Elevated maternal levels of the long pentraxin 3 (PTX3) in preeclampsia and intrauterine growth restriction

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Objective: The prototypic long pentraxin pentraxin 3 is a new candidate marker for inflammatory conditions reflecting the involvement of the vascular bed. Endothelial dysfunction is a prominent feature of preeclampsia as a result of excessive maternal systemic inflammation. We investigated pentraxin 3 levels in preeclampsia and intrauterine growth restriction, pregnancy conditions related to altered placentation.

Study design: We cross-sectionally studied nonpregnant women (n = 20); normal pregnancies in the first (n = 8), second (n = 10), and third (n = 26) trimester of pregnancy; 20 pregnancies complicated by preeclampsia; and 16 pregnancies complicated by intrauterine growth restriction. Maternal plasma samples were analyzed and pentraxin 3 determined by enzyme-linked immunosorbent assay. Pattern and site of placental expression of pentraxin 3 were studied by immunohistochemistry.

Results: In normal pregnancies pentraxin 3 concentrations were significantly higher than nonpregnant women and did not change among the 3 trimesters. Significantly higher levels of pentraxin 3 were found in preeclampsia (median values 13.8 versus 2.2 ng/mL; P < .001), compared with normal pregnancies. Intrauterine growth restriction pregnancies showed intermediate levels between normal and preeclamptic patients, but this difference was not significant, compared with normal pregnancies (median values 3.9 versus 2.2 ng/mL). No significant difference of pentraxin 3 levels was found between mild and severe preeclampsia.

Conclusion: Elevated maternal plasma levels of pentraxin 3 in preeclamptic versus normal pregnancies could represent a marker of altered endothelial function, typical of preeclampsia.

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Preeclampsia represents an important cause of maternal as well as perinatal morbidity and mortality. In spite of its relevant epidemiologic impact, the complete pathogenesis of this disease still remains unclear, underlining a multifactorial etiology. The typical clinical manifestations of preeclampsia, including hypertension, proteinuria, and the varying degrees of ischemic peripheral organ damage, which typically arise in the third trimester of gestation, might be late phenomena of the complex process of embryo implantation. Deficient remodeling of the spiral arteries during the interaction between maternal and fetal sides at the time of trophoblast invasion has been postulated as a cause of placental insufficiency. This would lead to the dismissal of inflammatory factors in the systemic maternal circulation. Endothelial dysfunction has been hypothesized to be part of an excessive maternal inflammatory response to pregnancy. Complement activation, activated circulating leukocytes, increased release of reactive oxygen species, and increased levels of various inflammatory cytokines in preeclampsia all agree with this hypothesis.

Intrauterine growth restriction (IUGR) is also an important cause of perinatal mortality and morbidity; infants with this disorder have a greater risk of handicaps in later childhood and cardiovascular-metabolic disease in adult life. IUGR and preeclampsia can occur together or alone sharing placental insufficiency as a common pathogenetic mechanism.

Pentraxin (PTX3) is a recently described inflammatory molecule that belongs to the same family of the well-known C-reactive protein (CRP). PTX3 differs from CRP in terms of cellular origin, molecular inducers, and kinetic of production. It is expressed by different cells like endothelial cells, monocytes, macrophages, and fibroblasts exposed to inflammatory stimuli. PTX3 plasma levels increase dramatically during endotoxic shock, sepsis, or other inflammatory conditions. Recent studies suggest that PTX3 plays an important role in innate immunity, female fertility, and inflammatory processes.

PTX3 is produced at high levels by vessel wall elements, binds to the angiogenic growth factor fibroblast growth factor 2 and tunes its action in vitro and in vivo. PTX3 plasma levels are increased in vascular disorders including myocardial infarction and small vessel vasculitis and correlate with outcome or disease activity. These biological and clinical properties of PTX3 prompted us to investigate this molecule in preeclampsia, a syndrome characterized by a prominent vascular component.

**Material and methods**

**Study population**

Patients were recruited at the Institute of Obstetrics and Gynecology “L. Mangiagalli,” University of Milan. The study protocol was approved by the medical ethics committee of the hospital, and written informed consent was obtained from each woman.

The study population consisted of 20 nonpregnant women, 44 normal pregnancies, 20 pregnancies complicated by preeclampsia, and 16 by IUGR, which were diagnosed and treated in our department.

Nonpregnant women were of reproductive age and were sampled in the follicular stage of the menstrual cycle. In pregnant women gestational age was determined according to the onset of the last menstrual period and by an ultrasonographic examination performed before 20 weeks of gestation.

We enrolled as normal pregnancies women with physiological pregnancies and normal intrauterine fetal growth. Exclusion criteria of this group were previous or current maternal diseases and pharmacologic treatment that could influence pregnancy outcome and fetal growth. Fetal growth was documented by ultrasound in uterus and confirmed by fetal weight at birth between the 10th and 90th percentile according to Italian standards for birth weight and gestational age. All normal pregnancies delivered at term, between 37 and 42 weeks of gestation. Maternal blood samples were cross-sectionally collected in the first (n = 8), second (n = 10), and third trimester (n = 26) of gestation in normal pregnancies. Table I presents the characteristics of normal pregnancies of the 3 trimesters. Maternal age and prepregnancy body mass index (BMI) were similar for the women of the 3 trimesters and were not different from nonpregnant women. Pregnancy outcome was normal for all the control normal pregnant women with delivery of a healthy, term neonate with appropriate weight for gestational age.

Preeclampsia was diagnosed as pregnancy-induced hypertension associated with proteinuria according to the current American College of Obstetricians and Gynecologists guidelines. Hypertension was defined as sustained arterial blood pressure readings of 140/90 mm Hg or greater (with reading taking place 6 h or more apart). Proteinuria was defined as urine protein concentrations of 30 mg/dL or greater (or 1+ on a urine dipstick) on 2 or more random specimens collected 4 hours or more apart or the presence of 0.3 g or more of protein in a 24-hour urine specimen. According to the American College of Obstetricians and Gynecologists practice bulletin criteria of 2002, cases were classified as mild (n = 9) and severe (n = 12) preeclampsia. Preeclampsia has been considered severe if 1 or more of the following criteria were present: blood pressure 160/110 mm Hg or greater on 2 occasions on bed rest; proteinuria 5 g or greater in a 24-hour urine specimen or 3+ or greater on 2 random urine samples; oliguria; cerebral or visual disturbances; pulmonary edema or cyanosis; epigastric or right-upper-quadrant pain; impaired liver function; thrombocytopenia; or fetal growth...
restriction. In 3 cases, severe preeclampsia was also complicated by IUGR.

Pregnancies complicated by IUGR were identified in utero by serial measurements of abdominal circumferences below the 10th percentile of reference values for fetuses of similar gestational ages and by a decrease of more than 40 percentiles from their growth curve. Growth restriction was confirmed at birth if the neonatal weight was below the 10th percentile according to Italian standards for birth weight and gestational age. At birth all IUGR fetuses had normal karyotype and none of them presented major malformations.

Three cases of IUGR and 1 case of preeclampsia were complicated by intrauterine fetal death (IUFD) in the second trimester. In these cases maternal blood samples were collected at 23.2 ± 1.4 (22.1-25.3) weeks of gestation, and fetal intrauterine death occurred at 24.1 ± 2.2 (22.4-27) weeks.

**Blood sampling and PTX3 measurement**

Maternal blood samples were collected in the first, second, and third trimester of gestation in normal pregnancies at the time of their routine obstetric visit at the hospital. Samples of preeclamptic and IUGR pregnancies were collected at the time of diagnosis of the gestational disease, before starting antihypertensive therapy. Maternal blood was drawn from a brachial vein under fasting conditions and centrifuged. Plasma was then stored at −80°C until analysis.

The sandwich enzyme-linked immunosorbent assay (ELISA) for PTX3 was performed as previously described. Briefly, ELISA plates (96 well; ImmunoPlate, MaxiSorp, Nunc, Roskilde, Denmark) were coated with 100 ng/well of rat monoclonal anti-PTX3 antibody MNB4 diluted in coating buffer (15 mM carbonate, Na2CO3 + NaHCO3, buffer pH 9.6) and were incubated overnight at 4°C. The plates were washed with washing buffer (Dulbecco’s phosphate-buffered medium containing 0.05% Tween 20) and 300 µL of 5% dry milk were added to block nonspecific binding sites. Fifty microliters of recombinant human PTX3 standards (100 pg/mL to 2 ng/mL) and unknown samples were added in duplicate and incubated for 2 hours at 37°C. After 3 washes with the washing buffer, 25 ng/well of biotin-conjugated PTX3 affinity-purified rabbit IgG were added for 1 hour at 37°C. Wells were extensively washed and incubated with 100 µL of streptavidin-peroxidase conjugated to dextran backbone (AmDex, Copenhagen, Denmark) diluted 1:4000 for 1 hour at room temperature. After incubation the plates were washed 4 times and 100 µL of Tetramethyl-Benzidine chromogen (BD Pharmingen, San Diego, CA) were added. Absorbance values were read at 405 nm in an automatic ELISA reader. The within- and between-assay coefficients of variation were both below 10%.

**Immunohistochemistry**

Pattern and site of expression of PTX3 were studied by immunohistochemistry on placental samples of normal, IUGR, and preeclamptic pregnancies collected at the time of cesarean section. The pathologist was blinded with regard to the groups from which the samples were derived.

Placental samples were fixed in neutral buffered formalin and embedded in paraffin; hematoxylin-eosin-stained sections were examined for histological evaluation. For immunohistochemical analysis, 3 µm paraffin-embedded sections were cut and mounted on Superfrost slides (Bio-Optica, Milan, Italy); after dewaxing in xylene and rehydrating in ethanol, the sections were pretreated in a microwave oven (2 cycles for 5 minutes each at 780 W in 0.01 M citrate buffer) and incubated for 2 hours with PTX3 affinity-purified rabbit immunoglobulin G against human PTX3. The reactions were revealed by nonbiotin peroxidase detection system with 3,3’-diaminobenzidine freebase as chromogen. Negative controls were obtained by omission of the primary antibody.

**Statistical analysis**

Sample size was calculated on the basis of the primary aim of the study (difference between pregnant and nonpregnant women). Stating as clinically relevant an
at least 2-fold increase in serum levels of PTX3 and setting type I and II errors at 0.05 and 0.20, respectively, we calculated the required sample size should be about 10 cases per group for each comparison (nonpregnant versus first, second, and third trimester).

Data concerning the clinical characteristics of the population are presented as mean ± SD and compared by unpaired Student’s *t* test. PTX3 data in pregnant women at different trimesters and in preeclampsia, IUGR, and normal pregnancies of the third trimester are presented as median with interquartile range and compared by nonparametric Mann-Whitney *U* test. PTX3 results are presented as median with interquartile ranges.

### Results

#### Characteristics of the third-trimester population

Table II shows the clinical characteristics of the preeclampsia and IUGR groups, excluding the cases of IUFD and of preeclampsia associated with IUGR, compared with normal pregnancies of the third trimester. Gestational age at sampling was similar in subjects and controls. IUGR mothers were significantly older than those with normal pregnancies (34.2 ± 3.7 versus 29.7 ± 5.9 years, *P* < .05).

Nine of the 26 normal third-trimester pregnancies were delivered by cesarean section because of breech presentation or previous cesarean section. Preeclamptic and IUGR pregnancies were delivered significantly earlier than normal pregnancies (32.1 ± 3.6 and 35.3 ± 2.5, respectively, versus 39.2 ± 1.0 weeks of gestation, *P* < .001) and always by cesarean section because of fetal compromise or maternal indications, according to our clinical protocol. Gestational age at delivery was significantly lower in preeclampsia versus IUGR (32.1 ± 3.6 versus 35.3 ± 2.5 weeks, *P* < .01). Fetal and placental weights were significantly lower in preeclampsia and IUGR groups, compared with normal pregnancies. As expected, all IUGR fetuses had a birth weight below the 10th percentile for gestational age.

#### PTX3 in nonpregnant women and normal pregnancies in first, second, and third trimester

In normal pregnancies PTX3 levels do not change significantly among the 3 trimesters (first trimester: 2.8 ng/mL [1.4-4.6]; second trimester: 2.1 ng/mL [1.4-2.2]; third trimester: 2.2 [1.2-3.8] ng/mL expressed as median values and interquartile range). These values are significantly higher than values in nonpregnant women (1.0 [0.6-1.4] ng/mL) (*P* < .001 versus first, second, and third trimesters).

Individual values and medians of PTX3 throughout gestation in normal pregnancies and in the follicular stage of nonpregnant women are shown in Figure 1.

#### PTX3 in pregnancies complicated by IUGR and preeclampsia

Table II and Figure 2 present PTX3 concentrations in preeclampsia and IUGR pregnancies versus normal pregnancies expressed as median and interquartile range, respectively. Preeclamptic patients show significantly higher levels of PTX3 than normal pregnancies of the same sampling gestational weeks. In Figure 2, subjects with only preeclampsia and subjects with both preeclampsia and IUGR are presented separately. Both groups present significantly higher levels of PTX3 than control cases (only preeclampsia: see Table II;
preeclampsia with IUGR 16.2 [8.9-25.0] ng/mL; \( P < .001 \) versus controls). IUGR pregnancies show intermediate values between normal and preeclamptic pregnancies (3.9 [2.2-8.2] ng/mL), but the difference failed to reach statistical significance. No significant difference was observed between mild and severe preeclampsia PTX3 levels (data not shown) or in subjects with preeclampsia and IUGR (Figure 2). Although some PTX3 values in the preeclampsia group are very high, at least half appear to be within the normal range (Figure 2). Also in the IUGR group, single data show overlap with the normal third-trimester group. PTX3 values in pregnancies with IUFD were not significantly different from normal pregnancies of the second trimester as shown in Figure 2. It is noteworthy that the patient who developed eclampsia showed the highest observed PTX3 value (106.5 ng/mL).

Immunohistochemistry conducted on placental tissues revealed no differences of PTX3 distribution in normal, IUGR, and preeclamptic placentas. In both control and study subjects, immunostaining for PTX3 was localized in the stromal tissue of the stem villi (Figure 3, panels a and c) and in the anchoring villi (Figure 3, panels b and d) in a predominant way. Lower immunostaining was found in the matrix of some terminal villi (not shown). PTX3 reactivity was also observed in placental macrophages (data not shown).

Comment

To our knowledge, this is the first report investigating PTX3 in normal and complicated pregnancies. Previous studies, both in vitro and in vivo, have focused on the role of this inflammatory molecule in conditions involving the systemic inflammatory process like infections, sepsis, fertility, atherosclerosis, and myocardial infarction.9-12

The aim of this study was first to define whether pregnancy itself, a condition associated with relevant involvement of inflammatory molecules at the implantation site, is associated with changes in maternal circulating PTX3 levels, compared with the nonpregnant condition. Our results show that PTX3 levels do not change significantly throughout the 3 trimesters of normal pregnancies. However, average values in the first, second, and third trimesters are slightly higher (2.1-2.8 ng/mL) than levels tested in normal nonpregnant women of reproductive age (ie, less than 1.5 ng/mL). This difference could be part of the physiologic maternal inflammatory response to pregnancy, when the immune and inflammatory systems are involved in deep changes.23

We also report higher maternal PTX3 levels in pregnancies complicated by preeclampsia.
Similarly, another acute-phase protein, CRP has been described to be elevated in third-trimester preeclampsia. Our results are in line with the hypothesis that preeclampsia represents the clinical manifestation of an endothelial dysfunction as part of an excessive maternal inflammatory response to pregnancy. This endothelial dysfunction would lead to the activation and dismissal of inflammatory factors, like cytokines and growth factors (tumor necrosis factor-alpha, interleukin-1), in the systemic maternal circulation. The increase of inflammatory molecules released in the first trimester causes the activation of a cascade of systemic factors leading to an unbalance between vasodilating and vasoconstrictor molecules giving rise to the clinical manifestation of preeclampsia in the third trimester.

On the basis of our results, PTX3 may represent an inflammatory molecule involved in this complex mechanism. Previous studies have demonstrated in vitro a relevant expression of PTX3 by macrophages, endothelial cells, and smooth muscular cells belonging to advanced atherosclerotic plaques of ill arteries versus healthy vessels. Moreover, PTX3 binds fibroblast growth factor 2 and tunes its action on the vessel wall.

In particular, a prognostic value of the long pentraxin PTX3 molecule in acute myocardial infarction has been hypothesized: the early plasmatic rise of PTX3 in patients affected by acute myocardial infarction seems to predict a worse long-term outcome in these patients. Because preeclampsia shares the inflammatory basis with the atherogenic process, we can hypothesize a role of this molecule in the endothelial dysfunction typical of preeclampsia.

If raised circulating PTX3 were to be prognostic, rather than reactive, one would expect some correlation with disease severity. In this study, however, we failed to observe differences in PTX3 maternal levels according to clinical severity, except for one case of maternal eclampsia, which showed the highest observed value.

On the contrary, the lack of significant differences observed in the IUGR group led us to hypothesize that the role of this protein is not specifically at the placental level because placental insufficiency is a common feature of both IUGR and preeclamptic disease. Besides a shallow trophoblast invasion leading to poor placental perfusion, these diseases are associated with high levels of apoptosis in placenta. However, whereas IUGR is a typical manifestation of placental damage on the fetal side, leading to reduced fetal growth, preeclampsia involves more specifically the maternal compartment. In our results the severity of the disease does not seem to be associated with maternal PTX3 levels, as shown by the 4 subjects complicated by IUFD. In all cases, IUFD occurred early, before viability, likely as expression of a severe placental damage. Our speculations are in line with the results obtained by immunohistochemistry on placentas of normal, IUGR, and preeclamptic pregnancies: pattern and site of expression of PTX3 revealed no differences between normal and pathologic placental tissues. These results suggest that PTX3 is increased in maternal blood because of an inflammatory response of the mother to placental debris released during preeclampsia.

In summary, the results of this study suggest a role whether causal or consequential of PTX3 in pregnancy complications. The elevated maternal plasma levels of PTX3 in preeclamptic versus normal pregnancies might be a marker of altered endothelial function, typical of preeclampsia.

Further studies are necessary to clarify its exact function in the etiopathogenetic mechanism of altered placentation as well as to define whether the main site of synthesis of PTX3 is the placental unit, the maternal-fetal interface, or the systemic endothelium. This evidence might have significant clinical implications in the early diagnosis of preeclampsia. Appropriately designed prospective studies are needed to evaluate whether PTX3 might represent an early marker of the disease for the development of adequate monitoring and therapies.

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References


