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Pyelonephritis during pregnancy: A cause for an acquired deficiency of protein Z

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Abstract

Objective—Pyelonephritis has a more severe course during pregnancy than in the non-pregnant state. This has been attributed to the increased susceptibility of pregnant women to microbial products. An acquired protein Z deficiency has been reported when there is excessive thrombin activity. The aim of this study was to determine whether pyelonephritis during pregnancy is associated with changes in maternal plasma protein Z concentrations.

Study Design—A cross-sectional study was conducted to compare plasma protein Z concentrations between normal pregnant women (n=71) and pregnant women with pyelonephritis (n=42). Protein Z concentrations were measured by ELISA. Parametric and non-parametric statistics were used for analysis.

Results—Patients with pyelonephritis had a significantly lower median plasma concentration of protein Z than did patients with normal pregnancies [median 2.14 μg/mL (0.4-3.4) vs. median 2.36 μg/mL (1.09-3.36); p=0.03]. There was no difference in the median plasma concentration of anti-protein Z antibodies between patients with pyelonephritis and those with normal pregnancies.

Conclusion—The median maternal plasma protein Z concentration was significantly lower in patients with pyelonephritis during pregnancy than in patients with normal pregnancies.

Keywords

bacteremia; inflammation; pregnancy; Factor X; coagulation

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Introduction

Pyelonephritis is a common complication of pregnancy, affecting 1-2% of pregnancies and is a frequent cause of antepartum hospitalization[1,2]. Pyelonephritis during pregnancy generally has a good prognosis[1,2]; however, it can be associated with severe maternal morbidity such as Gram-negative sepsis[3-6], renal failure[7], and acute respiratory distress syndrome[8-12].

Infection can induce a systemic inflammatory response syndrome (SIRS) and activation of the coagulation cascade[13-16] leading to multi-organ damage and failure. During systemic inflammation activated monocytes express tissue factor on their membrane[17-21]; and higher tissue factor concentrations in plasma[22] and bronchoalveolar lavage[23] have been associated with the pathophysiology of acute lung injury complicating sepsis. The generation of thrombin leads to amplification of inflammatory processes[24-30] which are, at least partially, mediated through the thrombin receptor, also known as protease-activated receptor (PAR). This receptor can be activated also by the complex of tissue factor, activated factor VII (FVIIa), and activated factor X (FXa[31-35]. Activation of PAR receptors leads to the synthesis and secretion of inflammatory mediators [e.g. interleukin (IL)-6 and IL-8] by monocyte and endothelial cells[36-45].

Exposure of tissue factor due to tissue damage leads to the formation of a complex between tissue factor and activated factor VII that activates factor X[19,46-48]. Activated factor Xa generates thrombin from prothrombin, and thrombin cleaves fibrinogen into fibrin and activates platelets, leading to clot formation (Figure 1a-1b)[46,48-51].

The coagulation cascade is tightly regulated, and the activation of prothrombin by factor Xa is inhibited by antithrombin III, tissue factor pathway inhibitor [50] and by the protein Z/protein Z-dependent protease inhibitor (ZPI) complex (Figure 1c)[52-54]. Protein Z is a vitamin-K-dependent plasma glycoprotein[55] that is an essential cofactor for ZPI activity. In the absence of protein Z, the activity of ZPI is reduced by more than 1,000-fold[54]. Patients with a factor V Leiden mutation and low protein Z plasma concentration have a higher risk for thrombosis than those with only a factor V Leiden mutation[56,57]. Moreover, low plasma protein Z concentrations have been associated with acute coronary syndrome[58], ischemic stroke[59] and central retinal vein or artery occlusion[60]; it has been proposed that protein Z deficiency is associated with a prothrombotic state[58].

Low plasma protein Z concentrations have been reported in women with adverse pregnancy outcomes such as unexplained fetal loss[61], intrauterine growth restriction[62,63], vaginal bleeding[63], preeclampsia[63] and fetal demise[62]. Moreover, women with complicated pregnancies (preeclampsia, intrauterine growth restriction, and fetal demise) have higher rates of low maternal plasma protein Z concentrations than patients with inherited thrombophilias (i.e. protein C deficiency, factor V Leiden mutations) and antiphospholipid antibodies[62].

The aims of this study were to: 1) determine whether there are changes in maternal plasma concentrations of protein Z during an acute systemic inflammatory state (pyelonephritis); and 2) examine whether there is an association between low maternal plasma concentration of protein Z and pyelonephritis with associated complications.

Material and methods

A cross-sectional study was conducted in patients with 1) acute pyelonephritis (n=42) and 2) uncomplicated pregnancies (n=71). Patients with multiple pregnancies and with fetuses with congenital and chromosomal anomalies were excluded.

The study was conducted at the Detroit Medical Center/Wayne State University in Detroit, Michigan. The utilization of samples for research purposes was approved by the Institutional Review boards of both the National Institute of Child Health and Human Development (Bethesda, MD) and Wayne State University (Detroit, MI). Patients provided written informed consent prior to enrollment in the study.

Clinical definitions

Pyelonephritis was diagnosed in the presence of the following criteria: 1) fever (temperature ≥38°C); 2) clinical signs of an upper urinary tract infection (e.g., back pain); 3) pyuria; and 4) a urine culture positive for microorganisms. Blood cultures were also performed in a subset of patients at the discretion of the treating physician. The control group consisted of pregnant women between 20 to 42 weeks of gestation who were not in labor and had no medical or obstetrical complications. Both groups were matched for gestational age. A small for gestational age (SGA) neonate was defined as having a birthweight below the 10th percentile[64].

Blood samples collection

All blood samples were collected with a vacutainer into 0.109M (3.2%) trisodium citrate anticoagulant solution (BD, San Jose, CA). The samples were centrifuged at *1300g* for 10 minutes at 4°C and stored at -70°C until assay.

Human Protein Z immunoassays—Concentrations of protein Z in maternal plasma were determined by sensitive and specific immunoassays obtained from Diagnostica Stago (Asnieres-sur-Seine, France). The assay was conducted according to the manufacturer's recommendations. The calculated inter- and intra-assay coefficients of variation (CVs) were 3.1% and 2.4%, respectively. The sensitivity of the protein Z immunoassay in our laboratory was 0.05μg/ml.

Measurements of anti-protein Z IgG and IgM isotypes—Immunoassays to quantify anti-protein Z IgG and IgM isotypes were obtained from HYPHEN BioMed (Neuville-sur-Oise, France). The assays were conducted according to the manufacturer's recommendations. The concentrations of anti-protein Z IgG or IgM in samples were determined by interpolation from individual standard curves composed of purified human anti-protein Z IgG or IgM (calibrators). The calculated inter- and intra-assay CVs for anti-protein Z IgG isotype immunoassay in our laboratory were 6% and 5.4%, respectively. The detection limit (sensitivity) for the anti-protein Z IgG isotype immunoassay was 1.11 arbitrary units (AU)/ml. The calculated inter-and intra-assay CVs for anti-protein Z IgM isotype immunoassay were 7%, and 2.2%, respectively. The sensitivity for the anti-protein Z IgM isotype immunoassay was 2.02 AU/ml.

Statistical Analysis

The Shapiro-Wilk test was used to assess normality. A Mann-Whitney U test was used to compare medians, t-test was used to compare means, and chi-square test and Fisher exact test were used to compare proportions. A p value <0.05 was considered statistically significant. Statistical analyses were preformed using SPSS, version 12 (SPSS Inc., Chicago, IL, USA).

Results

Demographic and clinical characteristics of the study groups are presented in Table I. The mean birth weight was significantly lower in the pyelonephritis group than in the control

group (3086 ± 584 g vs. 3340 ± 392 g, respectively, p=0.02). The frequency of SGA in the pyelonephritis group was 12.2% (5/41).

In the control group, there was no correlation between gestational age at blood collection and protein Z plasma concentrations (r^2=0.004, p=0.6) (Figure 2). The median maternal plasma concentration of protein Z was significantly lower in patients with pyelonephritis than in normal pregnant women [median: 2.14 μg/ml (range: 0.44-3.42) vs. median 2.36 μg/ml (range: 1.09-3.36), p=0.03] (Figure 3).

In contrast, the concentrations of anti-protein Z antibodies were not significantly different between the study groups [IgG (p=0.12) and IgM (p=0.76)] (Table II). In the pyelonephritis group, there was no difference in the median protein Z plasma concentration between patients who delivered an SGA neonate [median: 2.21 μg/ml (range: 1.89-3.25)] and those who delivered an AGA neonate [median: 2.09 μg/ml (range: 0.44-3.42)] (p=0.307).

E. Coli was the most frequent microorganism isolated in urine cultures [73.8% (31/42)]. Blood cultures were obtained in most patients with pyelonephritis [88.1% (37/42)], and 45.9% (17/37) were positive for microorganisms. Gram-negative bacteria constituted 70.6% (12/17) of the pathogens in blood cultures, and *E. Coli* was detected in 83.3% (10/12) of the Gram-negative organisms isolated. A higher proportion of patients with positive blood cultures had plasma protein Z concentrations below the 5th percentile of the control group (<1.59 μg/ml) when compared to patients with negative blood cultures [23% (4/17) vs. 0% (0/20), respectively; p=0.04]. However, there was no significant difference in the median plasma protein Z concentration between patients with pyelonephritis with positive and negative blood cultures [positive blood cultures: median: 2.13 μg/ml (range: 0.44-3.42) vs. negative blood cultures: median: 2.20 μg/ml (range: 1.69- 3.30); p= 0.8].

Thirteen patients in the pyelonephritis group had a chest x-ray, and 38.5% (5/13) had radiological signs of pulmonary congestion or mild to moderate pulmonary edema; 53.8% (7/13) had a normal chest x-ray, and one patient had chronic abnormal findings. Patients with abnormal chest x-ray results had a lower median plasma protein Z concentration than those with a normal chest x-ray. However, this difference was not statistically significant [abnormal chest x-ray: median: 1.12 μg/ml (range: 0.48-3.26) vs. normal chest x-ray: median: 1.89 μg/ml (range: 0.95-2.58); p= 0.34].

Discussion

Principal findings

1) Women with pyelonephritis during pregnancy have a significantly lower median plasma concentration of protein Z than those with normal pregnancies; 2) this finding cannot be attributed to differences in maternal plasma concentrations of anti-protein Z antibodies; and 3) a higher proportion of patients with pyelonephritis and a positive blood culture had a low plasma protein Z concentration (below the 5th percentile) than patients with pyelonephritis and a negative blood culture.

Protein Z: A vitamin K-dependent cofactor with anticoagulant properties

Originally described in 1977 as a circulating protein in bovine plasma[65], protein Z is a 62 kDa single chain glycoprotein[66]. The designation of “Z” referred to the fact that this was the last vitamin K-dependent protein to elute during anion exchange chromatography. Subsequently, this protein was found in humans in 1984[66]. This protein serves as a cofactor for the inhibition of factor Xa by another protein which is called protein Z-dependent protease inhibitor (ZPI)[52-54]. ZPI is a member of the serpin superfamily of

proteinase inhibitors[53]. Importantly, ZPI can inhibit other coagulation factors, such as XIa in the absence of protein Z[54,67].

The major source of protein Z is thought to be the liver, similarly to other vitamin K-dependent factors and patients with liver disease and newborns have lower concentrations of this protein in their plasma[68,69]. However, other sources are possible. We have demonstrated immunoreactivity to protein Z in the human placenta (Romero, Broze, Kim, unpublished observations). Patients receiving oral contraceptives have higher plasma concentrations of protein Z[70]. There is a controversy concerning the changes in protein Z plasma concentration between pregnant and non-pregnant women[62,63,71,72]. The plasma concentrations of protein Z in normal individuals vary widely and this appears to be under genetic control[73-75]. The same is the case for ZPI[70].

The precise functions of protein Z and ZPI remain controversial. However, accumulating evidence suggests that this complex inhibits, retards and reduces thrombin generation through inhibition of factor X activity[52-54]. Protein Z deficiency has been associated with a moderate risk for thrombosis[58] and this risk is magnified when protein Z deficiency is associated with a thrombophilic state[56,57]. Of interest is that some investigators have postulated that protein Z deficiency may predispose to hemorrhage[76]. However, these observations have not been replicated[77].

Protein Z deficiency has been associated with adverse pregnancy outcomes: 1) a higher proportion of patients with a previous fetal death (10 to 15 weeks of gestation) had protein Z deficiency (<1 mg/L) compared to the control group[61]; and 2) patients with adverse pregnancy outcome (including preeclampsia, IUGR, recurrent unexplained vaginal bleeding, and preterm parturition) had a significantly lower mean plasma concentration of protein Z than patients with normal pregnancy outcome. The authors proposed that changes in the maternal plasma protein Z concentration may have an important role in the regulation of thrombin generation during pregnancy[63]. This is relevant because excessive thrombin generation is associated with preterm parturition with intact or ruptured membranes[78], preeclampsia, and small for gestational age fetuses[79]. In contrast, a recent study[62] demonstrated that the median plasma concentration of protein Z in patients with preeclampsia, intrauterine growth restriction (IUGR), and late fetal demise (IUFD) was not significantly different than that of patients with normal pregnancy. However, the authors reported a higher frequency of protein Z deficiency (<1.2 mg/L) among patients with IUFD and IUGR compared to those with normal pregnancy[62].

Pyelonephritis, systemic inflammation and the coagulation system during pregnancy

Patients with pyelonephritis during pregnancy have phenotypic and metabolic changes in granulocytes and monocytes which are consistent with an exaggerated maternal systemic intravascular inflammation[80]. Systemic inflammation is associated with activation of the hemostatic system[14,81,82], particularly among patients with sepsis[22,42,83-86]. Three mechanisms have been proposed to explain this:

1. Activation of the extrinsic coagulation pathway by inflammation—

Proinflammatory cytokines such as IL-1β and TNF-α increase mRNA and protein expression of tissue factor by monocytes[18-22,87-89] and macrophages[90]. Increased bioavailability of tissue factor could lead to generation of the complex of tissue factor/FVIIa/FXa, which in turn generates thrombin and activates the protease-activated receptor, PAR-2. The latter enhances the production of IL-6[36,45,82,91]. Thus, an amplification loop between inflammation and the coagulation system is in place. For a more detailed account of the close relationship and the importance of the link between inflammation and coagulation, the reader is referred to recent reviews of this subject[14,82,90,92]. This interaction is

particularly important in obstetrics because placentas often have mixture of thrombotic and inflammatory lesions[93,94]. Evidence in support of this concept includes the observation that administration of antibodies against the binding site of FXa to tissue factor/FVIIa complexes attenuates tissue damage and thrombosis in baboons with *E. coli*-induced sepsis[95]. The proposed mechanism of action is that the complex tissue factor/FVIIa cannot activate protease-activated receptor-2 without the contribution of factor Xa[95]. Thus, during systemic inflammation FXa plays a dual role: 1) activation of the prothrombinase complex leading to the generation of thrombin; and 2) amplification of the inflammatory process by inducing interleukin-6 secretion from endothelial cells[96,97] and monocytes[36,91,95]. As demonstrated in a previous study[95], inhibition of factor Xa reduces the inflammatory and thrombotic complications of systemic inflammation during sepsis. Patients in the intensive care unit who developed acute respiratory distress syndrome had higher plasma concentrations of tissue factor but not tissue factor pathway inhibitor in comparison to intensive care unit patients who did not develop acute respiratory distress syndrome[22]. This observation suggests that the lung injury observed during systemic inflammation may result from a failure to maintain an adequate balance between the activated proteases of the coagulation cascade (i.e. tissue factor, FXa) and their natural inhibitors (i.e. tissue factor pathway inhibitor). One possibility is that the low maternal protein Z plasma concentrations observed in patients with pyelonephritis are due to the increased consumption of protein Z resulting from the increased activation of FX during systemic inflammation[98].

2. Activation of the extrinsic coagulation pathway through the complement system—Normal pregnancy is characterized by complement activation[99] and we have proposed that this phenomenon may be a compensatory mechanism aimed at protecting the host against infection[99]. Pregnant patients with pyelonephritis have significantly higher concentrations of the complement protein, C5a, than those without pyelonephritis[100]. C5a induces a 4.9-fold increase in tissue factor activity and a 3.75-fold increase in tissue factor mRNA expression by endothelial cells[101]. Moreover, administration of C5a to animals increases 5- to 6-fold the procoagulant activity of alveolar macrophages through tissue factor activation[102]. Collectively, these observations suggest that complement activation in pyelonephritis contributes to the activation of the coagulation cascade.

3. Inhibition of the anticoagulation mechanisms—During severe systemic inflammation, the physiologic anticoagulant pathways are down regulated[82,92]. The plasma concentration of anti-thrombin III is markedly decreased[103,104], and protein C activity is impaired[103,105-107]; resulting in decreased anticoagulant activity that further increases the hypercoagulable state observed during systemic inflammation.

A potential role for protein Z in acute maternal systemic inflammation during pregnancy

The lower median concentration of protein Z observed in patients with pyelonephritis during pregnancy is novel. One explanation for this finding is that the prothrombotic state of normal pregnancy[108-112], associated with increased generation of factor Xa, is exacerbated during the course of acute infection/inflammation. The complex of protein Z and ZPI participates in counteracting the effects of an excess of factor Xa and may be consumed in this context. It is important to note that in vitro experiments have reported consumption of ZPI but not of protein Z[54]. However, because protein Z circulates bound to ZPI, immunoreactive protein Z determinations reflect not only protein Z but also the concentration of protein Z/ZPI complexes[67]. Therefore, the lower median plasma concentration of protein Z may reflect decreased circulating concentrations of this complex. The extent to which this phenomenon occurs in the non-pregnant state requires further investigation.

In conclusion, patients with pyelonephritis during pregnancy have a lower median maternal plasma concentration of protein Z than normal pregnant women.

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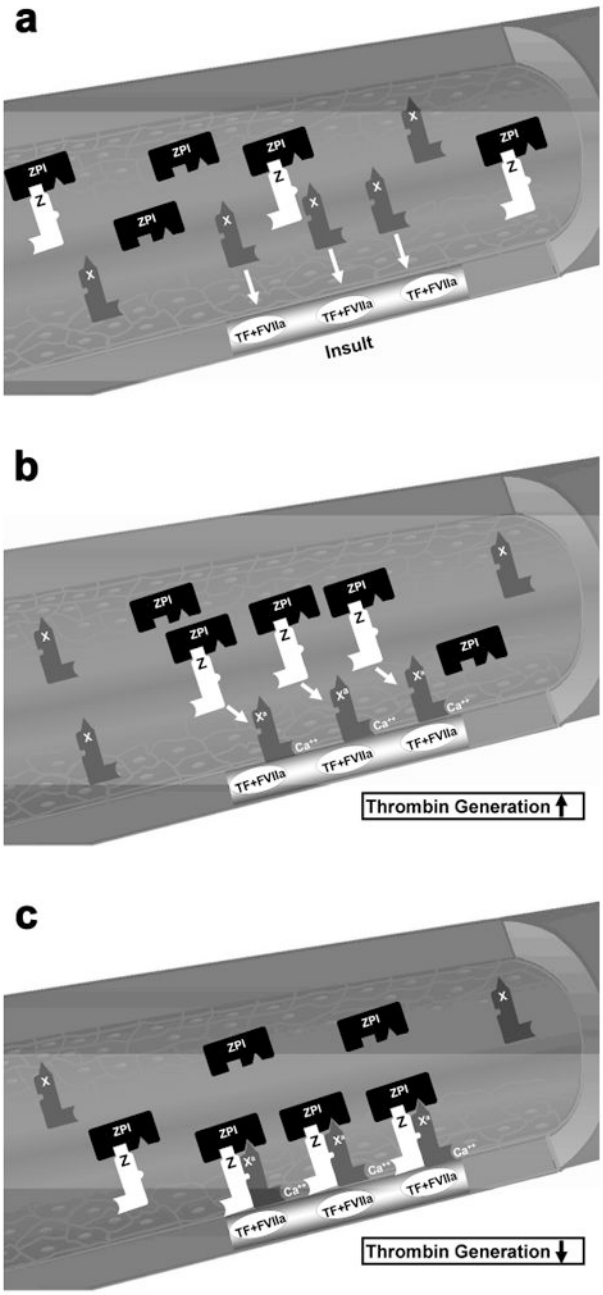

Figure 1.

Factor X activation and protein Z/ZPI inhibition of activated factor X. Figure 1a- the formation of the complex of tissue factor (TF) and factor VIIa at the site of injury and activation of extrinsic coagulation cascade. Figure 1b- Activation of circulating factor X by the TF+FVIIa complex in the presence of exposed phospholipids and Ca^{+2}. Figure 1c- Inhibition of FXa by the protein Z/ZPI complex by binding to its active site. Modified from Broze JG, The Lancet 2001;357:900-901.

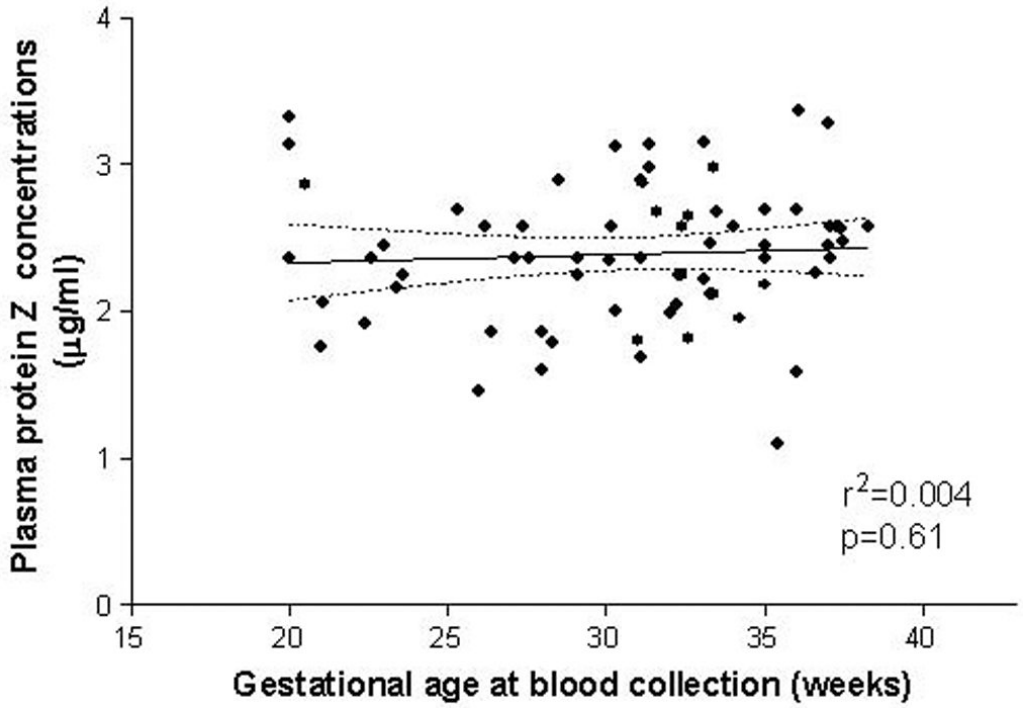

Figure 2.
Maternal plasma protein Z concentrations in patients with normal pregnancy (n=71) according to gestational age

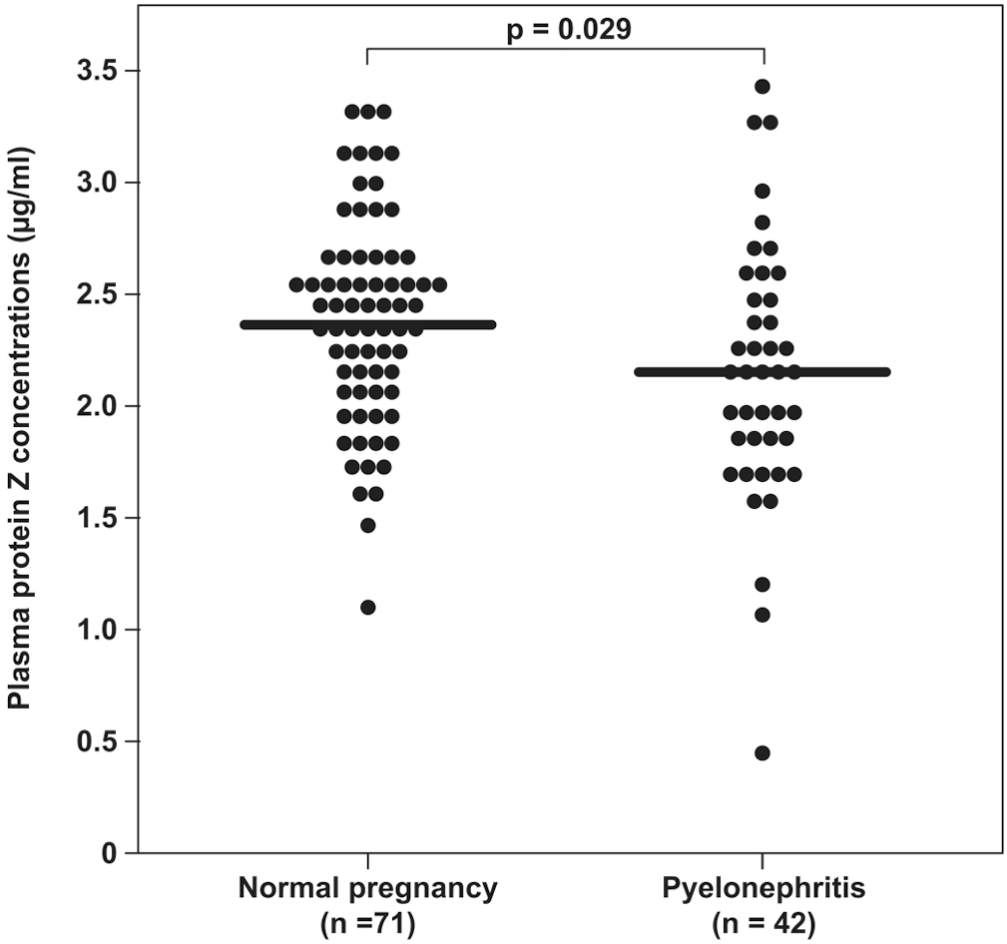

Figure 3.

Maternal plasma protein Z concentrations in patients with normal pregnancy and patients with pyelonephritis. The median maternal plasma concentration of protein Z was significantly lower in paitents with pyelonephritis than in normal pregnant women (2.14 µg/mL (range 0.44-3.42) vs. 2.36 µg/mL (range 1.09-3.36; p=0.029))

Table I

Demographic and clinical characteristics of the study population

	Normal pregnancy n= 71	**Pyelonephritis n=42**	**p**
Maternal age (year)	24.2±4.5	23.8± 6.7	0.2
Gravidity€			
1	14 (20.3%)	9 (21.43%)	
2-5	45 (65.2%)	30 (71.43%)	0.4
≥6	10(14.5%)	3 (7.14%)	
Parity §			
1	39 (55.7%)	30 (71.4%)	
2-5	30 (42.9%)	12 (28.6%)	0.3
≥6	1 (1.4%)	0	
Ethnic origin£			
African-Americans	53 (77.9%)	31 (75.6%)	
Caucasians	11 (16.1%)	6 (14.6%)	0.3
Hispanic	2 (3%)	4 (9.8%)	
Asian	2(3%)	0	
Gestational age at blood collection (weeks)	30.8 ± 5.1	29.8 ± 5.9	0.4
Gestational age at delivery (weeks)	39.4 ± 1.5	38.7 ± 2.6	0.3
Birthweight (grams)	3340 ± 392	3086 ± 584	0.02

Data are presented as mean ± standard deviation or numbers (%)

€ = Normal pregnancy (n=69)

§ = Normal pregnancy (n=70)

£ = Normal pregnancy (n=68); Pyelonephritis (n=41)

Table II

Plasma concentrations of anti-protein Z antibodies in both study groups

	Normal pregnancy n= 71	**Pyelonephritis n=42**
Antiprotein Z IgG (AU/ml)	2.93 (0, 500.5)	2.48 (0, 101.42)
Antiprotein Z IgM (AU/ml)	12.67 (0, 44.03)	10.71 (1.58, 61.28)

Data presented as median (minimum, maximum).