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Maternal Plasma Retinol Binding Protein 4 in Acute Pyelonephritis during Pregnancy

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Abstract

Objective—Adipokines have been implicated in metabolic regulation and the immune response thus providing a molecular mechanism for the interaction between these two systems. Retinol binding protein 4 (RBP4) is a novel adipokine that plays a role in the pathophysiology of obesity-induced insulin resistance, as well as in the modulation of inflammation. The aim of this study was to determine whether there are changes in maternal plasma concentrations of RBP4 in pregnant women with acute pyelonephritis.

Study design—This cross-sectional study included pregnant women in the following groups: 1) normal pregnancy (n=80); 2) pyelonephritis (n=39). Maternal plasma RBP4 concentrations were determined by enzyme-linked immunoassays. Non-parametric statistics were used for analyses.

Results—1) The median maternal plasma RBP4 concentration was lower in patients with acute pyelonephritis than in those with a normal pregnancy (3709.6 ng/mL, IQR 2917.7-5484.2 vs. 9167.6 ng/mL, IQR 7496.1-10384.1, p<0.001; 2) the median maternal plasma RBP4 concentration did not differ significantly between patients with acute pyelonephritis who had a positive blood culture and those with a negative culture (3285.3 ng/mL, IQR 2274.1-4741.1 vs. 3922.6 ng/mL, IQR 3126.8-5547.1, respectively, p=0.2); and 3) lower maternal plasma RBP4 concentrations were independently associated with pyelonephritis after adjustment for confounding factors.

Conclusions—In contrast to what has been reported in preeclampsia, acute pyelonephritis during pregnancy is associated with lower maternal plasma RBP4 concentrations than in normal pregnancy. This finding suggests that the acute maternal inflammatory process associated with pyelonephritis is fundamentally different from that of the chronic systemic inflammatory process suggested in preeclampsia, in which RBP4 concentrations were found to be elevated.

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Keywords

Adipokines; bacterial infection; pregnancy; RBP4; sepsis; systemic inflammation; urinary tract infection; UTI

Introduction

Adipose tissue, previously considered to be only a primary repository of triglycerides, has emerged as an active contributor to the regulation of the immune system through the production and secretion of a variety of cytokines, chemokines and hormones. Collectively, these adipocyte-derived proteins are termed adipokines. Adipokines have been implicated in the physiology and pathophysiology of glucose homeostasis, providing a molecular link between obesity, insulin resistance and type 2 diabetes mellitus (DM) [80]. In addition, adipocytokines play a role in the pathophysiology of inflammatory disorders such as asthma [27], inflammatory bowel disease [4,83], rheumatoid arthritis [5,72], and obesity [17,81,89,90]. Moreover, alterations in adipokine homeostasis (e.g., leptin) may predispose to increased susceptibility to infection [18,78,87].

Retinol binding protein 4 (RBP4), previously thought to be only a specific carrier for retinol and to be produced mainly by the liver, has recently been classified as an adipokine [89]. A growing body of evidence suggests that RBP4 plays a role in obesity-induced insulin resistance, as well as in the modulation of inflammation [3,7,36,73,91]. Indeed, in non-diabetic human subjects, RBP4 gene expression was found to be strongly associated with inflammatory markers of adipose tissue such as CD68 and monocyte chemoattractant protein-1 (MCP-1) [91].

Alterations in circulating concentrations of adipokines such as leptin, adiponectin, resistin, and visfatin, as well as RBP4, have been associated with physiologic metabolic adaptations during normal pregnancy [8,48,50,52,⁵⁴,56,63,64,76] and with complications of pregnancy including preterm labor [42,43,53], intra-amniotic infection/inflammation [35,46,84], preeclampsia [11,15,30,39,58,62,70,85], gestational diabetes and others [44,45,49,51,55,66].

Pregnancy is associated with a greater susceptibility to infection, especially of the lower urinary tract, than the non-pregnant state [16,21]. Pyelonephritis is a relatively common disease and affects 1-2% of pregnant women [21,31] with the majority of cases occurring in the second and third trimesters or during the postpartum period [22,31]. Pyelonephritis during pregnancy may be associated with transient renal dysfunction in 20-25% of patients [21,88] and occasionally with life-threatening maternal complications, including acute respiratory distress syndrome (ARDS) [10,12,14,26,28], sepsis [13,31,74], and septic shock [13,40,74].

Recently, we have reported that acute pyelonephritis is associated with alterations in the maternal plasma concentrations of adipocytokines such as visfatin [47], adiponectin [57], tumor necrosis factor (TNF)- α and interleukin (IL)-6 [9]. Thus, the aim of this study was to determine whether acute pyelonephritis during pregnancy is associated with changes in maternal plasma concentrations of RBP4.

Materials and methods

Study groups and inclusion criteria

A retrospective cross-sectional study was conducted comprised of pregnant women in the following groups: 1) normal pregnancy (n=80); and 2) patients with pyelonephritis (n=39).

Women with multiple pregnancies or fetuses with chromosomal and/or congenital anomalies were excluded.

Samples and data were retrieved from our bank of biological samples and clinical databases. Many of these samples have previously been used to study the biology of inflammation, hemostasis, angiogenesis regulation, and growth factor concentrations in normal pregnant women and those with pregnancy complications [9,24,34,65].

All participants provided written informed consent prior to the collection of maternal blood. The collection of maternal blood and its use for research purposes was approved by the Institutional Review Boards of Wayne State University (Detroit, Michigan, USA) and the Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD/NIH/DHHS).

Clinical Definitions

Women with a normal pregnancy were defined as those without medical, obstetrical, or surgical complications at the time of the study and who subsequently delivered at term (≥ 37 weeks of gestation) an appropriate-for-gestational age neonate [2], without neonatal complications. Pyelonephritis was diagnosed in the presence of fever (temperature $\geq 38^{\circ}\text{C}$), clinical signs of an upper urinary tract infection (e.g., flank pain, costo-vertebral angle tenderness), and a positive urine culture for microorganisms. The body mass index (BMI) was calculated using the formula: weight/height squared (kg/m^2). The study population was classified according to the pre-pregnancy BMI into two groups: normal weight (BMI $< 25 \text{ kg}/\text{m}^2$) and overweight/obese (BMI $\geq 25 \text{ kg}/\text{m}^2$) women [1].

Sample collection and determination of RBP4 in maternal plasma

Maternal blood samples were obtained from each normal pregnant woman at the time of a routine clinical visit and from women with pyelonephritis at the time of diagnosis. Samples were collected in vials containing ethylene-diamine-tetra-acetic acid, centrifuged at $1300 \times g$ for 10 minutes at 4°C , and the plasma was stored at -80°C until assayed. Maternal plasma concentration of RBP4 was determined by sensitive enzyme-linked immunoassays (Millipore Corporation, St. Charles, MO, USA). The RBP4 immunoassay was validated for human plasma in our laboratory prior to the conduction of this study. Immunoassays were carried out according to the manufacturer's recommendations. The calculated inter- and intra-assay coefficients of variation for RBP4 immunoassays in our laboratory were 5% and 5.1%, respectively. The sensitivity was calculated to be $0.1 \text{ ng}/\text{mL}$.

Statistical analysis

Normality of the data was tested using the Kolmogorov-Smirnov test. Since maternal plasma RBP4 concentrations were not normally distributed, Kruskal-Wallis tests with post-hoc analysis by Mann-Whitney U tests were used for comparisons of continuous variables. Comparison of proportions was performed using Fisher's exact test. Correlations between RBP4 concentrations and pre-pregnancy BMI and gestational age at blood sampling were determined using Spearman's rank correlation test. Logistic regression (stepwise backward) was applied to examine the relationship between maternal plasma RBP4 concentrations ($\mu\text{g}/\text{mL}$) and the presence of pyelonephritis, while adjusting for the following potential explanatory variables: maternal age (years), pre-pregnancy BMI (kg/m^2), smoking status (categorical), gestational age at blood draw (weeks), and sample storage time (years). A p -value < 0.05 was considered statistically significant. Analysis was performed with SPSS, version 14 (SPSS Inc., Chicago, IL, USA).

Results

Demographic and clinical characteristics of women with a normal pregnancy and those with pyelonephritis are displayed in Table 1. Compared to women with a normal pregnancy, patients with pyelonephritis had a significantly lower neonatal birthweight. The median sample storage time was significantly higher in the pyelonephritis group than in those with a normal pregnancy (8.5 years vs. 8.0 years).

Among patients with acute pyelonephritis, *Escherichia coli* was the most common microorganism isolated from urine cultures (31 cases). Other microorganism isolated from urine included *Klebsiella pneumoniae* (2 cases), *Streptococcus agalactiae*, *Enterobacter aerogenes*, *Proteus mirabilis*, *Citrobacter koseri*, Gram-negative bacilli, and *Streptococcus viridans* (1 case each). Blood cultures were obtained in 34 patients (87.2%) with pyelonephritis. Of those, 14 (41.2%) blood cultures were positive for one of the following microorganisms: *Escherichia coli* (n=9), Coagulase-negative *Staphylococcus* (n=2), *Enterobacter aerogenes* (n=1), *Klebsiella pneumoniae* (n=1), Gram positive cocci (n=1).

Maternal plasma RBP4 concentration in normal pregnancy

Retinol binding protein 4 was detected in the maternal plasma of all subjects included in this study. Among women with a normal pregnancy, maternal plasma RBP4 concentrations did not correlate with pre-pregnancy BMI (Spearman's rho 0.17, p=0.2), gestational age at blood sampling (Spearman's rho -0.13, p=0.3), or sample storage time (Spearman's rho 0.11, p=0.3). In addition, the median maternal plasma RBP4 concentration did not differ significantly between women with a normal pre-pregnancy BMI and those who were overweight/obese (median 9365.3 ng/mL, IQR 7441.9-10866.2 vs. 9168.8 ng/mL, IQR 7460.6-10101.1, respectively; p=0.6).

Maternal plasma RBP4 concentration in patients with pyelonephritis

The median maternal plasma RBP4 concentration was lower in women with acute pyelonephritis than in those with a normal pregnancy (3709.6 ng/mL, IQR 2917.7-5484.2 vs. 9167.6 ng/mL, IQR 7496.1-10384.1, p<0.001; Figure 1). Similar to the findings in women with a normal pregnancy, the maternal plasma RBP4 concentration in patients with pyelonephritis did not correlate with pre-pregnancy BMI (Spearman's rho 0.14, p=0.4), gestational age at blood sampling (Spearman's rho 0.05, p=0.8), or sample storage time (Spearman's rho -0.18, p=0.3).

As demonstrated in women with a normal pregnancy, the median maternal plasma RBP4 concentration was not significantly different between patients with pyelonephritis with a normal weight and those who were overweight/obese (3335.4 ng/mL, IQR 2536.1-5214.6 vs. 4470.1 ng/mL, IQR 3112.2-5505.2, p=0.4; Figure 2).

Among patients with acute pyelonephritis, the median maternal plasma RBP4 concentration did not differ significantly between women who had a positive blood culture and those with a negative culture (3285.3 ng/mL, IQR 2274.1-4741.1 vs. 3922.6 ng/mL, IQR 3126.8-5547.1, respectively, p=0.2; Figure 3).

Stepwise multiple regression analysis was applied to examine the relationship between maternal plasma RBP4 concentrations and pyelonephritis, while adjusting for possible explanatory variables, including maternal age, pre-pregnancy BMI, smoking status, gestational age at blood draw, and sample storage time. The final regression model suggested that maternal plasma RBP4 concentration was significantly associated with pyelonephritis (odds ratio 0.01, 95% confidence interval 0.03-0.33; p<0.001).

Discussion

Principal findings of the study

1) Patients with pyelonephritis had a lower median maternal plasma concentration of RBP4 than women with a normal pregnancy; and 2) the median maternal plasma RBP4 concentration in patients with acute pyelonephritis was not significantly different between patients who had a positive blood culture and those who did not.

What is RBP4?

Retinol binding protein [32] is a specific carrier of retinol from the liver to peripheral tissues. Although it is mainly synthesized by the liver [68], subsequent studies demonstrated that retinol binding protein is also produced by adipocytes [41,60]. Yang et al. [89] were the first to propose that RBP4 plays a role in the pathogenesis of insulin resistance. The authors demonstrated that circulating concentrations of RBP4 are elevated in insulin-resistant knockout mice for adipose specific glucose transporter 4 (GLUT4), as well as in obese individuals with type 2 DM [89]. Of note, similar to other adipokines [6,35,38,53-55,62-64,75,80,81,89,90], RBP4 has also been linked to inflammation and the immune system [3,7,36,73,91]. Gene expression of RBP4 is strongly associated with inflammatory markers (i.e. CD68 and MCP-1) of adipose tissue in non-diabetic human subjects [91]. In children, circulating concentrations of this adipokine correlated with inflammatory factors such as C-reactive protein and IL-6 as well as with metabolic indices [3].

We have reported that normal gestation is not associated with alterations in maternal plasma RBP4. In addition, maternal RBP4 concentrations were not correlated with pre-pregnancy BMI and did not differ significantly between normal weight and overweight/obese patients. Interestingly, among patients with preterm labor, amniotic fluid concentrations of RBP4 were significantly higher in pregnancies complicated by intra-amniotic infection and/or inflammation [84]. Consistent with this finding, we have recently reported that preeclampsia, an inflammatory disorder of pregnancy [71,86], is associated with higher maternal plasma RBP4 concentrations than normal pregnancy [85]. Taken together, these findings support a role for RBP4 in the host response against infection and in the regulation of the inflammatory response.

RBP4 concentration is decreased in acute pyelonephritis

The finding that the median maternal plasma concentration of RBP4 is significantly lower in patients with acute pyelonephritis during pregnancy than in women with a normal pregnancy is novel. This finding is in agreement with previous studies in non-pregnant subjects in which acute stressful conditions such as a critical illness of pulmonary origin, [36] sepsis, [36] elective surgery, [36] or burn injury [61] were all associated with lower circulating concentrations of RBP4 than in healthy controls. Furthermore, RBP was the only acute phase reactant protein able to discriminate in patients with gastric carcinoma between those infected by *Helicobacter pylori* and those who were not (significantly lower concentration in the infected group) [82]. Of note, while maternal RBP4 concentrations were significantly lower in patients with pyelonephritis than in the control group, we did not find a significant difference in RBP4 concentrations between patients with pyelonephritis with and without a positive blood culture. The reason for this is not clear. It is possible that the dramatic decrease in circulating RBP4 in the presence of pyelonephritis overwhelms other potential inhibitors of RBP4 production and/or secretion. In addition, a non-significant trend toward a lower median concentration of RBP4 in patients with bacteremia than in those with a negative blood culture was noticed and this may reflect a lack of power in the current study to detect such a difference.

The apparent contradictory results observed in pyelonephritis (lower RBP4 concentrations) and in early-onset preeclampsia (elevated RBP4 concentration)[85] may reflect the different inflammatory processes involved in these two complications of pregnancy. In the former, the inflammatory process is secondary to infection, while the origin of the exaggerated maternal inflammatory process that favors the production of Th1 type cytokines in preeclampsia is not clear [71,86].

Why is pyelonephritis during pregnancy associated with decreased maternal RBP4 concentrations?

The cross-sectional nature of our study does not allow us to discern a cause-effect relationship between lower maternal plasma RBP4 concentrations and pyelonephritis; however, several suggestions for this can be hypothesized.

The low circulating RBP4 in women with pyelonephritis during pregnancy can be the result of increased utilization, degradation, and/or excretion or decreased production and/or secretion, either by the liver, adipose tissue or both. Acute infection is associated with increased energy expenditure and insulin resistance [19,23,79]. Indeed, sepsis is associated with major metabolic alterations including increased glucose production, gluconeogenesis, and insulin resistance, as well as increased lipolysis and proteolysis [79]. While high concentrations of RBP4 have been associated with insulin resistance, the metabolic consequences of low RBP4 concentration has not been elucidated. Assuming that there is a linear correlation between insulin resistance and concentrations of this adipokine, a state of low RBP4 concentration, such as pyelonephritis during pregnancy, will be associated with increased glucose tolerance. Thus, it is possible that the low RBP4 concentration in maternal circulation observed in patients with pyelonephritis is a counter-action response to the generalized hypermetabolic/catabolic state observed in infected patients.

An additional possible explanation for the low RBP4 concentration in patients with pyelonephritis is inhibition of its production/secretion. Indeed, TNF- α strongly down-regulates the production of RBP4 in primary human adipocytes [73]. Moreover, differentiated human macrophages have been shown to express RBP4 [7]. Inhibition of gene expression and production of RBP4 by macrophages has been documented after exposure to bacterial endotoxin such as *Escherichia coli* lipopolysaccharide [7]. In our study, almost 80% (31/39) of cases of pyelonephritis were due to *Escherichia coli* and more than a quarter of patients (9/34) had a positive blood culture for *Escherichia coli*. Moreover, pyelonephritis in pregnancy is associated with increased TNF- α concentration [9]. Collectively, these data suggest that the lower RBP4 concentration found in pregnant patients with pyelonephritis can be the result of reduced production/secretion.

What is the possible role of the low RBP4 concentration observed in maternal circulation in acute pyelonephritis? Recent studies have suggested a link between insulin resistance and high serum and adipose tissue RBP4 concentrations [20,25,33,59,67,69,89]. In contrast, genetic deletion of RBP4 enhanced insulin sensitivity [89]. Furthermore, in apparently healthy subjects, circulating RBP4 correlates negatively with insulin sensitivity [77]. Interventions in humans such as exercise, lifestyle modification, or gastric banding surgery resulted in reduced serum RBP4 concentrations and improved insulin sensitivity [3,25,29,37]. From these data, it is tempting to propose that the lower RBP4 concentrations found in pyelonephritis are part of a counter-regulatory mechanism in response to the metabolic challenge and the insulin resistance described in acutely ill or stressed patients [19,23,79], as may be the case of pyelonephritis during pregnancy.

In conclusion, the present study is the first to report an association between lower maternal circulating RBP4 concentration and an acute infectious disease during pregnancy. Further

studies are needed to clarify whether low maternal RBP4 concentrations are the result of the infectious process or rather increased susceptibility of pregnant women to infection.

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Figure 1. Comparison of the median maternal plasma RBP4 concentration between women with a normal pregnancy and those with pyelonephritis

The median maternal plasma concentration of RBP4 was lower in patients with pyelonephritis than in those with a normal pregnancy (3709.6 ng/mL, interquartile range (IQR) 2917.7-5484.2 vs. 9167.6 ng/mL, IQR 7496.1-10384.1, $p < 0.001$).

Figure 2. Comparison of the median maternal plasma RBP4 concentration among normal weight and overweight/obese women with a normal pregnancy and with pyelonephritis
The median maternal plasma concentration of RBP4 was lower in patients with pyelonephritis than in those with a normal pregnancy, in both normal weight and overweight/obese patients ($p < 0.001$ for both comparisons). The median maternal plasma RBP4 concentration did not differ significantly between normal weight and overweight/obese women in both normal pregnant women ($p = 0.6$) and those with pyelonephritis ($p = 0.4$).

Figure 3. Comparison of the median maternal plasma RBP4 concentration among patients with pyelonephritis with and without a positive blood culture for microorganisms

The median maternal plasma concentration of RBP4 did not differ significantly between patients with pyelonephritis with and those without a positive blood culture (3285.3 ng/mL, interquartile range (IQR) 2274.1-4747.1 vs. 3922.6 ng/mL, IQR 3126.8-5547.1, respectively; $p=0.2$).

Table 1

Demographic and clinical characteristics of the study population.

	Normal Pregnancy (n=80)	Pyelonephritis (n=39)	p
Maternal age (years)	24 (21-29)	22 (19-26)	0.13
Ethnicity			
African American	76.2 (61)	79.5 (31)	0.4
Caucasian	12.5 (10)	10.25 (4)	
Hispanic	6.3 (5)	10.25 (4)	
Others	5 (4)	0 (0)	
Smoking	20 (16)	19.4 (7)	0.6
Nulliparity	32.5 (26)	33.3 (13)	0.5
Pre-pregnancy BMI (kg/m²)	26.4 (23.0-31.4)	26.7 (23.1-31.3)	0.9
Gestational age at blood sampling (weeks)	32.2 (27.1-36.4)	28.7 (24.4-35.7)	0.075
Gestational age at delivery (weeks)	39.1 (38.2-40.1)	39.1 (37.7-40.2)	0.6
Birth weight (grams)	3280 (3001-3537)	3130 (2673-3572)	0.039
Sample storage time (years)	8.0 (6.9-8.1)	8.5 (8.1-9.3)	<0.001

Values are expressed as median (interquartile range) or % (number);

BMI – Body Mass Index;