Can protein Z deficiency predict pregnancy outcome?

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Abstract

Objecive: The objective of this was to determine if preeclampsia (PE), intrauterine growth restriction "IUGR"; or intra-uterine fetal death "IUFD" are associated with changes in maternal plasma concentrations of protein Z.

Methods: This is a prospective case control study which comprised of 130 pregnant women. They were divided into 4 groupd : Severe preeclampsia group (n=40), unexplained IUGR group (n=25), Unexplained IUFD group (n=25) and 40 women with normal pregnancy as a control group. Cross sectional maternal plasma protein Z concentrations were measured by a sensitive and specific immunoassay.

Results: There was a statistical significant difference regarding protein Z between control group and complicated pregnancies (p=0.047). Protein Z levels were significantly lower in severe preeclamptic patients was than that of the other study groups p<0.0001. However, there was no significant difference in the maternal plasma protein Z concentration between patients with unexplained IUGR or intrauterine fetal demise.

Conclusion: Preeclampsia is associated with significantly lower maternal median plasma concentration of protein Z than normal pregnancy and also a high rate of protein Z deficiency was observed in patients with IUGR and fetal demise.

Keywords: preeclampsia, intrauterine growth restriction, fetal demise, protein Z dependent protease inhibitor (ZPI).

Introduction

During pregnancy the alterations in the coagulation system are considered to be adaptive mechanisms for prevention of bleeding at the time of delivery [1-2]. Normal pregnancy is associated with excessive thrombin generation [3] and a tendency for platelets to aggregate [4]. Thrombosis, however, has been proposed as a mechanism of disease in preeclampsia (PE) [5, 6], intrauterine growth restriction (IUGR) [7-8], stillbirth, recurrent pregnancy losses [9], and preterm delivery [10-11]. There is good evidence to support this view including an excessive rate of thrombotic lesions in the placental villi and decidual vessels in patients with these pregnancy complications [12-13]; and higher maternal plasma concentrations of thrombin-anti-thrombin complexes in patients with PE, and small-for-gestational age neonates [14-15].

Protein Z is a single chain vitamin-K-dependent plasma glycoprotein formed in the liver and contributes to the inhibition of activated factor X (FXa), limiting thrombin generation and thus inhibiting coagulation [16]. Protein Z is a co-factor of the protein Z-dependent protease inhibitor (ZPI) [17]. In the absence of protein Z, the activity of ZPI is reduced by more than 1,000-fold, thus, protein Z deficiency has been associated with a procoagulant state [17, 18]. Therefore, protein Z deficiency has been reported with poor pregnancy outcome as it is incriminated in the pathophysiology of early fetal losses between the 8th and 15th weeks' gestation, as well as late pregnancy complications including PE, IUGR, IUFD, preterm delivery (19). Anti-protein Z antibodies have also been proposed as a possible underlying mechanism leading to low plasma PZ concentrations that brought the patient to the previous mentioned pregnancy related complications (20).
In this prospective cross sectional controlled study, we aimed to compare the level of maternal plasma protein Z in normal and abnormal pregnancy and correlate that with the pregnancy outcome.

Patients and methods

This prospective case control cross sectional study comprised of 130 patients among those attending he obstetric unit in Mansoura University Hospitals, a tertiary referral center, in the period from May 2009 –September 2011. The study group included patients with severe PE (N=40); patients who had fetuses with unexplained IUFGR and delivered neonates small for gestational age (N=25); patients with unexplained fetal demise (N=25); and gestational age matched women with a normal pregnancy and normal outcome (N=40) which served as a control group. An informed consent was taken from all patients

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before being included in the study and the protocol of the study was approved by Mansoura University ethics committee. We excluded from the study al patients with multiple pregnancies, those who have lupus anti-coagulant, vitamin K deficiency, vitamin K antagonist treatment, chronic essential hypertension or chronic renal disorders, fetuses with congenital defects and/ or chromosomal anomalies.

In this study, severe PE was defined as diastolic blood pressure ≥110 mmHg or systolic blood pressure ≥160 mmHg and/or proteinuria ≥3+ by dipstick. Unexplained IUGR was defined as ultrasonographic fetal biometry less than the fifth percentile gestational age in the absence of apparent etiology. This is proved postpartum by SGA when a birth weight is below the 10th percentile [21]. Birth weight percentiles for gestational age were classified in four groups as follows: 1) ≤5th percentile; 2) 5th-10th percentile; 3) 10th – 90th percentile; and 4) >90th percentile [21]. Unexplained fetal demise was defined as a fetal death occurring after 19 weeks of gestation and proved by ultrasound examination when the fetus is visible without cardiac activity.

All assays were done on the first diagnosis of the condition (PE, IUGR or IUFD) after 20 weeks of gestation [22]. Assays were done at a corresponding mean gestational age in the control group. All venous blood samples were collected into plastic tubes containing anhydrous salt of trisodium citrate anticoagulant solution (3.2 at a ratio of 9 volumes blood to one volume citrate). The samples were centrifuged at 1300g for ten minutes at 4°C and the plasma was used to perform the global coagulation tests and a part stored at -70°C until assay. Prothrmbin time was estimated according to, using Thrombol S Kit from Dade Behring (Germany) (23). Activated partial thromboplastin time (APTT) was estimated according to the method reported by Procter and Rapaport (24), using pathromtin SL Kit from Dade Behring (Germany). Fibrinogen assay was done according to the method of Clauss (25) using multifibrin-U Kit from Dade Behring (Germany). Concentrations of protein Z in maternal plasma were determined by sensitive and specific immunoassays obtained from Diagnostica Stago (Asnieres-sur-Seine, France). The protein Z immunoassay utilizes the quantitative sandwich enzyme immunoassay technique. Protein Z deficiency was defined as maternal plasma concentrations ≤5th percentile [22] of the normal pregnancy group (≤ 1.59μg/mL).

Statistical analysis:

The statistical package used was SPSS (statistical package for social science) version 12 (SPSS Inc., Chicago, IL USA). As protein Z plasma concentrations were not normally distributed; thus, Kruskal–Wallis and Mann–Whitney U tests were used for comparisons among groups. The Chi-square was used to compare categorical variables and p value < 0.05 was considered statistically significant.

Results

The study comprised of 130 patients in total. They were divided into control group of 40 normal pregnancies and 3 case groups of 90 patients according to pregnancy complications. Age of patients in both groups ranged from 18-38 years with no statistical significant difference between both groups (27.6 + 6.27 vs 29.43+ 5.07). There were no differences between both groups as regards gravidity, parity or previous pregnancy complications and the mean gestational age at the time of examination (Table 1).
Table (2) for protein z assay shows a statistical significant difference between control group and complicated pregnancies (p=0.047). Protein Z levels were significantly lower in severe preeclamptic patients was than that of the other study groups p<0.0001. However, there was no significant difference in the maternal plasma protein Z concentration between patients with unexplained IUGR or intrauterine fetal demise.

Table (1): Clinical characteristics of the study and control groups

	Normal pregnancy group (n=40)	Complicated pregnancy group (n=90)	p
Age (years)	27.6 + 6.27	29.43+ 5.07	0.60
Gravidity	1.93 + 1.31	2.07 + 1.33	0.09
Parity	0.93 + 1.01	0.5 + 0.89	0.07
Gestational age (weeks)	30.2 + 4.31	28.9 + 5.11	0.11

Table (2): Protein Z concentration in normal and complicated pregnancies

	Control group (n=40)	Complicated pregnancies (n=90)			p
Protein Z (μgm/ml)	2.3± 0.31 (1.4 -3.2)	1.85± 0.41 (03 -5.4)			0.047
		PE	**IUGR**	**IUFD**	
		1.6 ± 0.43 (0.3-3.3)	2.3 ± 0.62 (0.3-3.8)	2.85 ± 0.21 (0.3-5.4)	< 0.0001

Discussion

There are inconsistent reports regarding the changes in plasma concentrations of protein Z in women with PE. While some authors have reported that there is no significant difference in the median plasma concentrations of protein Z between patients with PE and women with normal pregnancy, [20] others have reported that the median plasma protein Z concentrations are significantly lower in women with PE, SGA, and preterm delivery than those with normal pregnancies [26, 27]. However, the authors did not analyze each complication independently; therefore, the association between PE and changes in protein Z plasma concentration is not clear [27].

Normal pregnancy is characterized by an increased plasma concentration of protein Z, which has been proposed to be part of a compensatory mechanism for the increased concentration of factor X and perhaps for the increased thrombin generation [26]. Preeclampsia is associated with an exaggerated hypercoagulable state and excessive thrombin generation, [27] as determined by higher maternal plasma concentrations of TAT complexes [28-30] and lower antithrombin III concentrations [31, 32] than patients with a normal pregnancies. Moreover, patients with PE who delivered preterm have a higher rate of thrombotic lesions in the decidua and in the placental villi [12] than normotensive patients with indicated or spontaneous preterm delivery [10, 12]. Therefore, it is possible that an exaggerated procoagulant state may account for the lower plasma concentration of protein Z among women with preclampsia.

In this study, there was a significantly lower level of protein Z in women with PE than in women with normal pregnancy outcome (OR 22.65, 95% CI 6.79-116.82). In a previous report, [18] the rate of protein Z deficiency defined as the 10th percentile of the normal population in women with PE was not significantly different from the rate observed in women with normal pregnancy. In contrast, we found that 25% = 10/40 of the patients with PE

had protein Z deficiency; this difference may be attributed to the relatively larger sample size of patients with PE included in our study, different definition of protein Z deficiency (<5th percentile of the normal pregnant population) and differences in the study population. Protein Z deficiency has been reported in non-pregnant women [22] as well, suggesting that in some of the patients protein Z deficiency may precede the clinical presentation of PE, and a low maternal plasma concentration of protein Z can be a risk factor for the subsequent development of PE in a subset of patients. There were no significant differences between the median maternal protein Z plasma concentrations of patients who delivered an SGA neonate or had fetal demise in comparison to women with normal pregnancy; these results are consistent with a previous report by Bretelle et al. [18]. In contrast, a recent study reported that patients with adverse pregnancy outcome, including PE, SGA, recurrent unexplained vaginal bleeding, and preterm parturition, had lower mean plasma concentrations of protein Z than patients with normal pregnancy outcome in all three trimesters [27]. In our study we observed low protein Z level in 40% = 10/25 cases of IUGR.

The finding that women with fetal demise have a higher rate of protein Z deficiency than women with normal pregnancy is in harmony with Bretelle et al. report [18]. Of interest, the rates of protein Z deficiency that were observed in women with normal pregnancy (2.5%) and those with fetal demise (40%) in the current study, are similar to those reported in non-pregnant women with normal obstetric history, and with a history of previous fetal loss between 10-15 weeks gestation [33]. The similarity in the rate of protein Z deficiency between pregnant and non-pregnant women in both groups (those who had a normal pregnancy and those with fetal demise) suggests that a subset of women in the latter group might have a predisposing protein Z deficiency.

Moreover, we have proposed that pregnancy could be considered as a stress test to the hemostatic system [34]. Thus, the physiologic hypercoagulable state that accompanies pregnancy may facilitate the occurrence of thrombotic events of the placenta and adverse pregnancy outcome (i.e. fetal demise) in potentially thrombophilic patients that were clinically "silent" in the non-pregnant state [34]. In addition, Gris et al [22] reported that six out of eight patients with protein Z deficiency had one parent who is also protein Z deficient; [22] thus, the possibility that in some cases protein Z deficiency may be inherited. The process which is not searched in our study and needs further genetic investigations.

The results of this study indicate that PE, IUGR and fetal demise are associated with maternal protein Z deficiency; however, only patients with PE have a lower median maternal plasma concentration of protein Z that may be secondary to a higher activation of the coagulation system in patients with this pregnancy complication.

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