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Original Article

Low plasma levels of decoy receptor 3 (DcR3) in the third trimester of pregnancy with preeclampsia

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ABSTRACT

Objective: The pathophysiology of preeclampsia, a major threat during pregnancy characterized by excessive inflammatory status, remains unclear. Decoy receptor 3 (DcR3), a soluble member of the tumor necrosis factor receptor (TNFR) superfamily, is capable of inducing anti-apoptosis via binding with TL1A and anti-inflammation by driving Th2 immune reactions. DcR3 may, therefore, play a role in immune modulation during pregnancy. The purpose of this study is to explore the role of DcR3 in normal and preeclamptic pregnancies.

Materials and methods: Plasma samples from 104 normal pregnant women (26, 42, and 36 in the first, second, and third trimester, respectively) and 10 patients with preeclampsia in the third trimester were collected. Plasma DcR3 levels were determined by using commercial ELISA kits. ANOVA and linear regression analysis were performed to analyze the relationship between gestational age and DcR3 levels. After adjusting for gestational days, the levels of plasma DcR3 in preeclamptic and non-preeclamptic women in the third trimester were compared.

Results: The plasma levels of DcR3 gradually decreased as the gestational days increased during pregnancy ($p < 0.05$). In the third trimester, pregnant women with preeclampsia had significantly lower plasma DcR3 levels compared to non-preeclamptic women ($p < 0.05$).

Conclusions: We found that plasma DcR3 levels gradually decreased as gestation progressed. The levels of plasma DcR3 in preeclamptic women were significantly lower than those of normal pregnant women, suggesting that a potential involvement of DcR3 in normal pregnancy and decreased levels of DcR3 may be related to preeclampsia.

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Introduction

Successful pregnancy needs adequate maternal immune adaptation toward the semi-allogeneic fetus, although the underlying

mechanism of immune tolerance is still unclear [1–4]. It is generally accepted that the type 2-bias immunosuppressive environment at the maternal-fetal interface as well as the fact that the trophoblasts do not express rejection-associated classical major histocompatibility complex (MHC) molecules contribute to successful pregnancy and reproduction [5–9]. Imbalanced maternal immune modulation may lead to adverse pregnancy outcomes such as preeclampsia. Preeclampsia, manifested by hypertension and proteinuria after 20 weeks of gestation, is one of the leading causes of maternal and neonatal mortality and morbidity [10–13]. Preeclampsia is a systemic inflammatory disease that may lead to

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multiple maternal organ damage in the liver, kidneys, lungs, and the central nervous system [14–16]. Preeclampsia may also predispose affected women to subsequent cardiovascular diseases in later life [13]. Fetuses in pregnancies complicated with preeclampsia are at risk of placenta abruption, intrauterine growth restriction, and preterm birth [11,14,17]. Delivery of the placenta remains the only effective treatment for preeclampsia [14]. Most of the preeclampsia symptoms disappear within a week after delivery. However, termination of the disease process with delivery may contribute to iatrogenic prematurity. The pathogenesis of preeclampsia remains largely unknown [1,18]. Accumulating evidence suggests that preeclampsia is characterized by abnormal placentation, which involves shallow trophoblast invasion and impaired spiral artery remodeling early in pregnancy and subsequent systemic responses in later gestations [19]. Of all the concurrent hypotheses on the pathophysiology of preeclampsia, excessive inflammation is thought to play an important role [19]. Balanced maternal immune modulation to the semi-allogeneic fetus is essential for an uneventful pregnancy.

Decoy receptor 3 (DcR3), also known as tumor necrosis factor receptor (TNFR) superfamily member 6b (TNFRSF6B)/TR6/M68, is a soluble receptor capable of neutralizing known apoptosis and inflammation inducers, including Fas ligand (FasL/CD95L/TNFSF6), LIGHT (TNFSF14) and TNF-like molecule 1A (TL1A/TNFSF15) [20–22]. This anti-apoptotic soluble receptor is almost undetectable in non-pathologic conditions but may participate in immune suppression and play an important role in immune modulation [22,23].

Expression of DcR3 by tumor cells originating from various lineages has been considered to contribute to evasion of tumor cells from immune cytotoxic attack by blocking the Fas ligand and the LIGHT-mediated apoptosis [24,25]. In addition, LIGHT, as a costimulatory molecule for priming T cell responses, may promote T helper 1 (Th1)-type cytokine production [26]. Furthermore, TL1A was found to bind to death receptor 3 (DR3) (TNFRSF25) on immune cells and skews T-cell differentiation favoring Th1 and Th17 subsets [27,28]. DcR3 may play a role in regulating T-cell activation through its action as a decoy receptor for LIGHT and TL1A [27,28]. Additionally, DcR3 may drive macrophages towards an M2 phenotype and regulate dendritic cell differentiation leading to Th2 polarization and a Th1-suppressing effect [23]. These results are consistent with the finding that transgenic mice over-expressing DcR3 exhibit attenuated Th1 differentiation [23]. In addition to Th2 polarization effects, DcR3 has also been found to mediate down-regulation of MHC-II expression [29] and in dampening T-cell responses to alloantigens through interfering with the LIGHT–TR2/HVEM interaction [22], which might collectively contribute to the maintenance of successful pregnancies.

In addition to immune regulation, DcR3 has been shown to promote angiogenesis through antagonizing the autocrine angiostatic effects of TL1A on endothelial cells [30]. Furthermore, DcR3 is also suggested to play a role in human placentation and in follicular developments of vertebrate ovaries [31,32]. Previous studies have shown that DcR3 is expressed in placenta throughout the gestation period [31]. However, decreased DcR3 expression was found in preeclamptic placenta [32]. Taken together, DcR3 is postulated to play a role in modulating the immune balance during pregnancy and dysregulation of DcR3 may have induced in the excessive inflammation reactions of preeclampsia. To obtain further evidence to support the proposed immune modulation role of DcR3 in pregnancy, the purpose of the current study was to determine the plasma levels of DcR3 in normal pregnancy compared to pregnancies complicated with preeclampsia.

Materials and methods

Participants and samples

The participants of this study were healthy pregnant women ranging from 18 to 45 years of age. Pregnant women with labor signs, proven malignancy or autoimmune diseases including rheumatoid arthritis or systemic lupus erythematosus (SLE) were excluded. With written and verbal informed consent, blood samples were taken during routine outpatient prenatal visits and at the delivery room. Preeclampsia was defined in our subjects as having a maternal blood pressure of more than 140/90 mmHg and proteinuria more than 2 + by dipstick, or 300 mg/day. Blood samples from preeclamptic women were taken at the time of diagnosis. Plasma samples from 104 normal pregnant women (26, 42, and 36 in the first, second, and third trimester, respectively) and 10 patients with preeclampsia in the third trimester were collected. The study was approved by Institutional Review Board of Taipei Veterans General Hospital (TVGH) (VGHIRB No: 2015-03-015B), and was conducted at TVGH.

Decoy receptor 3 ELISA

Plasma DcR3 levels were analyzed by an Enzyme-Linked Immunosorbent Assay (ELISA) kit (Biolegend Inc., San Diego, CA). The plates were pre-coated with a capturing antibody. Standards and aliquots of samples were pipetted in triplicates into the wells and incubated for 2 h at room temperature. After washing, detection antibody was added and incubated for 2 h. After another washing, avidin-horse radish peroxidase E solution was added and incubated for 30 min. After further washing, a substrate solution was added for colorimetric detection with incubation in the dark for 25 min. On stopping the reaction, the optical density was measured in a spectrophotometer. The amount of DcR3 in the standards and samples were determined by using a 4-parameter logistics curve-fitting algorithm. A mean value of triplicate determination for each sample was obtained for further analysis.

Statistical analysis

All statistical analysis was performed by SPSS for Windows, version 22.0. We reported our samples descriptive data summarized with count and percentage for nominal data and mean and standard deviation for continuous data. An analysis of variance (ANOVA) was used to compare differences in mean DcR3 levels during different trimesters. Additionally, linear regression analysis was conducted to find the association of gestational age in pregnancy as a continuous variable and DcR3 levels. Finally, pregnancy type (normal and preeclamptic) was compared by the mean plasma DcR3 levels in the third trimester using a t-test. An alpha cut-off value of $p < 0.05$ was set *a priori* for determining significance.

Table 1
Demographic data of pregnant women in normal pregnancy.

Trimester	First	Second	Third
Number	26	42	36
Age (years)	33.2 ± 4.9	32.9 ± 4.9	33.1 ± 3.6
Primigravida	15 (57%)	24 (57%)	22 (61%)
Gestational days	79.5 ± 8.3	159.1 ± 32.3	268.4 ± 19.0

Results

A total of 114 pregnant women were recruited for this study. The number of blood draws from 104 normal pregnant women in the first, second, and third trimesters were 26, 42 and 36, respectively. Demographic data of the patients are listed in Table 1. There were 10 patients with preeclampsia enrolled in the third trimester (Table 2).

Plasma DcR3 levels by trimesters were reported (Fig. 1). Analysis of Variance (ANOVA) was conducted to detect differences in mean DcR3 levels. ANOVA results indicated that there was not a significant difference in overall mean between trimesters ($F(2, 101) = 2.83$; $p = 0.063$). However, further sub group *post-hoc* analysis revealed a significant difference in mean DcR3 levels between the first trimester and third trimester ($p < 0.05$). Upon conducting a simple linear regression analysis, the plasma levels of DcR3 were found to be negatively associated with gestational days in pregnancy ($p < 0.05$) (Fig. 2).

Plasma level of DcR3 in the 10 preeclamptic and 36 non-preeclamptic patients were compared. T-test findings indicated that the plasma DcR3 levels were significantly lower in patients with preeclampsia ($t(44) = 2.53$; $p < 0.05$) (Fig. 3). Our study, thus, demonstrated a gradual decrease of plasma DcR3 during pregnancy. Furthermore, the DcR3 levels were lower in preeclampsia than in normal pregnancy in the third trimester.

Discussion

Since DcR3 may participate in immune modulation and contribute to successful pregnancy, circulating DcR3 levels in three different trimesters of normal pregnant women were determined in this study. Our results showed significant progressive decrease in plasma DcR3 levels, starting from the first trimester, during normal pregnancy as the gestational days progressed. However, a higher degree of variations in the DcR3 levels was found in the first trimester of pregnancy. Our results are in contrast to those of Chen et al. who showed that the serum DcR3 levels remained relatively low (0.2–0.4 ng/ml) with no major fluctuation throughout normal gestation [33]. In their study, the serial serum samples were collected at different trimesters from each pregnant woman ($n = 20$). In our study, the number of pregnant women in the first, second and third trimester were 26, 42, and 36, respectively, and triplicates plasma samples from each subject were used to determine the levels of circulating DcR3. Data were analyzed using ANOVA for nominal variable (trimester), and simple linear regression for continuous variable (gestational days) to compare plasma DcR3 level. Further studies using serial serum samples from the same pregnant individual could be performed to clarify this discrepancy.

Immune modulation at the beginning of pregnancy at the maternal-fetal interface is crucial for a successful pregnancy.

Table 2

Demographic data of preeclamptic and non-preeclamptic pregnant women in the third trimester.

	Non-Preeclampsia	Preeclampsia
Number	36	10
Age (years)	33.1 \pm 3.6	33.1 \pm 3.1
Primigravida	14	10
Gestational days	268.4 \pm 19.0	259.5 \pm 17.0
No. of Cesarean section (%)	13 (36%)	3 (30%)
Systolic BP (mmHg)	118.2 \pm 6.8	161.9 \pm 5.4
Diastolic BP (mmHg)	73.2 \pm 5.5	98.7 \pm 9.2
Proteinuria (mg) per day	None	1776.3 \pm 456.2
Birth weight (gm)	3224.1 \pm 463.7	2685.6 \pm 538.4

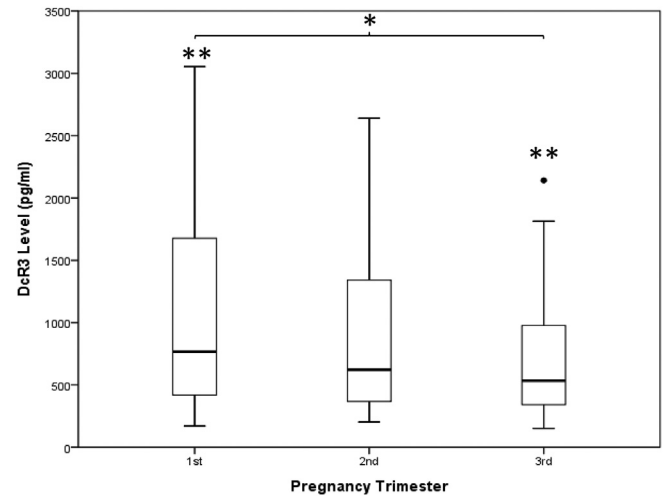


Fig. 1. Association between Decoy Receptor 3 and total days pregnant in normal pregnancies ($n = 104$). Notes. 1. ANOVA analysis: $F(2, 101) = 2.83$; $p = 0.063$. 2. Post-hoc LSD test: 1st Trimester vs. 3rd Trimester; $p = 0.021$.

Results from Shima et al. showed that Treg cells are important in mediating maternal tolerance to the allogeneic fetus during the implantation phase and the early stage of pregnancy, but might not be necessary for maintenance during the late stage of allogeneic pregnancy [34]. In addition, results from Raghupathy et al. showed that significantly higher levels of Th2 cytokines were produced in the first trimester among normal pregnancy group than among the recurrent spontaneous abortion (RSA) group [35]. Furthermore, type 2 cytokines may also induce the release of human chorionic gonadotrophin (hCG) from extravillous trophoblast cells to promote Treg cell migration to the site of contact between paternal antigens and maternal immune cells and to scheme immune tolerance toward the fetus [36]. Furthermore, DcR3 levels in decidua were reported to be significantly lower in anembryonic than in normal pregnancies [30]. Our findings of a higher circulating level of DcR3 during the first trimester of pregnancy may,

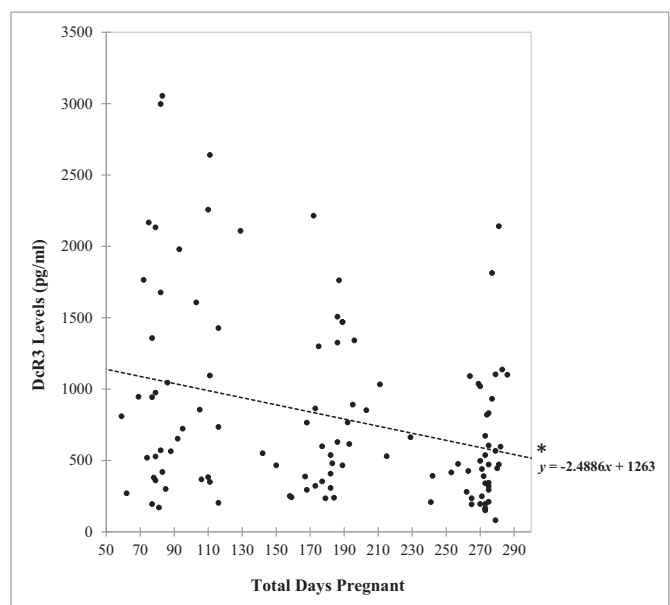


Fig. 2. Association between decoy receptor 3 levels and total days pregnant ($n = 104$). Notes. * Simple Linear Regression: $\beta = -2.05$; 95% CI = -3.66 to -0.44 ; $p = 0.013$.

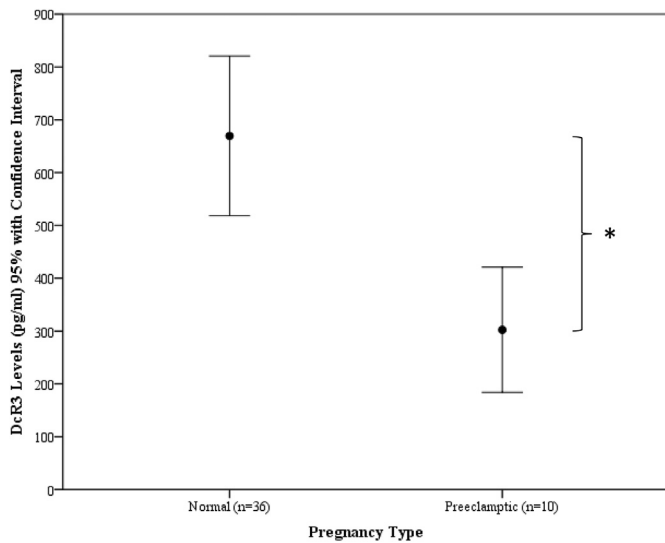


Fig. 3. Mean difference in DcR3 levels between normal and preeclamptic pregnancy types in the third Trimester (n = 46). Notes. *T-test: $t(44) = 2.53$; $p = 0.015$.

thus, contribute to a skew of the immune system towards a Th2-type suppressive responses and a successful pregnancy.

The environment during a pregnancy induces a bias away from the potentially harmful Th1-oriented, or cell-mediated immunity, and in favor of the development of maternal suppressive and Th2-biased immunity, nevertheless, restores a Th1-oriented response that would be required for delivery [37]. The subjects recruited in this study were not in labor. The observed lowest circulating DcR3 level for the third trimester women may contribute to a less suppressive and a more Th1-oriented response for successful delivery. Further studies are required to explore possible relationship between DcR3 and parturition.

The limitation of the current study included (1) a relatively small sample size in the current study (only 10 patients with preeclampsia); (2) no additional T cells-related cytokines tested in the current study; (3) absence of DcR3-associated receptors evaluated in the current study; and (4) absence of a significant trend of decreasing plasma levels of DcR3 in relation to the advanced gestational age in the current study. In fact, there are many studies available to test the relationship between T cell responses and preeclampsia, and results are still conflicted [38]. Some studies showed that a shift towards inflammation in the Th1/Th2 and Th17/Treg balances, because of significant decrease of Th2 and Treg populations in women with preeclampsia [39]; however, some reported that immature dendritic cells (DCs) undergo maturation and initiate a pro-inflammatory response [40]. Excessive LIGHT expression and decreased DcR3 expression in preeclamptic placenta was also demonstrated before [41]. The strength of current study included that (1) the study population was relatively homogeneous and consistent; (2) a longitudinal study from the first trimester to the third trimester. Early prediction of women with preeclampsia is beneficial because early treatment of the high-risk population (for example, use of aspirin) is highly effective in the prevention of the disease [42–45].

Conclusion

Our current study demonstrated a gradual decrease in plasma DcR3 levels from the first to the third trimester during normal pregnancy. Importantly, the circulating DcR3 is lower in pregnancy complicated with preeclampsia than without preeclampsia. Results

obtained from this study may provide information on underlying mechanisms of normal pregnancy and on factors contributing to complications, such as preeclampsia, in pregnancy.

Conflict of interests

The authors declare that they have no competing interests.

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