ± 269	1.00
RBC ($\times 10^6$/µL)	4.85 ± 0.05	4.93 ± 0.06	0.117
HGB (g/dL)	13.07 ± 0.10	12.93 ± 0.13	0.376
HCT (%)	38.84 ± 0.44	39.19 ± 0.54	0.714
MCV (fL)	80.86 ± 0.49	80.88 ± 0.50	0.733
MCH (pg)	27.23 ± 0.17	27.02 ± 0.26	0.737
MCHC (g/dL)	33.69 ± 0.21	33.30 ± 0.19	0.13
RDW (%)	13.64 ± 0.19	13.50 ± 0.14	0.81
PLT (/µL)	308621 ± 932	282100 ± 131	0.58
MPV (fL)	8.97 ± 0.86	9.21 ± 1.01	0.17

Data are given as mean ± standard deviation.
WBC: white blood cell, RBC: red blood cell, HGB: hemoglobin, HCT: hematocrit, MCV: mean erythrocyte volume, MCH: mean erythrocyte hemoglobin, MCHC: mean erythrocyte hemoglobin concentration, RDW: red cell distribution width, PLT: platelet, MPV: mean platelet volume

p = 0.002], maximum aggregation value [OR; 95% CI 1.33 (1.07–1.76); p = 0.003], and slope [OR; 95% CI 1.06 (1.01–1.13); p = 0.03] in collagen-induced PLT aggregation were associated with PA in the patient group (Table 6).

Discussion

To the best of our knowledge, there is no study in the literature concerning PLT counts and PLT aggregation in girls with PA. In this study, we provide evidence of increased collagen-induced PLT aggregation in girls with PA.

Girls with a history of PA display a hyperandrogenic hormonal environment which may lead to increased cardiovascular risk (19,20,21,22,23,24,25). Çelik et al. (20) detected early atherosclerotic changes and subclinical deterioration in cardiac functions in children with PA. However, it was not fully explained why children with PA tend to show early cardiovascular changes. The authors showed that PA increased the risk of coronary heart disease, and this result was attributed to increased carotid intima-media thickness and epicardial adipose tissue measurements in PA patients. The increased risk of coronary heart disease in girls with PA in general was partly associated with the excess

Table 3. DHEAS, DHEA, LH, 17-OH progesterone, 11-deoxycortisol, ferritin level, PT, aPTT, and fibrinogen level in the study and control groups

	Study group	Control group	
Parameters (± SD)	**n = 40**	**n = 30**	**p value**
Ferritin (ng/mL)	29.18 ± 1.71	36.97 ± 4.73	0.61
PT (s)	13.66 ± 0.16	13.71 ± 0.22	0.808
aPTT (s)	30.00 ± 0.27	30.48 ± 0.29	0.222
Fibrinogen (mg/dL)	290 ± 6.40	293 ± 7.88	0.980
DHEAS (µg/dL)	69.1 ± 13.20	26 ± 8.30	***0.002***
DHEA (ng/dL)	149.2 ± 12.57	106.6 ± 9.45	***0.003***
LH (mlU/mL)	0.1 ± 0.03	0.1 ± 0.02	0.79
17-OH progesterone (ng/mL)	0.79 ± 0.07	0.51 ± 0.05	0.09
11-deoxycortisol (ng/mL)	3.1 ± 0.3	2.2 ± 0.4	0.11
Cortisol (µg/dL)	12.1 ± 3.12	14.3 ± 3.25	0.35

Data are given as mean ± SD.
PT: prothrombin time, aPTT: activated partial thromboplastin time, DHEAS: dehydroepiandrosterone-sulfate, DHEA: dehydroepiandrosterone, LH: luteinizing hormone, 17-OH progesterone: 17-hydroxyprogesterone, SD: standard deviation

Table 4. Platelet aggregation parameters of the study and control groups

	Study group	Control group	
Agonist	**n = 40**	**n = 30**	**p value**
ADP (10 µmol)			
Maximum aggregation time (s)	342.50 ± 15.49	339.75 ± 12.89	0.975
Maximum aggregation value (%)	74.73 ± 2.07	70.63 ± 2.35	0.16
Slope (%/min)	114.30 ± 7.36	98.98 ± 3.98	0.09
Collagen (5 µg/mL)			
Maximum aggregation time (s)	405.00 ± 14.64	337.83 ± 10.54	***0.001***
Maximum aggregation value (%)	74.95 ± 1.27	66.20 ± 2.33	***0.002***
Slope (%/min)	127.46 ± 4.69	111.30 ± 6.44	***0.04***

Data are given as mean ± standard deviation.
ADP: adenosine diphosphate

Table 5. Correlation analysis of androgen levels and platelet aggregation parameters in patients with PA

	DHEAS	DHEA
Agonist	**r (p)**	**r (p)**
ADP		
Maximum aggregation time	0.044 (0.65)	0.036 (0.71)
Maximum aggregation value	0.087 (0.22)	0.073 (0.31)
Slope	0.151 (0.08)	0.136 (0.09)
Collagen		
Maximum aggregation time	***0.446 (0.013)***	***0.356 (0.024)***
Maximum aggregation value	***0.397 (0.018)***	***0.308 (0.029)***
Slope	***0.263 (0.034)***	***0.217 (0.039)***

ADP: adenosine diphosphate, DHEAS: dehydroepiandrosterone-sulfate, DHEA: dehydroepiandrosterone, PA: premature adrenarche

Table 6. Multivariable logistic regression analysis for PA in the study group

Collagen	OR	95% CI	p value
Maximum aggregation time	**1.42**	**1.04-2.11**	**0.002**
Maximum aggregation value	**1.33**	**1.07-1.76**	**0.003**
Slope	**1.06**	**1.01-1.13**	**0.03**
ADP			
Maximum aggregation time	1.01	0.92-1.13	0.39
Maximum aggregation value	1.09	0.96-1.22	0.27
Slope	1.04	0.93-1.16	0.15

ADP: adenosine diphosphate, OR: Odds ratio, CI: confidence interval, PA: premature adrenarche

adipose tissue found in these patients in adulthood (5). It is thought that a process beginning with childhood obesity may ultimately lead to cardiovascular diseases in later life, in association with PA and adult obesity (21,22,23). The relationship of an atherogenic abnormal lipid profile such as increased serum triglyceride and low-density lipoprotein cholesterol with PA has also been demonstrated (24,25). Moreover, Topaktaş et al. (26) showed that cholesterol and triglyceride-related arterial involvement caused by obesity and metabolic syndrome are effective in the pathogenesis of arterial stiffness in PA, rather than increasing androgens.

Along with obesity and abnormal lipid status, it was found that a hyperandrogenic hormonal environment is also associated with PLT aggregation. It is known that sex steroids are absorbed at PLT membranes, and they modify the surface properties of these membranes. These modifications induce permeability changes (27). Sex steroids may also interact with fibrinogen, plasminogen, or fibrinolytic inhibitors (28). For instance, it was reported that testosterone increases the concentration of plasma prostaglandins (29). Furthermore, it was demonstrated that PLT aggregation induced by arachidonic acid (30) is enhanced after androgens are added under *in vitro* conditions (31). This effect of testosterone on PLT aggregation leads to an increase in thrombus formation and mortality. In a rat study, arterial thrombosis development was observed after the administration of testosterone (32). Human studies have also demonstrated that increased testosterone levels affect the induction of PLT aggregation. It was speculated that this effect might have resulted via testosterone receptors in PLTs (33). The decrease in the incidence of acute coronary syndrome in men with prostate cancer due to flutamide use was associated with these results (34).

Although sexual maturity has been shown to affect PLT aggregation in pigs, no study has investigated the role of PLT aggregation in the increased risk of cardiovascular disease in later life, either in girls or older women with a history of PA (35). In our study, we demonstrated a relationship between PA and collagen-induced PLT aggregation. In PA patients, mainly DHEA and DHEAS levels are increased, and these androgens are known as weak androgens. However, it is known that the most potent androgens have the greatest aggregation-increasing effect (33). This effect was more significant in collagen-induced PLT aggregation than ADP-induced PLT aggregation (36). In studies with weak androgens, inhibition, rather than an increase in PLT aggregation, has been observed. In a previous study, the *in vitro* administration of DHEAS showed dose- and time-dependent inhibition in arachidonate-induced PLT aggregation (37). In another study, the administration of DHEAS at physiological doses with thrombin or supraphysiological doses with collagen, thrombin, and TxA2 analog U-46619 also inhibited PLT aggregation (38). The difference in the results of our study may be due to the different potencies of DHEA and DHEAS in children.

In our study, we showed that collagen-induced PLT aggregation was increased in those girls with PA, and this may be due to increased DHEAS levels. Collagen is a strong agonist, but ADP is a weak one (39). In our study, ADP was used at a concentration of 10 µmol, which is sufficient for PLT aggregation (39). We did not detect any changes in aggregation at this level of ADP, but there was an increase in collagen, suggesting selectivity for collagen-induced PLT activation pathways. Similar to our findings, Leng et al. (40) achieved an increase in collagen-induced PLT aggregation, but not thrombin-induced PLT aggregation, by giving different estrogen derivatives to ovariectomized mice, which was done to examine the effects of estrogens on arterial thrombosis. In their study, estrogens acted in an agonist-specific fashion which changed collagen-induced PLT surface glycoprotein-VI expression, which then initiated adhesion followed by aggregation. Collagen, unlike ADP, also stimulates the release of thromboxane A2 (TxA2), a strong aggregation agent (41). Several studies have shown that adrenal androgens increase the number of TxA2 receptors (42,43). This may indicate that the increased collagen-induced PLT aggregation in our study may be due to increased adrenal androgens via glycoprotein-VI and/or TxA2. In order to explain this, studies showing the effects

of androgens on PLT surface glycoprotein-VI expression are needed.

There are several studies which revealed normal and increased PLT counts in healthy people using anabolic androgenic steroids (43,44,45). In a study of 25-month-old Sprague-Dawley rats, a slight increase in PLT numbers was found with DHEAS treatment (46). In our study, we found no significant difference between the patient and control groups in terms of PLT counts and volumes.

Although an increased risk of coronary heart disease in girls with PA history is partly associated with the excess adipose tissue and the increased atherogenic lipid profile found in adulthood in these individuals, the increased PLT aggregation we found in girls with PA may ultimately lead to cardiovascular disease.

Study Limitations

Some limitations of our study were 1) the small sample size of our study may have been insufficient in the evaluation of PLT aggregation; 2) other high-risk factors for cardiovascular events were not discussed in detail in our study, and 3) it is not known how long the increase in PLT aggregation detected in these children with PA would last and whether this increase in aggregation would continue when they reach the normal age of puberty as we did not study the results of this test again in these children.

Conclusion

In conclusion, increased collagen-induced PLT aggregation was detected in girls with PA. As PA is associated with a higher risk of cardiovascular events later in life, close follow-up of PA may be beneficial. Repeated studies of PLT aggregation in patients with PA are needed in order to demonstrate whether the increase in collagen-induced PLT aggregation persists later in life.

Ethics

Ethics Committee Approval: The study was approved by the Gülhane Training and Research Hospital Local Ethics Committee (date: 30.06.2009, no: 135).

Informed Consent: Written informed consent from the families of the patients.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: Onur Akın, Concept: Orhan Gürsel, Mehmet Emre Taşçılar, Design: Ahmet Bolat, Cengiz Zeybek, Data Collection or Processing: Ahmet Bolat, Analysis or Interpretation: Ahmet Bolat, Literature Search: Ahmet Bolat, Cengiz Zeybek, Onur Akın, Writing: Ahmet Bolat, Cengiz Zeybek.

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