Taiwanese Journal of Obstetrics & Gynecology 55 (2016) 60–63

Contents lists available at ScienceDirect

Taiwanese Journal of Obstetrics & Gynecology

journal homepage: www.tjog-online.com

Original Article

Levels of thrombin-activatable fibrinolysis inhibitor and platelet-activating factor in recurrent pregnancy loss patients

Ayla Eser [a,*], Ilknur Inegol Gumus [a], Husamettin Erdamar [b], Ikbal Kaygusuz [a], Melahat Yildirim [c], Betul Usluogullari [d], Muzeyyen Duran Erdolu [e], Serap Aynur Simavli [f], Ramazan Yigitoglu [b], Nilgun Ozturk Turhan [g]

[a] Department of Obstetrics and Gynecology, Turgut Ozal University Medical School, Ankara, Turkey
[b] Department of Biochemistry, Turgut Ozal University Medical School, Ankara, Turkey
[c] Department of Obstetrics and Gynecology, Ataturk Education and Research Hospital, Ankara, Turkey
[d] Department of Obstetrics and Gynecology, Cengiz Gokcek Hospital, Gaziantep, Turkey
[e] Department of Obstetrics and Gynecology, Sevket Yilmaz Education and Research Hospital, Bursa, Turkey
[f] Department of Obstetrics and Gynecology, Pamukkale University Medical School, Denizli, Turkey
[g] Department of Obstetrics and Gynecology, Sitki Kocman University Medical School, Mugla, Turkey

ARTICLE INFO

Article history:
Accepted 30 June 2015

Keywords:
abortion
habitual
carboxypeptidase B2
platelet activating factor
pregnancy

ABSTRACT

Objective: The aim of this study was to investigate factors associated with thrombosis that may contribute to recurrent pregnancy loss (habitual abortion), specifically differences in serum levels of platelet-activating factor and thrombin-activatable fibrinolysis inhibitor (carboxypeptidase B2) between women with a history of recurrent miscarriage and those with no recurrent miscarriage history.
Materials and methods: A case-controlled, prospective study design was adopted to compare women with a history of two or more first-trimester miscarriages ($n = 42$) with those with no history of recurrent miscarriage ($n = 36$). Participants were recruited from the Department of Obstetrics and Gynecology of Turgut Ozal University Hospital. Platelet-activating factor and thrombin-activatable fibrinolysis inhibitor levels in serum samples were measured by an enzyme-linked immunosorbent assay.
Results: Platelet-activating factor levels were significantly ($p = 0.018$) higher in the recurrent miscarriage group. There was no difference in levels of thrombin-activatable fibrinolysis inhibitor expression between the groups.
Conclusion: Platelet-activating factor is significantly higher in serum of patients with a history of recurrent miscarriage than in those without such a history, with potential implications for placental function and fetal growth, which could be relevant to miscarriage recurrence. Larger studies are indicated to further examine these findings.

Introduction

Loss of pregnancy in the first trimester is relatively common, occurring in 10–20% of clinically recognized pregnancies and in many more that are not yet clinically recognized [1–3]. Recurrent miscarriage (habitual abortion) is defined by the United Kingdom's Royal College of Obstetricians and Gynaecologists as three or more consecutive pregnancy losses [1]. Risk of subsequent miscarriage is estimated to be 30% after two pregnancy losses as opposed to 33% after three losses [2]. This suggests that an evaluation after two pregnancy losses is advisable in women who have not had previous live births, as recommended by the American College of Obstetricians and Gynecologists [4]. The incidence of recurrent miscarriage has been estimated at 0.5–3% among fertile couples of reproductive age [3]. However, while there are various possible causes, in more than 50% of cases no clear cause can be identified [1–3]. Among the accepted etiologies are parental chromosomal abnormalities, untreated hypothyroidism, diabetes, antiphospholipid antibody syndrome, and some congenital uterine abnormalities [2,5]. Other

* Corresponding author. Department of Obstetrics and Gynecology, School of Medicine, Turgut Ozal University, Ciftlik Cad. Number 57, Emek 06510, Ankara, Turkey.
E-mail address: aylaacar76@yahoo.com.tr (A. Eser).

http://dx.doi.org/10.1016/j.tjog.2015.06.013

suggested etiologies include endocrine disorders, immunological causes, or infections. Both inherited thrombophilias and acquired thrombophilias such as hyperhomocysteinemia, or activated protein C resistance have also been implicated [2,5].

One hypothesis put forward to explain recurrent miscarriage is that it is the result of exaggerated hemostatic responses owing to the existence of a prothrombotic state prior to pregnancy [6]. Normal embryonic implantation involves elements of the coagulation and fibrinolytic pathways. Thus, pregnancy in itself is associated with higher levels of procoagulants, lower levels of anticoagulants, and a reduced fibrinolytic activity with an increased risk of thrombosis [7]. A current theory suggests that defects in hemostatic mechanisms contribute to placental microthrombi and placentation defects in recurrent miscarriage [6–8]. However, the subject of whether an underlying prothrombotic state contributes to recurrent miscarriage is controversial. Some studies dispute this link and the use of prophylactic heparin during pregnancy in women with idiopathic recurrent miscarriage without any known inherited thrombophilia [9,10].

Evidence from other studies, on the other hand, supports the idea of an underlying prothrombotic state, at least in a subgroup of women with recurrent miscarriage. For example, increased tissue factor activity, procoagulant phospholipids, and levels of thrombomodulin, an activator of both protein C and thrombin-activatable fibrinolysis inhibitor (TAFI), has been shown in patients with two or more miscarriages compared with in women with normal pregnancies or nonpregnant women [8]. Other studies have shown increased platelet aggregation in response to arachidonic acid [6], increased levels of procoagulant microparticles derived from platelets and other cell types [7,11], increased thrombin levels or endogenous thrombin potential [12,13], and increased clot strength and stability measured by thromboelastography in women with unexplained recurrent miscarriage [14].

Some of the controversies surrounding this subject are caused by differences in techniques between studies and poor control of some patient studies in terms of inclusion criteria and definition of recurrent miscarriage [10]. Given the implication of platelet reactivity and activation factors in thrombosis and recurrent pregnancy loss, the authors aim to determine the levels of platelet-activating factor (PAF) and of TAFI in women who had suffered two or more unexplained first-trimester miscarriages without any diagnosed thrombotic disorder in a well-designed, case-controlled prospective study. PAF is associated with maintenance of healthy pregnancy [15], while TAFI is a procarboxypeptidase (carboxypeptidase B2) involved in fibrinolysis inhibition and contributes to thrombosis [16].

Materials and methods

This case-controlled, prospective study was carried out with women recruited from the Department of Obstetrics and Gynecology of Turgut Ozal University Hospital in Ankara/Turkey.

This study was approved by Fatih University Ethical Committee and it complied with the Helsinki Declaration. All women provided written informed consent prior to the start of the study.

Participants

A total of 78 nonpregnant women were recruited in this study. Exclusion criteria included smoking and use of hormonal medication such as oral contraceptive pills. The study group consisted of 42 Caucasian patients with a history of two or more pregnancy losses prior to 12 weeks of gestation and normal thrombophilia panel tests. Three participants of the study group had a history of preeclampsia and two had chronic hypertension. The control group consisted of 36 healthy Caucasian women matched for age (Table 1) who had no history of miscarriage or obstetric morbidity. No patient or control had any aspirin, steroid, or anticoagulant intake. Participants were recruited a minimum of 6 months after the last miscarriage event.

Sample preparation

Venous blood samples were collected after overnight fasting using a 21-gauge butterfly needle and placed in no-additive-containing tubes. The serum fraction was obtained by centrifugation (2000*g*, 10 minutes, 4°C) after storing the whole blood at room temperature (approximately 10 minutes). All samples were stored at −80°C prior to assays.

Measurement of PAF and TAFI levels

Enzyme-linked immunosorbent assay kits were used to measure the levels of PAF and TAFI according to the manufacturer's instructions (USCN Life Science Inc., Houston, TX, USA). All samples were analyzed in duplicate. Serum TAFI and PAF levels were presented as ng/mL and pg/mL, respectively.

Statistical analysis

All statistical analyses were performed using the SPSS 16.0 (SPSS Inc., Chicago, IL, USA) statistical package. Distributions were evaluated using one-sample Kolmogorov–Smirnov test. Student *t* and Mann–Whitney *U* tests were used for testing differences between groups. The results were expressed as mean ± standard deviation. Spearman rho correlation test was used to indicate relationships between variables. A probability level of $p < 0.05$ was considered statistically significant. Correlation between body mass index (BMI) and PAF levels was evaluated using Pearson correlation. The results were evaluated within 95% confidence interval, and $p < 0.05$ was accepted as the level of significance. Logistic regression analysis was performed to discriminate between contributions of BMI and PAF for recurrent abortus.

Results

Table 1 shows the biochemical and clinical characteristics of the study participants. The BMI was significantly higher in the recurrent miscarriage group than in controls ($p = 0.019$). The number of gravidity and abortions was also higher in the study group than in the control group ($p < 0.001$ for both), while the parity was higher in the study group ($p < 0.001$).

With respect to the mediators tested, serum PAF levels were significantly higher in the recurrent miscarriage group than in the control group ($p = 0.018$) (Table 1). No difference was detected in serum TAFI levels between groups (Table 1).

Given the significantly higher BMI in the study group compared with that in the control group (Table 1), a correlation analysis was carried out to determine if there was any correlation between BMI and PAF levels (Table 2). The analysis did not indicate any correlation ($p = 0.829$; Table 2). This indicated that BMI and PAF levels were independent of each other. When using BMI as a covariate in a logistic regression analysis, the association with recurrent miscarriage group was attenuated to $p = 0.105$ for PAF level association and $p = 0.998$ for BMI association. This suggests a higher contribution of PAF than BMI to recurrent miscarriage.

Table 1
Clinical and laboratory data of the study participants.

	Recurrent miscarriage ($n = 42$)	Control ($n = 36$)	p
Age	33.3 ± 7.9	32.7 ± 4.0	0.646
BMI	25.5 ± 3.5	22.8 ± 2.2	**0.019**
Gravidity (number of pregnancies)	4.0 (2.0)	3.0 (1.0)	**<0.001**
Parity (number of deliveries at ≥20 wk)	1 (1)	3 (1)	**<0.001**
Abortion (number of pregnancy losses at ≤20 wk)	2 (1)	0 (1)	**<0.001**
PAF (pg/mL)	23,022 ± 10,978	15,809 ± 10,773	**0.018**
TAFI (ng/mL)	27.4 ± 2.3	26.4 ± 4.0	0.257

A p value of <0.05 was considered statistically significant. Gravidity, parity, and abortion reported as medians (interquartile range) for pregnancy number, delivery number, and abortion number, respectively.
BMI = body mass index; PAF = platelet-activating factor; TAFI = thrombin-activatable fibrinolysis inhibitor.

Table 2
Analysis of correlation between PAF and BMI.

		PAF	BMI
PAF	Pearson correlation	1	−0.044
	Significance. (2-tailed)		0.829
	N	62	26
BMI	Pearson correlation	−0.044	1
	Sig. (2-tailed)	0.829	
	N	26	26

BMI = body mass index; PAF = platelet-activating factor.

Discussion

A pre-existing prothrombotic state is hypothesized to contribute to unexplained recurrent miscarriage in at least a subgroup of women, although the association is controversial [5–14]. In this context, we analyzed the expression of PAF and TAFI in serum of women with a history of two or more recurrent miscarriages compared with women with no miscarriage history. We found that levels of PAF but not TAFI were significantly higher in women with recurrent miscarriage.

Pregnancy in itself is a highly hypercoagulable state, making it difficult to distinguish between cause and effect when considering the contribution of prothrombotic factors in recurrent miscarriage. The lack of explanation for more than 50% of cases of recurrent pregnancy makes the need to investigate and define possible underlying causes all the more urgent.

PAF is a signaling phospholipid that is produced by numerous cell types and is involved in mediation of inflammation and allergy [15]. PAF is also associated with maintenance of healthy pregnancy and has been implicated in ovulation, implantation, and parturition in both humans and domestic animals [15,17]. It exerts its effects via a high-affinity receptor on the cell membrane of many cell types including platelets. While no previous study, to our knowledge, has implicated PAF in recurrent miscarriage, other studies have suggested that increased PAF activity may contribute to other complications of pregnancy. An elevated PAF level has been associated with fetal growth restriction in rats [18,19] and a decline in placental function [19]. By contrast, increased levels of PAF-acetyl hydrolase (PAF-AH), an enzyme that degrades PAF, have been observed in cord plasma from human fetuses with fetal growth restriction [20]. Alterations in distribution and activity of PAF-AH have also been observed in women with preeclampsia [21], and increased PAF-AH has been associated with reproductive disorders in dairy cows [22]. Gestational diabetes mellitus has also been associated with high levels of PAF-AH activity compared with women with healthy pregnancies, and within a gestational diabetes mellitus cohort, higher PAF-AH levels are associated with an increased risk of metabolic syndrome [23]. The observation of elevated levels of PAF in women with recurrent miscarriage warrants further analysis, especially in the context of rat studies showing effects of elevated PAF levels on fetal growth and placental function [18,19]. Studies should be extended to larger patient groups incorporating sufficient numbers to compare women with two or less miscarriages with those with three or more, for example. It would also be of interest to examine levels of PAF-AH in these patients as PAF-AH levels are vital in maintaining the balance between biosynthesis and degradation of PAF [15]. Increased PAF-AH expression or alterations in distribution could represent a compensatory mechanism aimed at controlling PAF levels.

Our study did not indicate any change in levels of TAFI in women with recurrent miscarriage. TAFI is a carboxypeptidase (carboxypeptidase B2) with a central role in the inhibition of fibrinolysis, which has been increasingly implicated in thrombosis [16]. TAFI is suggested to contribute to hypercoagulability in pregnancy, but its role in recurrent miscarriage is uncertain. Some studies suggest that it has no role [24,25], while others suggest that high levels of TAFI are protective against recurrent miscarriage and levels of TAFI are lower in women with recurrent miscarriage [16,26], and yet others suggest that high TAFI levels may contribute to recurrent miscarriage due to disordered fibrinolysis [27]. Our study does not support a role for altered TAFI levels in women with a history of two or more miscarriages. Again, studies with larger patient groups are indicated.

One limitation of our study was that BMI was significantly higher in women with recurrent miscarriage. The World Health Organization has defined BMI cutoff points for classification of individuals as underweight (<18.5 kg/m^2), healthy weight (18.5–24.9 kg/m^2), overweight (25.0–29.9 kg/m^2) and obese (≥30.0 kg/m^2) [28]. Our results indicate that the mean BMI for women suffering recurrent miscarriage is in the overweight range, as opposed to the control group whose mean BMI lies in the healthy weight range. This is consistent with the published evidence suggesting that overweight and obesity increase the risk of recurrent miscarriage [29,30]. Overweight and/or obesity are also associated with thrombotic complications in pregnancy such as venous thrombosis and thromboembolism [31,32]. A recent study in mice suggests that PAF may have an antiobesity function [33]. It remains to be seen whether this function is present in humans, and whether there could any connection between increased PAF levels and attempted compensatory antiobesity mechanisms in overweight or obese women with recurrent miscarriage. In our current study, correlation analysis did not indicate any correlation between PAF and BMI in our relatively small group of participants. However, some BMI effects may be present, as indicated by the attenuated significance of PAF level association on correction for BMI. Future studies with larger numbers of participants should focus on any possible relationship between PAF and BMI, particularly in light of the mice studies suggesting an antiobesity function for PAF [33].

In conclusion, we have observed significantly increased levels of PAF in serum samples from women who have suffered two or more recurrent miscarriages. This could have implications for placental function and fetal growth, and suggests that larger studies should be carried out to further investigate the role of elevated PAF level in recurrent miscarriage.

Conflicts of interest

The authors have no conflicts of interest relevant to this article.

Acknowledgments

Fatih University financially supported this research (grant no: 1337).

References

[1] Royal College of Obstetricians and Gynaecologists. The investigation and treatment of early miscarriage. Guideline No. 17. London, UK: RCOG Press; 2003.
[2] Ford HB, Schust DJ. Recurrent pregnancy loss: etiology, diagnosis and therapy. Rev Obstet Gynecol 2009;2:76–83.
[3] American College of Obstetricians and Gynecologists. Management of recurrent early pregnancy loss, ACOG practice bulletin number 24, February 2001. Int J Gynecol Obstet 2002;78:179–90.
[4] American College of Obstetricians and Gynecologists. Repeated miscarriage. Frequently asked questions FAQ100 pregnancy. Washington DC, US: ACOG; 2013.
[5] Kavalier F. Investigation of recurrent miscarriages. BMJ 2005;331:121–2.
[6] Flood K, Peace A, Kent E, Tedesco T, Dicker P, Geary M, et al. Platelet reactivity and pregnancy loss. AJOG 2010;203:281.e1–5.
[7] Greer IA. Procoagulant microparticles: new insights and opportunities in pregnancy loss? Thromb Haemost 2001;85:3–4.
[8] Van Dreden P, Woodhams B, Rousseau A, Favier M, Favier R. Comparative evaluation of tissue factor and thrombomodulin activity changes during normal and idiopathic early and late foetal loss: the cause of hypercoagulability? Thromb Res 2012;129:787–92.
[9] Bennett SA, Bagot CN, Appiah A, Johns J, Ross J, Roberts LN, et al. Women with unexplained recurrent pregnancy loss do not have evidence of an underlying prothrombotic state: experience with calibrated automated thrombography and rotational thromboelastometry. Thromb Res 2014;133:892–9.
[10] McNamee K, Dawood F, Farquharson RG. Thrombophilia and early pregnancy loss. Best practice & research. Clin Obstet Gynaecol 2012;26:91–102.
[11] Shetty S, Patil R, Ghosh K. Role of microparticles in recurrent miscarriages and other adverse pregnancies: a review. Eur J Obstet Gynecol Reprod Biol 2013;169:123–9.
[12] Vincent T, Rai R, Regan L, Cohen H. Increased thrombin generation in women with recurrent miscarriage. Lancet 1998;352:116.
[13] de Saint Martin L, Duchemin J, Bohec C, Couturaud F, Mottier D, Collet M, et al. Increased thrombin generation measured in the presence of thrombomodulin in women with early pregnancy loss. Fertil Steril 2011;95:1813–5. e1.
[14] Rai R, Tuddenham E, Backos M, Jivraj S, El'Gaddal S, Choy S, et al. Thromboelastography, whole-blood haemostasis and recurrent miscarriage. Hum Reprod 2003;18:2540–3.
[15] Tiemann U. The role of platelet-activating factor in the mammalian female reproductive tract. Reprod Domest Anim 2008;43:647–55.
[16] Legnani C, Bovara M, Valdrè L, Cosmi B, Caniato A, Palareti G. Risk of early recurrent fetal loss and levels of thrombin-activatable fibrinolysis inhibitor. Thromb Res 2012;130:237–41.
[17] Schaefer-Somi S, Sabitzer S, Klein D, Reinbacher E, Kanca H, Beceriklisoy HB, et al. Vascular endothelial (VEGF) and epithelial growth factor (EGF) as well as platelet-activating factor (PAF) and receptors are expressed in the early pregnant canine uterus. Reprod Domest Anim 2013;48:20–6.
[18] Neerhof MG, Khan S, Synowiec S, Qu X, Thaete LG. The significance of endothelin in platelet-activating factor-induced fetal growth restriction. Reprod Sci 2012;19:1175–80.
[19] Thaete LG, Neerhof MG, Jilling T, Caplan MS. Infusion of exogenous platelet-activating factor produces intrauterine growth restriction in the rat. J Soc Gynecol Investig 2003;10:145–50.
[20] Ohshige A, Yoshimura T, Maeda T, Ito M, Okamura H. Increased platelet-activating factor-acetylhydrolase activity in the umbilical venous plasma of growth-restricted fetuses. Obstet Gynecol 1999;93:180–3.
[21] Fan P, Liu X, He G, Zhang S, Zhang J, Bai H. Maternal and fetal plasma platelet-activating factor acetylhydrolase activity and distribution in pre-eclampsia. Pediatr Res 2012;72:426–31.
[22] Turk R, Juretić D, Geres D, Bacić G, Milesević M, Flegar-Mestrić Z, et al. Bovine platelet-activating factor acetylhydrolase (PAF-AH) activity related to fertility. Anim Reprod Sci 2008;105:344–53.
[23] Derbent A, Kargili A, Koca C, Gümüş İİ, Sevgili S, Simavli S, et al. Serum platelet-activating factor acetylhydrolase activity: relationship with metabolic syndrome in women with history of gestational diabetes mellitus. Gynecol Endocrinol 2011;27:128–33.
[24] Sezer SD, Baz A, Küçük M, Odabaşi AR, Serter M, Yüksel H. Thrombin activatable fibrinolysis inhibitor (TAFI) is not associated with recurrent miscarriage. Clin Exp Obstet Gynecol 2011;38:228–31.
[25] Folkeringa N, Korteweg FJ, Veeger NJGM, Middeldorp S, Hamulyak K, Prins MH, et al. Thrombin-activatable fibrinolysis inhibitor (TAFI) is not associated with fetal loss: a retrospective study. Thromb Res 2009;123:511–4.
[26] Knol HM, Veeger N, Middeldorp S, Hamulyak K, van der Meer J. High thrombin-activatable fibrinolysis inhibitor levels may protect against recurrent fetal loss. J Thromb Haemost 2009;7:903–6.
[27] Martínez-Zamora MA, Creus M, Tassies D, Bové A, Reverter JC, Carmona F, et al. Thrombin activatable fibrinolysis inhibitor and clot lysis time in women with recurrent miscarriage associated with the antiphospholipid syndrome. Fertil Steril 2010;94:2437–40.
[28] World Health Organization. Physical status: the use and interpretation of anthropometry: report of a WHO expert committee. Geneva, Switzerland: World Health Organization; 1998.
[29] Sugiura-Ogasawara M. Recurrent pregnancy loss and obesity. Best Pract Res Clin Obstet Gynaecol 2015;29(4):489–97.
[30] Metwally M, Saravelos SH, Ledger WL, Li TC. Body mass index and risk of miscarriage in women with recurrent miscarriage. Fertil Steril 2010;94:290–5.
[31] Jensen TB, Gerds TA, Grøn R, Bretler DM, Schmiegelow MD, Andersson C, et al. Risk factors for venous thromboembolism during pregnancy. Pharmacoepidemiol Drug Saf 2013;22:1283–91.
[32] Jacobsen AF, Skjeldestad FE, Sandset PM. Ante- and postnatal risk factors of venous thrombosis: a hospital-based case-control study. J Thromb Haemost 2008;6:905–12.
[33] Sugatani J, Sadamitsu S, Yamaguchi M, Yamazaki Y, Higa R, Hattori Y, et al. Antiobese function of platelet-activating factor: increased adiposity in platelet-activating factor receptor-deficient mice with age. FASEB J 2014;28:440–52.