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Pentraxin-3 concentration in the amniotic fluid of women at term, in spontaneous preterm labor and when not in labor

Laura F. Martin [a], Natália P. Moço [a], Bruna R.A. Ramos [a], Rodrigo P.S. Camargo [b], Márcia G. Silva [a,*]

[a] Department of Pathology, Botucatu Medical School, São Paulo State University, UNESP, Botucatu, São Paulo, Brazil
[b] Jundiaí Medical School, Jundiaí, São Paulo, Brazil

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ABSTRACT

Objective: To evaluate the concentration of PTX3 in amniotic fluid (AF) during the final weeks of normal pregnancies and in pregnancies complicated by preterm delivery (PTD).
Study design: A cross-sectional study was conducted with 95 pregnant women followed to term and 25 who presented with PTD. Samples of AF from all patients were obtained during cesarean section and the PTX3 concentration was determined by enzyme immunoassay (ELISA). Maternal characteristics were compared by ANOVA and the Kruskal–Wallis and Chi square tests. Comparison between PTX3 concentrations in the "PTD in labor" and "PTD not in labor" groups were performed using the Mann–Whitney test. A p value <0.05 was considered statistically significant.
Results: Regarding term pregnancies, PTX3 concentrations were not statistically different across the period studied (37 weeks to 40 weeks). Among preterm pregnancies, those in preterm labor (PTL) presented higher PTX3 levels than those not in labor ($p = 0.001$) and the risk of occurrence of PTL increased by 1% with a rise of 1 pg/mL in PTX3.
Conclusion: PTX3 is a physiological constituent of the AF, and its concentration is elevated in the presence of spontaneous PTL, reinforcing the theory that PTX3 plays a role in the innate immune response during gestational complications associated with infectious/inflammatory conditions.

Introduction

The innate immune system is the first line of defence against potentially harmful pathogens. Components of the immune innate system recognize infection through pattern recognition receptors (PRRs), which bind to pathogen-associated molecular patterns (PAMPs) and lead to an inflammatory response [1,2]. Pentraxin-3 (PTX3) is a soluble PRR of humoral immunity that is produced and released by several cells, in particular mononuclear phagocytes, dendritic cells, fibroblasts and endothelial cells [3–6], in response to inflammatory mediators, such as interleukin (IL)-1β and tumor necrosis factor α (TNF-α), activating Toll-like receptors (TLR). PTX3 recognizes microbial products, opsonized fungi, selected gram-positive and gram-negative bacteria and viruses, and activates the complement system [7,8].

PTX3 is constitutively produced in normal circumstances and increases considerably and rapidly under inflammatory and infectious conditions [9]. Elevated concentrations of PTX3 have been reported in pregnant women compared with non-pregnant women [10] and although changes in these concentrations throughout pregnancy are not well established, plasma PTX3 levels are significantly elevated during labor at term [11,12].

Numerous studies have demonstrated the association between intra-amniotic infection and prematurity [13–20]. The principal pathway by which microorganisms access the amniotic cavity is the ascent of bacteria present in the lower genital tract. These microorganisms can traverse the endocervical canal, penetrate the membranes and invade the amniotic cavity. Thus, bacteria can proliferate in the amniotic fluid and spread within the amniotic membranes, activating the TLR signaling pathway, which leads to the production and release of cytokines, prostaglandins and other inflammatory mediators which are strongly associated with the early activation of parturition [21].

Spontaneous preterm labor (PTL) and preterm premature rupture of membranes (pPROM) have been associated with significant increases in maternal plasma concentrations of PTX3 [12,22]. Moreover, pregnancies with these complications and the concomitant presence of intra-amniotic infection have been

* Corresponding author. Tel.: +55 14 3880 1580; fax: +55 14 3815 2348.
E-mail address: mgsilva@fmb.unesp.br (M.G. Silva).

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associated with higher levels of PTX3 in the amniotic fluid (AF) at term compared with uncomplicated pregnancies [14,23]. The aim of this study was to determine the concentrations of PTX3 in amniotic fluid in the final weeks of the third trimester of normal pregnancy and in pregnancies complicated by preterm labor, and to evaluate whether there are any associations between PTX3 concentrations and preterm labor.

Material and methods

A cross-sectional study was conducted in Jundiaí Medical School, State of São Paulo, Brazil and in the Laboratory of Immunopathology of the Maternal–Fetal Relationship of the Department of Pathology of Botucatu Medical School, São Paulo State University (UNESP), from 2011 to 2013.

A total of 95 pregnant women who delivered at term and 25 women who had preterm delivery (PTD) were included in this study. Gestational age was calculated from the first day of the last menstrual period and/or by first-trimester ultrasound examination. Term samples were obtained from patients undergoing elective cesarean section in the following groups: 37 weeks ($n = 15$), 38 weeks ($n = 34$), 39 weeks ($n = 31$) and 40 weeks ($n = 15$). Preterm samples were obtained from patients in labor (PTD in labor, $n = 10$) and not in labor (PTD not in labor, $n = 15$). PTD patients not in labor underwent elective cesarean section due medical indications such as acute fetal distress, diabetes and preeclampsia.

Exclusion criteria for the term and PTD in labor groups were multiple pregnancies, diabetes, preeclampsia, fetal anomalies, intrauterine growth restriction, placental abruption, placenta previa and patients who were HIV positive. The Institution's Human Research Ethics Committee approved the study (DOC-CEPFMJ 0325-13) and written informed consent was obtained from all the participants.

Biological sample collection

Amniotic fluid was collected from all patients during cesarean section following uterotomy and before amniotomy, using a sterile syringe. The samples were frozen at −80 °C until analysis.

Enzyme-linked immunoassay (ELISA)

PTX3 concentration in AF was measured using a commercially available ELISA kit (R&D Systems, MN, USA), following the manufacturer's instructions. A standard curve was obtained in parallel to each assay and the results were converted to pg/mL. Absorbance values were read at 450 nm in an automatic ELISA reader (Biotek Instruments Inc, BE, USA). All the samples were tested in duplicate and those with values above the standard curve range were diluted (1:5) and retested. The minimum detectable level for PTX3 assays was 44 pg/mL. Intra- and inter-assay variability remained <10.0% for PTX3.

Statistical analyses

Maternal age and gestational age at delivery were compared between groups using ANOVA and Kruskal–Wallis tests, respectively. Marital status and parity were compared by the Chi square test. The normality of the data was checked using the Kolmogorov–Smirnov test. Comparisons of PTX3 concentrations among the term groups were performed using the nonparametric Kruskal–Wallis test. Comparisons between PTX3 concentrations in the PDT in labor and PDT not in labor groups were performed using the nonparametric Mann–Whitney test. A p value <0.05 was considered statistically significant. The software used was SigmaStat Software version 3.1. A logistic regression model was employed to examine the association between PTX3 concentration and PTD in labor. These data were processed using the SPSS processing software.

Results

Sociodemographic and obstetric characteristics

The characteristics of the pregnant women included in the study are presented in Table 1. Maternal age, marital status and parity were similar between the groups. Gestational age at delivery was similar between the PTD in labor and PTD not in labor groups.

PTX3 concentration in amniotic fluid

In this study, PTX3 was detected in 91.5% of the term AF samples and in 88.0% of the preterm samples. Regarding term pregnancies, PTX3 levels increased slightly over time, but these levels were not statistically different ($p = 0.321$) during the final weeks of normal pregnancies (Fig. 1).

Considering only the PTD groups, higher PTX3 concentrations were determined for those presenting in spontaneous preterm labor than those not in labor ($p = 0.001$) (Fig. 2). Moreover, the risk of occurrence of preterm labor increased by 1% with a rise of 1 pg/mL in PTX3 in the AF samples (Table 2).

Comment

Inflammation and the activation of the innate immune system are important to assure the success of pregnancy, since complex interactions between the maternal immune system and fetal cells promote the survival and proper development of the fetus. Fetal cells express paternal alloantigens that are not recognized as foreign by the mother due to the physical and immunological barrier represented by the maternal–fetal interface. This role of the maternal–fetal interface is essential as it ensures the proper development of the pregnancy by promoting tolerance to allografts, while maintaining the maternal immune response against invasion of the amniotic cavity by pathogens [24,25].

Table 1
Sociodemographic and obstetrical characteristics of the pregnant women included in the study.

Characteristics	Preterm in labor ($n = 10$)	Preterm not in labor ($n = 15$)	Term not in labor ($n = 95$)	p-Value
Maternal age*	22.9 ± 8.4[a]	29,3 ± 7.8[a]	26.6 ± 6.2[a]	0.06
Marital status				
Single	20.0% (2/10)[a]	13.3% (2/15)[a]	13.7% (13/95)[a]	0.95
Married	80.0% (8/10)[a]	86.7% (13/15)[a]	86.3% (82/95)[a]	0.95
Gestational age at delivery (days)**	231 (184–257)[b]	250 (216–258)[b]	272 (259–288)[a]	<0.001
Nulliparity	60.0% (6/10)[a]	13.3% (2/15)[a]	40.2% (37/92)[a]	0.39

Values followed by the same letter do not differ.
* Values expressed as mean ± SD.
** Values expressed as median (range).