Activated protein C resistance in preeclampsia

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Summary

Objective: Recently, hereditary and acquired diseases that lead to thromboembolic events by changing the hemostatic balance have attracted interest as a cause of preeclampsia. In this study the incidence of activated protein C resistance (APCR) in preeclamptic women was evaluated.

Methods: Activated protein C sensitivity ratio (APC-SR) was measured by the modified activated partial thromboplastin time (APTT) method in 19 preeclamptic and 12 healthy pregnant women and 26 normal women as the controls. Results below the levels of 2 were accepted as the presence of APCR.

Results: Median APC-SR values of 2.12 and 2.01 in preeclamptic and healthy pregnant women, respectively, were found significantly lower than the normal control values of 2.31 ($p = 0.0005$, $p = 0.001$). APCR was detected in 31% of preeclamptic patients, 16.6% of healthy pregnant women and 7.6% of normal controls.

Conclusion: APCR was found significantly higher in preeclamptic women and it may play an important role in the pathogenesis of preeclampsia.

Key words: Activated protein C resistance; Preeclampsia; Pregnancy.

Introduction

Placental perfusion defect is thought to be responsible for obstetrical complications, including preeclampsia, HELLP syndrome (hemolysis, elevated liver enzymes and low platelets) and fetal growth retardation. Coagulation factors found in excess amounts in the maternal or fetal circulation may play a role in the pathophysiology of the increasing thrombotic events during pregnancy [1, 2]. Even though, hypercoagulable events were not encountered clinically, increased coagulation activities were demonstrated with specific laboratory tests in preeclamptic women [3].

Recently, the place of hereditary and acquired diseases which cause thromboembolic events in the pathogenesis of preeclampsia have gained much attention, and many studies have been performed related to this subject [4-7]. Dekker *et al.* [4] demonstrated the presence of hemostatic and metabolic defects associated with the occurrence of vascular thrombosis in more than half of preeclamptic patients. These defects were defined as protein S deficiency, activated protein C resistance, hyperhomocysteinemia and anticardiolipin antibodies.

Resistance to activated protein C is caused by a point mutation in the factor V (FV) gene that leads to an inability of FV to bind with activated protein C, defined by the Dahlback in 1993 [8]. FV Leiden is the most common genetic defect that causes thrombophilia [9]. Acquired defects can also be a reason for activated protein C resistance (APCR). APCR tests made with plasma that was diluted with FV-deprived-plasma are very specific tests for the detection of the FV Leiden mutation [10, 11].

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Although most hemostatic changes have been shown in preeclamptic patients [8-10], the exact cause of preeclampsia is still unknown. The role of FV Leiden mutations and APCR in the pathogenesis of preeclampsia has been demonstrated in recent studies [12-15]. In the present study, we investigated the incidence of activated protein C resistance in preeclamptic pregnant patients by the modified activated partial thromboplastin time test.

Methods

Subjects: Three groups of women were studied: Group I: preeclamptic pregnant patients consisting of 19 women with the median age of 23 (range from 19 to 42), group II: 12 healthy pregnant women with the median age of 24 (range from 21 to 30), group III: non-pregnant female volunteers with the median age of 29 (range from 19 to 39) as a control. Study groups were selected from consecutive pregnant women who were admitted to SSK Ege Obstetrics and Gynecology Hospital between January 1998 and January 2000.

Preeclampsia was defined in accordance with the American Association of Obstetricians and Gynecologists Terminology Committee as pregnancy-induced hypertension occurring after the 20th week of pregnancy with increased blood pressure > 140/90 mmHg at least twice during a 24-hour period and proteinuria > 0.3 g/l [11]. Women in the control group had no history of hormonal contraceptive use and anticoagulant medication before or during the study.

Blood Sampling: Blood was obtained by fresh venopuncture from antecubital veins of patients and controls, and added to 0.106 mmol/l trisodium citrate (9 ml blood: 1 ml anticoagulant). Samples were mixed gently and centrifuged at 2000 rpm for 15 minutes to obtain platelet

poor plasma, then centrifuged for a further ten minutes at 2000 rpm and the plasma was separated into 1.5 ml aliquots. Plasma samples were then stored at –80°C until analysis.

Assays: Activated protein C resistance was measured by using a kit supplied by Instrumentation Laboratory Company (Chromogenix AB, Mölndal, Sweden) with ACL Futura coagulometer (Instrumentation Laboratory Company, 1998). The activated protein C resistance test is essentially a modified activated partial thromboplastin time (APTT). Plasma samples were diluted to a ratio of 1:5 with poor plasma from FV mutations, before the measurement of APTT. The results are expressed as the APC-sensitivity ratio (APC-SR) of two APTT values, determined in the presence and absence of activated protein C (APC). Resistance to APC was diagnosed when the APC-SR was below 2.

Statistical Analysis: Statistical analysis was carried out with GraphPad Prism version 3.03 statistical package program prepared for Windows (GraphPad Software, San Diego California). Differences in the parameters between the study groups were assessed with the Mann-Whitney U test after the ANOVA test was applied to the three groups.

Results

Median values of APC-SR were assessed as 2.12 (1.43-2.39), 2.07 (1.51-2.28), 2.31 (1.53-2.52) in groups I, II and III, respectively. When the results of preeclamptic and healthy pregnant patients were compared to normal women's a statistically significant difference was found ($p = 0.0005$ and $p = 0.001$, respectively) (Figure 1). As seen in Table 1, APCR was higher in the preeclamptic women (31%) than the healthy pregnant women (16.6%) and the normal controls (7.6%).

Table 1. — *APC resistance and the APC-SR results of the groups.*

Groups	Subject number	APC-SR*	APCR	
			number	%
I	19	2.12 (1.43 - 2.39)	6	31
II	12	2.07 (1.51 - 2.28)	2	16.6
III	26	2.31 (1.53 - 2.52)	2	7.6

* = median values.

Discussion

Preeclampsia is a major cause of maternal and neonatal morbidity and mortality. The etiology and pathophysiologic mechanisms of preeclampsia are still unknown. Recently, inadequate uteroplacental vascular impairment due to thrombosis has been investigated as a leading cause [16, 17]. Also, inherited and acquired thrombophilic disorders such as protein C, S and antithrombin III deficiency, hyperhomocysteinemia and presence of activated protein C resistance have been investigated [4, 15, 18, 19]. In our study, we evaluated the presence of APCR in preeclamptic women with the modified APTT

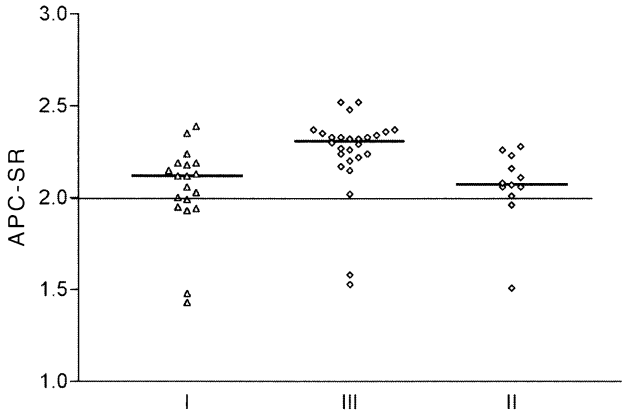

Figure 1. — APC sensitivity ratios of the study groups.

method, which was found significantly higher in the pregnant women with preeclampsia than the normal women. The modified APTT method is a highly specific test to demonstrate the presence of APCR, thus in accordance with the present study APCR may be a risk factor associated with preeclampsia.

APCR has recently been identified as the most common predisposing factor in venous thromboembolic events [20, 21]. APCR is associated with a point mutation in the factor V gene [22]. The prevalence of APCR in the general population varies widely in different populations and ethnic groups and appears to be approximately 5% [20, 23]. In Western countries resistance is ten times more common than the other genetic anticoagulant deficiencies [24, 25]. The prevalence of APCR in Turkey was reported as 7.1% which is a high rate [26]. This abnormality was found in 20-40% of patients with venous thrombosis and 60% of cases of gestational thrombosis [27, 28]. Interest has lately been focused on the role of APCR in thromboembolic complications during pregnancy.

The first study related to APCR in preeclampsia was published by Dekker *et al.* [4] in 1995. They showed APCR among 16% of women with a history of preeclampsia which was three times higher than the normal population. However, analysis of hereditary disposition to the FV gene mutation was not performed in this study. Most other studies following also demonstrated an increased incidence of APCR and FV Leiden mutation in preeclampsia [12, 13, 18]. In our study we found a higher incidence of APCR (30%) in preeclampsia than those reports did. We also demonstrated the presence of APCR in healthy pregnant women with a 16.6% incidence which was a much higher rate than the controls. In pregnancy, there are many different alterations that increase the risk of thromboembolic events. Activation of the coagulation system with the inhibition of fibrinolysis has been regarded as part of the physiologic adaptation to pregnancy [29]. Hemostatic changes in preeclampsia may represent an exaggerated form of this process.

Cetin *et al.* [14] demonstrated an increased APCR rate in pregnant women with preeclampsia of 84.4%, 71.9%,

and 15.6% in the third trimester of gestation and, three and nine months after childbirth, respectively, which was higher than the healthy pregnant women at the same time (28.1%, 14.8%, and 3.2%, respectively). This temporary increase of APCR during pregnancy advocated them to think that the pre-existing acquired APCR rather than the genetic defect was playing a role in the etiology of preeclampsia. Likewise, Pampus *et al.* [19] suggested the presence of acquired APCR in preeclampsia. They found a significant increase of APC-SR but no increase in the incidence of FV mutation in preeclamptic women. Paternoster *et al.* [15] also demonstrated the temporary and progressive increase of APCR in preeclampsia from the first to the third trimester of pregnancy which was not associated with the presence of FV gene mutation. With regard to the above studies and according to our results a screening test for APCR during pregnancy could be useful to detect the risk of thrombotic complications. In contrast to other studies, Linqvist *et al.* [30] reported that there was no difference between the APCR and non-APCR subgroups in the incidence of preeclampsia. Thus, they disagree with the view that APCR testing might be useful for screening the predisposition to preeclampsia.

In summary, early studies evaluating the prevalence of APCR in preeclampsia showed an increase of both APCR and FV gene mutations and proposed that there was a genetic predisposition for preeclampsia [18, 31]. Recently, the term temporary and acquired APCR, defined as lower ratios in the classic APCR test without presence of FV gene mutations during pregnancy, was addressed [32-34].

According to the present results, the incidence of APCR in preeclamptic women was approximately four times higher than the control groups. Alhough there was no statistically significant difference between the results of preeclamptic and healthy pregnant women, the incidence of APCR in preeclampsia was also high for the healthy pregnant women. We could not say that there is an acquired or a genetic pre-existing risk of thrombophilia but the presence of APCR during pregnancy may be a possible risk for preeclampsia. There is contradictory knowledge in the literature about the role of acquired or genetic APCR in the pathogenesis of preeclampsia. Further studies are needed to clarify the temporary increment or genetic predisposing of APCR and the causal relationship with the adverse pregnancy outcome.

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