

Association of Deficiency of Coagulation Factors (Prs, Prc, ATIII) and FVL Positivity with Preeclampsia and/or Eclampsia in Pregnant Women

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Received: 9, Apr, 2014

Accepted: 27, Apr, 2014

ABSTRACT

Background: Thrombophilia is a pathological state of increased blood coagulability. It causes problems during pregnancy including preeclampsia, stillbirth, repeated abortions, and detached pair. Out of the most prevalent factors causing inherited thrombophilia, protein S (Prs), protein C (Prc), and antithrombin III (ATIII) deficiency, and Factor V Leiden (FVL) mutation could be mentioned. This study aimed to investigate association of these parameters with preeclampsia.

Methods: In this case-control study, 142 pregnant women with preeclampsia referred to Obstetric Clinic of Hajar Hospital, southwest of Iran, were assigned to the case group after clinical laboratory tests and according to specialist point of view and 142 pregnant women with normal blood pressure were assigned to the control group. After obtaining consent and completing relevant questionnaire, a 4-cc blood sample was taken from the patients. Coagulation factors and FVL rate were measured and after 6 months patients were followed-up. Data analysis was done by SPSS software using t-test.

Results: In view of deficiency of Prs, Prc, and ATIII, no statistically significant association was observed between case and control groups ($P > 0.05$). Statistical t-test indicated that the rate of FVL deficiency in pregnant patients with preeclampsia was significantly different from that in the control group ($p = 0.03$). In addition, the body mass index of case group was significantly higher than that of control group prior to pregnancy ($P = 0.001$). In case group, preeclampsia history contributed to development of current preeclampsia in contrast to control group ($p < 0.001$). The patients of case group were followed up after 6 months in view of blood pressure and all had a normal mean blood pressure at the completion of the study.

Conclusion: Measurement of FVL deficiency could help to decrease the unpleasant complications of vascular disorders during pregnancy. But, screening test for pre-eclampsia does not seem necessary to determine the deficiency of coagulation factors, Prs, Prc, and ATIII.

KEYWORDS: Coagulation Factors; Eclampsia; Factor V Leiden; Pre-eclampsia; Thrombophilia

INTRODUCTION

Thrombophilia is the increase in blood coagulability, which could be inherited or acquired. Inherited thrombophilia is a genetic predisposition, which was described by Egberg in 1965 for the first

time.¹ During pregnancy, the predisposition to inherited thrombophilia increases because the changes associated with pregnancy in some coagulation factors cause increase in resistance to activated protein C (Prc) during second and third

trimesters and decrease in activity of protein S (Prs) because of estrogen effects; also, factors 2, 7, 8, 9, and 10 increase. All these lead to increase in coagulability in healthy women. Therefore, an individual with inherited thrombophilia is at high risk of thromboembolic problems during pregnancy, which is often followed by repeated abortions, preeclampsia, stillbirth, intrauterine growth restriction (IUGR), detached pair, increase in cardiovascular events, deep vein thrombosis (DVT) and pulmonary embolism.^{2,3}

Awareness of these genetic disorders (genetic thrombophilias) contributes greatly to determining the need for antithrombotic factors for prophylaxis in special conditions and the risk during pregnancy. The majority of these disorders are developed by genetic mutations, causing deficiency of an anticoagulation factor or increase in a pre-coagulation factor.¹

Out of the most prevalent factors causing genetic thrombophilia, Prs deficiency, Prc deficiency, factor V Leiden (FVL) mutation, and antithrombin III (ATIII) deficiency could be mentioned.⁴ In several studies in Turkey,⁵ Canada,⁶ Italy⁷ and in a study by Lockwood and Wendel,² inherited thrombophilia was identified as a risk factor for pregnancy complications such as repeated abortions and preeclampsia.

Prs is a glycoprotein associated with vitamin K and Prc system cofactor, which is developed by hepatocytes and megakaryocytes. Prs deficiency was described for the first time in 1984 and found to be transmitted in the form of a predominant etozome. Its familial prevalence has been reported 3-13%. This anticoagulation substance is activated by Prc in circulation and decreases thrombin production.²

Prc is a type of coagulation glycoprotein. When thrombin is linked to thrombomodulin in microvascular endothelial cells, its pre-coagulation activities are neutralized. More than 160 mutations have been described for Prc with the prevalence of 2-3 per 1000. For estimating the prevalence rate of Prc deficiency, the threshold level of functional activity of this protein is considered 50-60%.^{2,8}

The most prevalent inherited disorder which leads to coagulation incidence is FVL mutation.⁴ A research in Australia indicated that heterozygosity for FVL increased the risk of intrauterine fetal

death, detached pair and preeclampsia, but generally no clear association was obtained between inherited thrombophilia and unpleasant pregnancy outcome.⁹

Inability to deactivate mutated FVL allows prothrombin complex to remain relatively uninhibited, causing increase in thrombin production and thrombophilic phenotype.⁴

ATIII is a type of natural anticoagulation factor which is combined with endogenous heparin sulfates, inhibiting both the formed thrombin and the activated factor X.¹⁰ Antithrombin deficiency could cause resistance to heparin although in most cases, its deficiency does not lead to resistance to heparin.⁴ The homozygous factor deficiency is fatal.¹⁰

Prs deficiency, Prc deficiency, ATIII deficiency, and FVL mutation increase the risk of preeclampsia, repeated abortions, and intrauterine fetal death.² High blood pressure during pregnancy (preeclampsia) is hypertension accompanied with proteinuria after 20th week of pregnancy in the women with normal blood pressure prior to pregnancy. Preeclampsia accompanied with a seizure not attributable to other reasons is referred as eclampsia.¹¹ Preeclampsia is diagnosed in 3.9% of total pregnancies and is the reason for 16% of maternal mortalities.¹¹

Although the reason for preeclampsia is still unknown, some evidence is being obtained, suggesting that this disease is manifested during early pregnancy and that hidden, pathophysiologic changes are initiated from insemination. These are most probably due to vascular spasm consequences, disorder in endothelial function, and ischemia.¹¹ Cagigrans et al. in a study in Turkey found that resistance to activated Prc in the women with preeclampsia was considerably higher and could be a pathogenesis for developing this disease during pregnancy.¹²

In a study in Romania, the association of Prc deficiency, Prs deficiency, and FVL deficiency with brain's vascular events rate as well as with inherited thrombophilia was investigated, and these tests were found valuable for timely diagnosis of these events and inherited thrombophilia which may be developed in these individuals. But, for confirming this association further research is required.¹³

In a study in Poland, polymorphism G691A of FVL and polymorphism G20210A of prothrombin were investigated and a pronounced effect of these two polymorphisms was found on progression of patients' condition toward severe preeclampsia.¹⁴

In Kapfremine et al.'s study in University of Delhi¹⁵ and studies conducted in Germany^{16,17} and Canada,¹⁸ the unpleasant complications in the women with inherited thrombophilia decreased remarkably after treatment with heparin and aspirin. However, the definite treatment of preeclampsia in all cases is termination of pregnancy.¹¹

In Chaharmahal and Bakhtiari province, southwestern Iran, regarding ethnographic characteristics of region, frequency of intrafamilial marriages which increases the likelihood of inherited diseases such as inherited thrombophilia, and frequency of rural population with cultural beliefs in these marriages and a low level of health care during pregnancy, research on inherited thrombophilia is much needed. Evidence indicates an association of pregnancy complications, including pregnancy abortions of prior to 20th week and afterwards, preterm deliveries, and preeclampsia, with inherited thrombophilia. It is expected that, through determining the value of tests measuring thrombophilia including FVL, Prc, Prs, and ATIII in predicting development of eclampsia and preeclampsia, unpleasant complications of pregnancy is prevented as much as possible. Therefore, regarding the significance of issue in this province, we aimed to investigate the association of Prc deficiency, Prs deficiency, FVL positivity, and ATIII deficiency in diagnosing pregnancy complications such as preeclampsia and eclampsia in comparison with healthy pregnant women as controls.

MATERIALS AND METHODS

The sample population of this case-control study is all pregnant women admitted to Obstetric Clinic and Labor Unit of Hajar Hospital, southwest of Iran from 21 April, 2011 to 21 August, 2012. The development of eclampsia and preeclampsia had been already confirmed based on clinical symptoms, routine tests, and diagnosis of the gynecologists. The pregnant women having multiple

pregnancies, receiving treatment (heparin, aspirin, and enoxaparin) during pregnancy and declining to participate in the study were excluded.

It is assumed that deficiency of coagulation factors is 15% in the pregnant women with pre-eclampsia and eclampsia and 5% in pregnant women with no complication. Also, the confidence interval (CI) is 95% and test power is 80%. The study included 284 patients assigned to two groups of 142 each (142 pregnant women with pre-eclampsia and eclampsia were considered as the case group and 142 healthy pregnant women were considered as the control group). Having justified the patients, given explanations to the patients, obtained written consent, done complete physical examination, and obtained detailed description, we registered the required data in a questionnaire (including demographic characteristics, coagulation events, body mass index [BMI], smoking, the medications taken, history of surgery, hematological and rheumatoid diseases, pregnancy and abortion status, gestational age, and history of diseases during pregnancy such as pre-eclampsia, eclampsia, and detached pair) to measure the blood pressure of patients after six months of follow-up. Then, a 4-cc blood sample was taken from the patients and then was tested for the rate of ATIII, Prs, Prc and FVL in laboratory within half an hour.

FVL level was measured per coagulant assay (Hyphen Biomed, France). FVL measurement was done on two PTT tests obtained from patient. In one of the tests, activated Prc was added to the sample containing patients' plasma and then the relative time of thromboplastin was assessed. In patients with FVL mutation, Prc introduction could not suitably extend the time of thromboplastin compared to normal individuals.¹⁰ If the patient has no FVL resistance to Prc (no FVL mutation), PTT of the first tube is half PTT of the second tube in the presence of Prc. But, if the patient is FVL positive, there is little difference between the two tubes. The patients with index of two and higher are considered FVL-negative and with index of less than two are considered FVL-positive.¹⁰

ATIII was measured per chromogenic assay (Biophen Antithrombin 2.5, France). Antithrombin measurement by this method is synthetic, based on factor XA inhibitor, which involves formation of a

stable, increased concentration by antithrombin in the presence of heparin. Normal range of ATIII activity is 80-120%.²

Prc and Prs levels were measured using ELISA (AESKU, Germany). Normal range of Prs activity is 60-150% and that of Prc is 65-140%.² After collecting data and conducting secondary examinations, we analyzed them by means of SPSS software using t-test.

RESULTS

To examine deficiency of coagulation factors, Prc, Prs, and ATIII, and FVL positivity, the following data were obtained in this study. In total, 142 qualified pregnant women with pre-eclampsia (case group) and 142 healthy pregnant women (control group) were enrolled. The age range of patients in the case and control groups was 18-42 (mean: 28.8±5.7) and 18-38 (mean: 25.8±4.8) years, respectively. Independent t-test indicated no difference in age between the two groups. No difference in education levels was observed between the two groups ($P>0.05$) (Table 1).

Table 1. Occupation status in the case and control groups

Occupation status	Group		Total
	Case	Control	
Employed	6 4.2%	6 4.2%	12 4.2%
Housekeeper	136 95.8%	136 95.8%	272 95.8%
Total	142	142	284

In terms of education, 13 patients (46%) were illiterate, 51 (18%) had elementary education, 63 (22.2%) guidance school education, 113 (39.8%) high school education, and 64 (15.5%) academic education. In terms of the number of pregnancies, 151 patients (53.2%) experienced the first pregnancy, 62 (23.2%) the second, 37 (13%) the third, 20 (7%) the fourth, and 10 (3.5%) the fifth and more. In this regard, no difference was observed between the two groups, as well ($P>0.05$).

The pre-and post-pregnancy weight, height and BMI are shown in Table 2. In view of thrombosis, only two patients in the case group had thrombosis of DVT type, showing no significant difference between the two groups. Table 3 indicates the

history of unpleasant complications in the two groups.

Table 2. Weight, height and BMI of the women under study

Variable	Group (Mean ± SD)		Level of significance
	Case	Control	
Pre-pregnancy weight	63.6±12.3	61±11.1	0.041
Post-pregnancy weight	77.3±12.5	73.6±11.1	0.009
Height	160.7±5	161.7±6.2	0.123
Pre-pregnancy BMI	24.6±4.5	23.2±4.1	0.008
Post-pregnancy BMI	30±4.5	28.1±4	0.001

Table 3. History of disease in the case and control groups

Group/Disease	Case		Control		P value
	No.	%	No.	%	
Fetal loss	2	1.4	0	0	0.156
Preeclampsia history	21	14.8	1	0.7	<0.001
Abortion history	24	23	23	16.2	0.873
Thrombosis history	2	0	0	0	0.156

As Table 4 indicates, no significant difference in deficiency of coagulation factors (Prc, Prs, and ATIII) was observed between the two groups. In FVL deficiency, a unidirectional significance was observed.

Table 4. The rate of deficiency of coagulation factors in pregnant women with preeclampsia in comparison with healthy pregnant women

Deficiency Type	Case		Control		Level of significance
	No.	%	No.	%	
Prs Deficiency	2	1.4	6	4.2	0.282
Prc Deficiency	0	0	0	0	-
ATIII Deficiency	0	0	0	0	-
FVL Deficiency	5	3.5	0	0	0.03

*Level of significance is unidirectional (It is assumed that this is higher in the case group).

DISCUSSION

Mean age was 26.8±5.7 years in case group, which is not considered an age for high risk pregnancies,² and it could be argued that pregnancy conditions in this mean age is more tolerable in view of structural physiology and involves fewer underlying diseases.²

Occupational status and educational level are thought to have no effect on disease development because in the investigations conducted, there was

no association between occupational status and housekeeping as well as educational level with incidence rate of preeclampsia and eclampsia.

Thirty nine participants in the case group and 45 in the control group had Prs deficiency. After statistical analysis of data, no statistical association was found between the case and control groups.

Prc deficiency was observed in 6 and 3 participants in the case and control groups, respectively. No statistically association was found between Prc deficiency and preeclampsia and/or eclampsia incidence in this study. No significant association was observed between this deficiency and disease incidence. Protein decrease less than 50% and 30%, defined as the least natural rate of Prc and Prs, is considered as Prc and Prs deficiency, respectively. Based on this definition, no Prc deficiency was observed in the case and control groups, while 2 women in the case group and 6 in the control group were diagnosed with Prs deficiency.

Moreover, 22 women in the case group and 33 in the control group showed ATIII deficiency, but there was no significant association between ATIII deficiency and disease incidence.

In this study, 5 women in the case group were diagnosed with FVL deficiency, while no healthy pregnant woman in the control group showed FVL deficiency. The association was statistically unidirectional ($P=0.03$). No significant difference was seen in deficiency of coagulation factors, Prs, Prc and ATIII between the two groups. Therefore, based on the results of this study, FVL measurement is essential to diagnose unpleasant pregnancy complications. Some studies have revealed that FVL deficiency is prevalent among some ethnic groups but rare in others.²⁰

Similar results were obtained in studies conducted in Poland²¹ and Italy.²² FVL mutation has a direct and dependent relationship with unpleasant outcomes of pregnancy. In Klis et al.'s study, FVL factor mutation (except O blood type) and Prc deficiency were found to have the highest prevalence in the patients suffering from unpleasant pregnancy outcomes.²³ In a study conducted in Australia, unpleasant pregnancy outcomes were six times higher in the patients with FVL mutation compared to normal population of

the community.¹⁹ In an investigation conducted in London on vascular complications of the pair and its association with FVL, prothrombin G20210A, MTHFR C677T and beta fibrinogen, only FVL had a contribution to increase in incidence rate of pair's vascular events compared to the control group,²⁴ which is in agreement with the result of the present study. FVL deficiency could have a role in eclampsia and/or preeclampsia incidence. Through identifying the patients at risk of this factor's deficiency which, in turn, could cause coagulation events during pregnancy and eclampsia and/or preeclampsia exacerbation, development of pregnancy complications could be notably prevented by means of timely and efficient medical therapeutic interventions and even screening of pregnant women for FVL deficiency. In a study by Lund et al. in Denmark on thrombophilia and repeated abortions, FVL mutation and prothrombin had no value in predicting live birth in mothers with repeated abortions, but the present study indicates no effect of other factors on preventing pregnancy failures and treatment with anticoagulants improves pregnancy prognosis in the women with FVL mutation and prothrombin mutation with repeated pregnancy failure.²⁵ Pabinger conducted a study in Vienna, Austria and expressed screening mothers with repeated pregnancy failures, thrombophilia and timely treatment with anticoagulants such as enoxaparin could lead to pleasant pregnancy outcome. A strong association has been found between phospholipid antibodies and antithrombin deficiency, FVL, prothrombin G20210A mutation, Prc deficiency, Prs deficiency, inherited thrombophilia, and by extension, positive culmination of pregnancy.²⁶

However, different results could be due to ethnic differences, study criteria and the sample size in different studies.

The results of the present study demonstrated the effect of preeclampsia history on the incidence of preeclampsia in the case group compared to the control group ($P<0.001$), meaning that the risk of disease could be decreased by identifying patient's disease history, developing a comprehensive training program, more detailed care during pregnancy and medical treatments .

Treatment of venous thromboembolism patients in terms of FVL is similar to patients without a recognized thrombophilia. No long-term prophylaxis is needed unless the patient experiences thrombosis event more than once. Asymptomatic patients need no treatment, but the women with FVL mutation should be made aware of increased possibility of thrombosis during pregnancy and also of the fact that prophylaxis is necessary for these patients with high risk of thrombosis like surgery.² Presence of thrombosis in preeclampsia suggests that endothelial function contributes greatly to pathogenesis of thrombosis development in pre-eclampsia.²⁷

In the present study, the blood pressure of 142 pregnant women (control group) was measured after six months of follow-up and their blood pressure was 125/85 at the completion of the study. It is worth mentioning that no special intervention except routine standard treatments for eclampsia and preeclampsia based on scientific resources was adopted in this study. In a study in the USA, the administration of anticoagulants such as heparin and enoxaparin was the first choice for preventing pregnancy difficulties like stillbirth, IUGR, preeclampsia, and detached pair as they do not pass pair, decrease the risk of these diseases, and are not teratogenic. But, warfarin was better to be used post delivery because of the effects which it exerts on fetus.²⁸

In this study, prepregnancy BMI of case group was significantly higher than that of control group; preeclampsia incidence could be prevented through prepregnancy weight loss.

CONCLUSION

Preeclampsia/eclampsia is one of the most dangerous syndromes during pregnancy, being one of the members of fatal triads together with bleeding and infection, and is the reason for 16% of maternal mortalities. No significant difference was seen in deficiency of coagulation factors, Prs, Prc and ATIII between pregnant women with preeclampsia and healthy pregnant women in the studied region. No clear evidence of the association between above-mentioned factors and unpleasant pregnancy complications was found, so it did not

seem necessary to test the patients with preeclampsia for the rate of Prs, Prc and ATIII.

However, the study indicated a significant association between FVL deficiency and unpleasant pregnancy complications. FVL measurement was also shown to help to decrease the development of vascular disorders derived from FVL deficiency during pregnancy. Meanwhile, maternal mortalities could be notably reduced through appropriate training and formulation of interventional plans such as weight loss, etc. However, other coagulation factors, genetic traits and racial qualities of different populations should not be ignored and further research is needed to obtain more inclusive findings.

ACKNOWLEDGEMENTS

This study was obtained from Maryam Al-e-Rasul PhD thesis (No.1059). We gratefully thank to Research and Technology Deputy of Shahrekord University of Medical Sciences and all people who assisted in conducting this research.

CONFLICT OF INTEREST

All authors declare no conflict of interest.

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