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Low Levels of Protein Z Are Associated With HELLP Syndrome and its Severity

Isik Kaygusuz, MD,[1] Tulin Firatli-Tuglular, MD,[1] Tayfur Toptas, MD,[1] Vedat Ugurel, MD,[2] and Muzaffer Demir, MD[3]

Abstract

Protein Z (PZ) was found to be associated with pregnancy complications. There are no data implying an association between hemolysis (H), elevated liver enzymes (EL), and low platelet counts (LP) (HELLP) syndrome and changes in plasma levels of PZ. The aim of this study is to investigate whether HELLP syndrome is associated with plasma concentrations of PZ. Protein Z levels in 29 women with HELLP syndrome were compared with 29 healthy, nulliparous and 25 normal pregnant women. The median PZ levels in patients with HELLP syndrome were found to be significantly lower than those of pregnant women. No significant difference was found between HELLP and healthy groups. Protein Z levels correlated with platelet counts, lactate dehydrogenase (LDH), and aspartate aminotransferase (AST) levels in patients with HELLP syndrome. Median PZ level was higher in partial HELLP than in complete HELLP. We calculated 1330 ng/mL as a cutoff value for PZ level to discriminate HELLP syndrome from normal pregnancy. Low PZ levels are associated with the pathobiology of HELLP syndrome.

Keywords

protein Z, HELLP syndrome, pregnancy, consumptive coagulopathy, preeclampsia, fetal demise

Introduction

Pregnancy is an acquired hypercoagulable state. Several factors contribute to hypercoagulability during pregnancy. Compression of enlarged uterus to inferior vena cava causes venous stasis. Synthesis of coagulation factors II (FII), FVII, FVIII, FX, FXII and generation of thrombin increase. Concentrations of natural anticoagulant factors and fibrinolytic system components also change during pregnancy. Levels of antithrombin III and protein C remain same with an increased resistance of activated protein C. Tissue factor pathway inhibitor changes minimally and protein S, plasminogen activator inhibitor 1 and 2 levels decrease.[1,2]

The association between preeclampsia and hemolysis (H), elevated liver enzymes (EL), and low platelet counts (LP) (HELLP) syndrome is still controversial. HELLP syndrome that has been defined as a severe form of preeclampsia by some authors can be seen with or without preceding preeclampsia. It complicates 0.2% to 0.6% and 2% to 12% of all pregnancies and pregnancies with severe preeclampsia, respectively.[3,4] As many as 15% to 20% of patients with HELLP syndrome do not have antecedent preeclampsia. Perinatal mortality is about 7% to 20%.[5] One of the suggested hypotheses about the development of HELLP syndrome is impairment of uteroplacental circulation and subsequent placental hypoxia caused by endothelial injury, platelet activation, coagulopathy, and fibrin deposition in blood vessels.[6] Increased fibronectin, decreased antithrombin, protein C and S levels, increased platelet activation and aggregation by β-thromboglobulin, and decreased platelet survival are contributory factors for coagulopathy in preeclampsia and HELLP syndrome.[7-10]

Human protein Z (PZ) was first isolated in 1984.[11] PZ is a vitamin K-dependent plasma glycoprotein. It is homologous to the blood coagulation factors FVII, FIX, FX, and protein C. The coagulation factors are generally found in circulation in an inactive state. They are activated through proteolysis by an upstream factor. However, unlike other coagulation proteins, PZ lacks the histidine and serine residues of the catalytic triad and is therefore not a zymogen of a serine protease. Thus, it does not have a proteolytic activity.[12,13] Protein Z that is a cofactor circulates as a complex with PZ-dependent protease inhibitor (ZPI). In the presence of PZ, phospholipids, and calcium, ZPI rapidly inhibits FXa. In the absence of cofactors, ZPI also inhibits FXIa.[14,15] Protein Z-dependent protease inhibitor activity decreases during coagulation through proteolysis mediated by FXa with PZ and FXIa.[16] Warfarin reduces both the PZ antigen

[1] Division of Hematology, Department of Internal Medicine, Marmara University Hospital, Istanbul, Turkey
[2] Kartal Lutfi Kirdar Training and Research Hospital, Clinic of Gynecology and Obstetrics, Istanbul, Turkey
[3] Division of Hematology, Department of Internal Medicine, Trakya University School of Medicine, Edirne, Turkey

Corresponding Author:
Isik Kaygusuz, Altaycesme Mh. Begonya Sk. Yasam Kent Sitesi Mercan Ap. No: 16/2, 81530 Maltepe, Istanbul, Turkey.
Email: isikkaygusuz@yahoo.com

level and its degree of L-carboxylation much more than other vitamin K-dependent factors.[17]

Protein Z deficiency is associated with prothrombotic events. It increases mortality in mice with factor V Leiden mutation, suggesting that PZ deficiency may be a risk factor for thrombotic disease in humans.[18] Protein Z is associated with thromboembolic events involving both arterial and venous vasculature in brain, mesenteries, eyes, and limbs.[19-24] The procoagulant effect of PZ is implied in Behçet disease in which arterial and venous thrombosis is in part due to vasculitic process.[25] Protein Z levels were found to be lower in patients with preeclampsia than in healthy pregnants in a previous study.[26]

We conducted this cross-sectional study to test the hypothesis that PZ would be associated with the pathogenesis and the severity of HELLP syndrome, and a cutoff level of PZ might be helpful for the prediction of disease and its severity.

Design and Methods

Patients and Controls

Between June 2005 and September 2008, 29 women with HELLP syndrome and 25 pregnant women seen at Marmara University Hospital, Istanbul; Trakya University, Medical Faculty Hospital, Edirne; and Kartal Lutfi Kirdar Training and Research Hospital, Istanbul, were consecutively enrolled into the study. Protein Z levels in patients and healthy pregnant women were compared with 29 age- and sex-matched nonpregnant women. The study was approved by the Marmara University Hospital Institutional Review Board. Informed consent was obtained from all participants in accordance with the Declaration of Helsinki. Clinical data, including patient characteristics, and blood samples were collected at the time of study enrollment. Women with a history of vitamin K deficiency, recent infection, thrombosis, and current anticoagulant use including vitamin K antagonists were planned to be excluded, but none of the study participants fulfilled the exclusion criteria.

Assessment of PZ Levels

Blood samples were drawn using vacutainer containing 0.109 mol/L sodium citrate immediately after delivery in all pregnant women, centrifuged at 2500*g* for 10 minutes and stored at −80°C until used. Protein Z levels were assessed by a commercial enzyme-linked immunosorbent assay kit (Asserachrom Protein Z, Diagnostica Stago and Serbio, Asnieres-sur-Saine, France) using methods previously described.[27]

Definition of HELLP Syndrome and Its Severity

HELLP syndrome was diagnosed according to "The Tenessee Classification." "True or complete" HELLP syndrome was defined if all criteria were met: (1) thrombocytopenia with platelets 100 000/μL or less, (2) hepatic dysfunction with aspartate aminotransferase (AST) 70 IU/L or greater, and (3) evidence of hemolysis on peripheral blood smear with lactate dehydrogenase (LDH) 600 IU/L or greater. Patients who exhibited some but not all these criteria were termed as "partial or incomplete" HELLP syndrome: ELLP syndrome (severe preeclampsia, elevated liver enzymes, and thrombocytopenia), EL syndrome (severe preeclampsia with only mildly elevated liver enzymes), HEL syndrome (severe preeclampsia with hemolysis and elevated liver enzymes), and LP syndrome (severe preeclampsia with only thrombocytopenia. Fetal demise was defined as fetal death by 19th week or after.[5,28]

Statistical Analysis

Values were presented as mean ± SD or median and range. Protein Z levels in all groups were not normally distributed. Differences between groups were evaluated by Mann-Whitney *U* and Kruskal-Wallis tests for skewed variables. Fisher exact test was used to compare categorical variables. When the result of Kruskal-Wallis test was significant, Mann-Whitney *U* test with Bonferroni correction was used as a post hoc test. Relationships between skewed variables were explored using Spearman rank correlation coefficient. Receiver operating characteristic (ROC) curves were used to analyze PZ levels to determine a cutoff point that yielded highest combined sensitivity, specificity, and area under the ROC curve estimate for predicting HELLP syndrome, clinical severity, and fetal outcome. All tests were 2 sided. Statistical significance was defined as $P < .05$. All statistical analyses were performed with the use of SPSS (Statistical Package for Social Sciences Inc, Chicago, Illinois) software for Windows version 15.0.

Results

The median age was 29 (range: 22-40), 29 (range: 21-40), and 28 (range: 22-47) years for nonpregnant, pregnant, and HELLP groups, respectively ($P = .893$). Median parity, gravidity, and mean gestational age in patients with HELLP syndrome were 2 (range: 0-9), 3 (range: 1-10), and 32.62 ± 3.95 weeks, respectively; 41.38% (12/29) of patients with HELLP had normal vaginal delivery. Fetal mortality in pregnancies with HELLP syndrome was about 17.24% (5/29); 83% (24/29) of patients with HELLP syndrome had complete syndrome.

Median PZ level in patients with HELLP syndrome was found to be significantly lower than that of pregnant women ($P < .001$). Protein Z levels were higher in pregnant women than those in nonpregnant women ($P < .001$). However, no difference was observed between HELLP and nonpregnant groups ($P = .597$; Figure 1).

Despite comparable levels of PZ in patients with HELLP syndrome and nonpregnant women, we found that PZ levels correlated with disease severity in HELLP syndrome. Although a positive correlation exists between PZ levels and platelet counts ($r_s = .498$, $P = .006$), PZ levels correlated negatively with LDH ($r_s = -.458$, $P = .012$) and AST levels ($r_s = -.374$, $P = .046$) (Figure 2A-C). The median PZ level was higher in partial than in complete HELLP syndrome (1334 [range: 600-2220] ng/mL vs 550 [range: 423-750] ng/mL, respectively, $P < .001$). Women with fetal demise had a lower

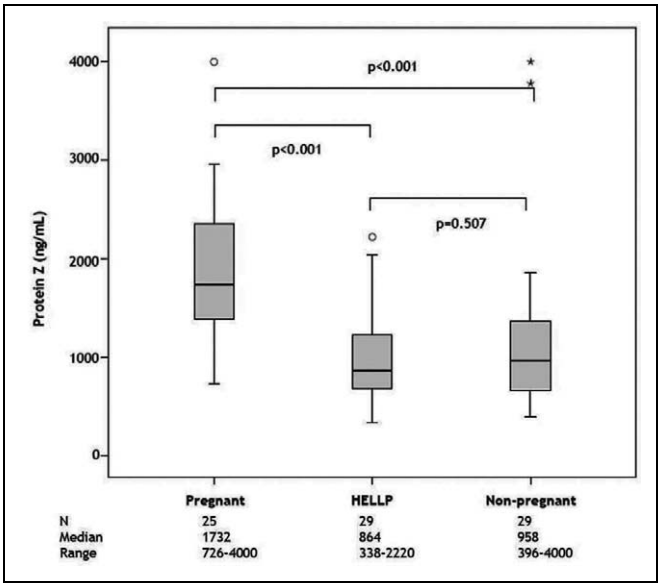

Figure 1. Median protein Z (PZ) concentrations in patients with HELLP syndrome, pregnant women, and healthy nonpregnant women.

median PZ concentrations than those with healthy fetus in HELLP syndrome (798 [range: 338-1287] ng/mL vs 1430 [range: 423-2220] ng/mL, respectively, $P = .03$).

There was no statistically significant correlation between PZ levels and maternal age ($r_s = .184$, $P = .339$), postconceptional week ($r_s = .115$, $P = .575$), gravidity ($r_s = -.019$, $P = .927$), parity ($r_s = .048$, $P = .803$), and alanine transaminase (ALT) levels ($r_s = -.270$, $P = .157$). Protein Z levels were comparable in patients with HELLP, who had vaginal delivery and cesarean section (1054 [range: 338-2220] ng/mL vs 1170 [range: 423-2040] ng/mL, respectively, $P = .842$).

We calculated 1330 ng/mL as a cutoff value for PZ to diagnose and discriminate HELLP syndrome from normal pregnancy. Estimates of ROC curve for this threshold were 0.79 for sensitivity and 0.76 for specificity with an area under the curve of 0.83 ($P = .0001$). Six of 25 healthy pregnants and 23 of 29 women with HELLP syndrome had a PZ value of less than 1330 ng/mL (odds ratio [OR]: 12.14, confidence interval [CI]: 3.36-43.85, $P = .0001$; Figure 3). However, PZ levels under this cutoff point did not correctly discriminate pregnancies with fetal demise from those with healthy fetus in HELLP syndrome (80% [4 of 5] vs 79% [19of 24], respectively, $P = 1.00$]. However, 88% (21 of 24) of patients with complete and 40% (2 of 5) of those with partial HELLP syndrome had a PZ concentration less than this level ($P = .046$). However, discriminative power of any PZ value for complete versus partial HELLP syndrome was not statistically significant with an area under the curve of 0.52 ($P = .91$).

Discussion

In normal pregnancy, PZ levels increase through the gestational weeks and correlate positively with gestational age. At

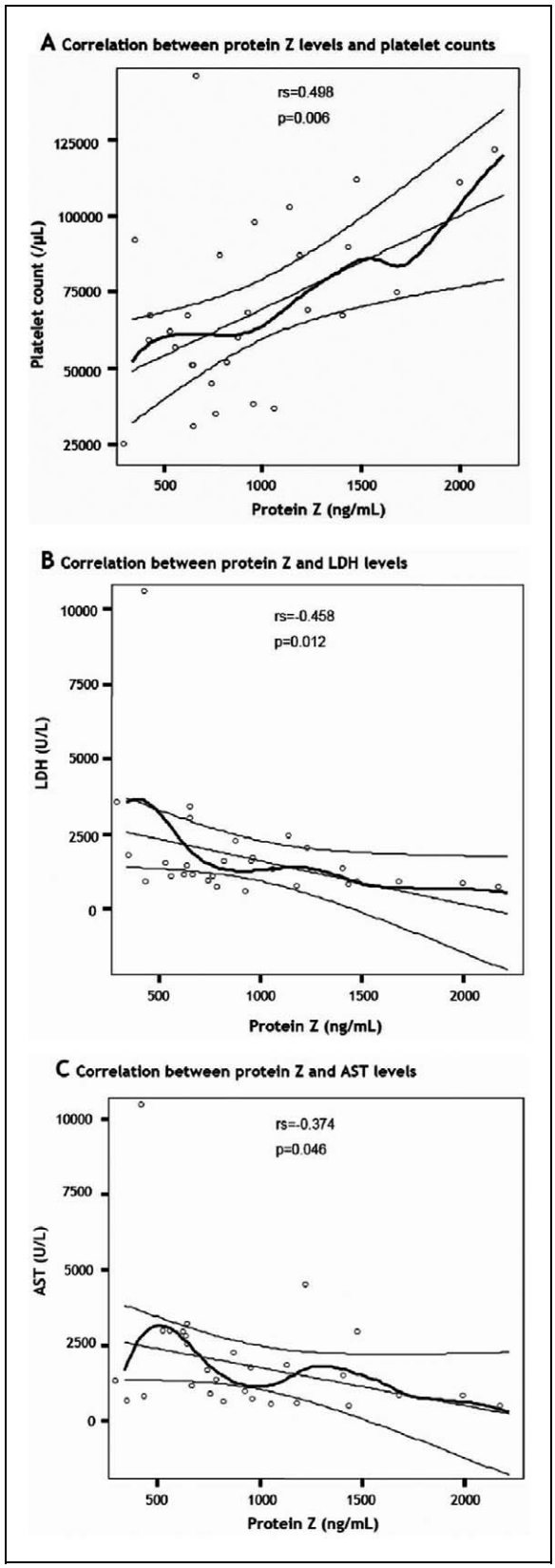

Figure 2. A-C, Correlations between protein Z, platelet counts, lactate dehydrogenase (LDH), and aspartate aminotransferase (AST) levels.

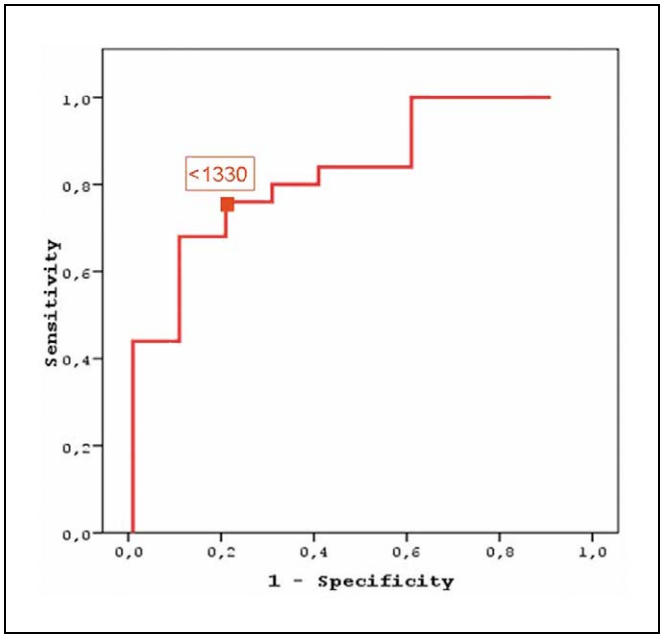

Figure 3. Receiver operating characteristic (ROC) curve estimate for <1330 ng/mL cutoff point of protein Z in discriminating patients with HELLP syndrome from women with normal pregnancy.

the time of delivery, PZ concentrations are almost 20% higher than those in the first trimester. However, those decrease 30% from delivery concentrations at the sixth week of postpartum period, even below the levels of first trimester. Because pregnancy is an acquired hypercoagulable state and hypercoagulability is partially associated with the increment in certain coagulation factors, PZ levels in pregnancy increases in a compensatory manner against the increased levels of FXa and thrombin to limit coagulum formation in case of a possible activation of coagulation cascade.[27] The major physiological function of ZPI is to attenuate the coagulation response prior to the formation of the prothrombinase complex. Protein Z is a cofactor of ZPI, and ZPI inhibits FXa in a calcium-dependent manner.[14] In the presence of PZ and prothrombin, thrombin generation was significantly delayed and the peak thrombin concentration was reduced >50%.[18]

Individuals with disseminated intravascular coagulation (DIC) and liver disease have low plasma levels of PZ.[5,29] Low levels of PZ in HELLP syndrome is probably due to low-grade consumptive coagulopathy. An endothelial injury specifically involving placenta and liver occurs as a result of immunologic mechanisms, enzymatic inborn errors of fatty acid metabolism, or acute inflammatory conditions in HELLP syndrome.[6] This endothelial damage leads to breakdown of endothelial cells and exposes blood to collagen and tissue factor beneath the endothelial cells. Subsequent activation, adhesion, and aggregation of platelets occur as a result of platelet and collagen interaction. Tissue factor activates coagulation cascade through forming a complex with FVII. This complex activates FXa and results in generation of thrombin, which is the most crucial feature of coagulation pathway.[14] Factor Xa (in the presence of PZ) is responsible for the consumption of ZPI with tissue factor–induced coagulation.[17]

Indeed, pathogenesis of low PZ levels in HELLP syndrome is probably multifactorial. Protein Z is synthesized mainly in liver and in a lesser degree in endothelial cells. Low PZ levels were found to be associated with liver diseases. Because the main targets of HELLP syndrome are liver and endothelial cells, it is likely that liver and endothelial dysfunction may impair the synthesis of PZ in HELLP syndrome.[29,30] Because trophoblast invasion exposes maternal immune system to fetus, HELLP syndrome can be considered an acute maternal immune rejection of the genetically foreign fetus.[31] HELLP has been described as a placenta-provoked, liver-targeted systemic inflammatory response syndrome (SIRS)-like inflammatory form of severe preeclampsia. The liver occupies a central role in the HELLP syndrome, and a major pathogenic mechanism for liver disease is CD95 (Fas)-mediated apoptosis of hepatocytes.[28] Moreover, PZ was defined as a negative acute phase reactant and relationship between certain inflammatory cytokines and PZ levels was demonstrated. Possible role of acute inflammatory conditions on pathogenesis of HELLP syndrome may also have an effect on PZ levels.[28,32] As we did not evaluate whether these factors have an impact on PZ levels, it is not possible to predict the magnitude of these effects on low PZ levels, which was found in our study. However, we think that some of these factors may only have a minor impact on low PZ levels in HELLP syndrome. HELLP syndrome is generally not accompanied by acute liver failure, although it is not a strict rule.[33] Coagulopathy that is seen in HELLP syndrome is mostly due to DIC rather than acute liver failure.[3]

Platelet counts, LDH, and AST levels are laboratory markers, which are used to diagnose HELLP syndrome and to predict disease severity. Perinatal and maternal outcomes are worse in complete HELLP syndrome than those in partial HELLP syndrome.[28] We found a correlation between PZ levels and these markers and showed that patients with complete HELLP syndrome had lower PZ levels than those with incomplete HELLP syndrome in our study. In women, PZ deficiency may induce an enhanced risk of severe placental insufficiency soon after the connection of maternal and fetal circulations.[34-36] In a previous study, PZ levels in pregnancies with fetal demise were found to be lower than those with healthy fetus.[26] Consistently, our study also support this findings. Because perinatal and maternal mortality is higher in more severe disease, suggested pathogenetic mechanisms for low PZ levels in HELLP syndrome would be expected to be more severe. Moreover, fetal demise may further exaggerate coagulopathy through the release of tissue factor, hepatic and endothelial dysfunction, inflammation, and immune responses.

Our data would suggest that a cutoff value for PZ levels of less than 1330 ng/mL would have correctly identified 23 of 29 (79%) patients with HELLP syndrome. Only 6 of 25 (24%) healthy pregnants had PZ levels lower than this cutoff point. This cutoff level may be useful for the prediction of pregnancies with HELLP syndrome. It, however, does not discriminate pregnancies with fetal demise from those with healthy fetus

and has a statistically nonsignificant discriminative power for differing complete from partial HELLP syndrome. Because more pregnants with complete HELLP syndrome have a PZ value lower than 1330 ng/mL, it will also be helpful for the prediction of disease severity.

Protein Z is relatively abundant in humans, with a wide plasma concentration range.[15] In our study, PZ levels in healthy pregnant women were found to be lower than those of previous studies.[26,27] This may be due to the use of different assay kits in each study. Moreover, ethnic and genetic diversities, maternal age, and conceptual date of blood sampling might also be an explanation for the different median PZ levels in various studies. However, our study population is homogenous for the mentioned factors. Despite differences between median PZ levels, results are similar showing healthy pregnants has higher PZ concentrations.

In conclusion, this cross-sectional study demonstrates that patients with HELLP syndrome have a lower PZ levels than healthy pregnants do. Biochemical and clinical indicators of severe disease are associated with lower PZ levels. A cutoff value of 1330 ng/mL might be helpful for the prediction of HELLP syndrome and its severity. These data are consistent with a role for PZ in the pathobiology of HELLP syndrome.

Authors' Note

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Declaration of Conflicting Interest

The authors declared no conflicts of interest with respect to the authorship and/or publication of this article.

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