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Insulin-like Growth Factor Binding Protein 1 (IGFBP-1) in Vaginal Fluid in Pregnancy

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Abstract. *Objective: To examine vaginal insulin-like growth factor binding protein 1 (IGFBP-1) as a marker of preterm delivery, amniotic fluid leakage or vaginal infection. Materials and Methods: The material consisted of a nested case-control study (67 with idiopathic preterm delivery and 406 randomly selected women with term deliveries) from a prospective cohort of 2,846 women. Results: Vaginal npIGFBP-1 was weakly associated with preterm delivery. Elevated npIGFBP-1 gave an increased risk of preterm delivery (OR 2.8 [1.4-6.7]). Elevated IGFBP-1 provided a sensitivity of 13% at a false-positive rate of 4.4% in a low-risk population. When adjusting for other co-variates associated with preterm delivery the association between npIGFBP-1 and preterm delivery disappeared; there was an interaction between alcohol consumption and npIGFBP-1. Conclusion: The current study found vaginal npIGFBP-1 to be weakly associated with gestational age at delivery. However, in this study the association may potentially be explained by alcohol consumption.*

Preterm birth (delivery before 37 weeks gestation) is the leading cause of neonatal morbidity and mortality. Attempts to predict preterm delivery have largely been unsuccessful and improvements in the rates of preterm delivery have not been achieved in recent decades.

The insulin-like growth factor (IGF) system comprises low molecular weight peptides that promote growth and differentiation. In the circulation and in various tissues, the IGFs are bound to specific IGF-binding proteins (IGFBPs) of which six high-affinity forms (IGFBP-1 to -6) have been characterized (1). In the circulation, these IGFBPs are believed to prolong the half-life and to regulate the endocrine effects of the IGFs. IGFBP-1 is believed to be important for the biologically active IGF-I as the free fraction of IGF-I is inversely correlated to IGFBP-1 (2).

Non-phosphorylated IGFBP-1 (npIGFBP-1) is found in 100- to 1000-fold higher concentrations in amniotic fluid compared to serum values and, as npIGFBP-1 leaks through the cervix, elevated concentrations of npIGFBP-1 can be used as a test for preterm pre-labor rupture of membranes (PPROM) (3). Elevated *phosphorylated* IGFBP-1 (pIGFBP-1) in cervical secretions has been described as a predictor of preterm delivery among women in labor (4,5). Further, increased levels of pIGFBP-1 in cervical secretions have been found to predict cervical ripening at term (6).

Our primary hypothesis was that in early pregnancy npIGFBP-1 in vaginal fluids was associated with preterm delivery. As npIGFBP-1 is found in high concentrations in amniotic fluid it can be used as a test for PPROM. We hypothesized that npIGFBP-1 could be an early marker of amniotic leakage caused by micro-ruptures of the fetal membranes, and/or that npIGFBP-1 was a marker of vaginal infection.

Thus, npIGFBP-1 was examined in vaginal fluids in a nested case-control study to evaluate the association with gestational age, PPROM and vaginal infections.

Materials and Methods

Patient population and recruitment. Pregnant women attending prenatal care at the Department of Obstetrics and Gynaecology, Odense University Hospital, Denmark, were invited to participate in the study during the period from November 1992 to February 1994. The pregnant women were enrolled at their first antenatal hospital visit before 24 full weeks of gestation. The inclusion criteria required the participants to be at least 18 years of age, able

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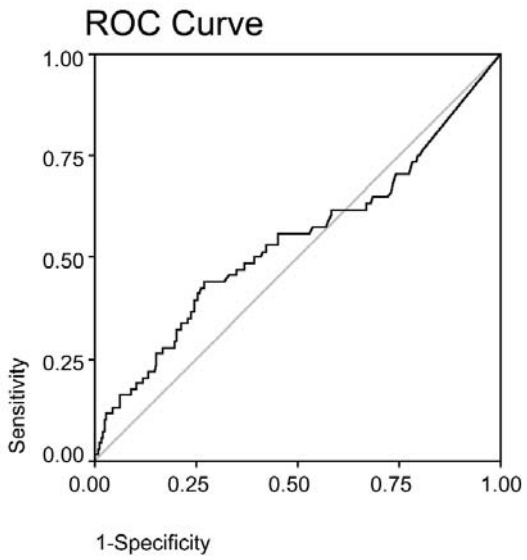

Figure 1. *ROC curve depicturing the predictive abilities of vaginal npIGFBP-1 as a single early predictor of preterm delivery (< 37 weeks gestation). Area under the curve (0.54 [95% CI: 0.46-0.62]) was not significantly different from 0.5. N=473.*

to understand Danish, and intending to deliver at Odense University Hospital. Women with multiple gestations, diabetes or a history of severe congenital malformations from previous pregnancies were excluded.

Upon enrolment, a vaginal swab was obtained. Samples were collected from the cervical os and the posterior fornix after the vault of the vagina had been exposed to a sterile non-lubricated vaginal speculum. The sample for npIGFBP-1 was collected with a sterile, cotton-tipped wooden swab and was inoculated directly into 1 ml of sodium chloride solution (0.9%) containing 2% sterile calf-serum (Life Technologies Inc., Gaithersburg, MD, USA). Immediately after collection, the sample was frozen to -80˚C until thawed for npIGFBP-1 testing. The methods for collecting cervical or vaginal samples for individual microorganisms have been described previously (7). Participants were asked to complete self-administered questionnaires by filling in basic demographic information and medical history at enrolment, at 30 weeks gestation and at delivery. At the time of delivery, the attending midwife completed a brief form concerning the clinical aspects of the delivery and course of the pregnancy. A total of 2,846 women with singleton pregnancies completed the study with a participation rate of 81.4%. The study has been described elsewhere (8).

Vaginal swabs were obtained at enrolment from 84 out of 89 participants who later experienced idiopathic preterm delivery. Samples from 67 cases were available, whereas the remaining samples either contained too little liquid for analysis or were missing. Idiopathic preterm delivery is here defined as non-medically indicated delivery before 37 weeks' gestation. The control samples (n=406 found out of 420 selected) were found among the women delivering at term by computer-generated random selection. The women (n=17 preterm, 32 term), from whom vaginal samples were not available, did not differ in gestational age at delivery or sampling, when compared with their group.

All women gave written informed consent as required by the local Regional Scientific-Ethics Committee for the counties of Vejle and Funen, Denmark, and the Institutional Review Board for research on human subjects, Centers for Disease Control and Prevention, Atlanta, USA. Data collection was approved by the Danish Data Surveillance Authority in accordance with their standards for multi-purpose register studies.

Gestational age and estimated date of delivery were calculated from the last menstrual period (LMP), adjusted to a cycle length of 28 days according to Nägele's rule, and verified by ultrasound scanning (fetal biparietal diameter) before 21 completed weeks of gestation. If discrepancies were found, the ultrasound data were used. The gestational age was based solely on LMP calculations for 13 women who had no available ultrasound measurements; 11 with term deliveries and 2 women delivering preterm.

Gestational age at enrolment was 16 weeks + 4 days [range 7+4–24+2] and did not differ between the cases and controls.

Laboratory techniques. npIGFBP-1 was measured by a commercially available assay (IGFBP-1 test kit 10851ETMB, Medix Biochemica, Kauniainen, Finland). This assay predominantly recognizes the less and non-phosphorylated forms of IGFBP-1 present in amniotic fluid, but not the entirely phosphorylated tissue form found in *e.g.* cervical secretions. No cross-reactions with IGFBP-2, IGFBP-4, IGFBP-5 or IGFBP-6 have been observed. By optimizing the ELISA, the detection limit of the assay was 0.05 μg/l, and the intra and interassay coefficients of variation were less than 5 and 10%, respectively.

Albumin was measured by an in-house radioimmunoassay (9) with a detection limit of 0.1 mg/l. The intra and interassay coefficients of variation were less than 5% and 10%, respectively. The assay is specific for human albumin.

Samples for microbiological culture were collected with a sterile, cotton-tipped stick from the cervical os and the posterior fornix to test for aerobe and anaerobe bacteria. Bacterial vaginosis was defined using the criteria by Amsel (10).

Statistics. npIGFBP-1 did not follow a normal distribution and was not easily transformable as 21% had immeasurable npIGFBP-1 levels. Therefore, only non-parametric and Cox-regression analyses were applied. Statistical analysis was performed in the statistical software package, SPSS system version 9.0.

Results

npIGFBP-1 was immeasurable in 14 samples from cases (21%) and 94 samples from controls (21%). In order to control for potential dilution problems, the concentration of albumin was measured. Albumin was evenly distributed among cases and controls (median 4.9 mg/l both for cases and for controls (range=detection limit-0.5 mg/l). Twenty-two (3 cases and 19 controls) had immeasurable albumin levels and, of these, five also had immeasurable npIGFBP-1 levels (23%), which is a comparable fraction to the women with measurable albumin. Thus, there is no reason to believe that a small sample volume caused immeasurable npIGFBP-1. Subsequently, all samples were used for all following analyses.

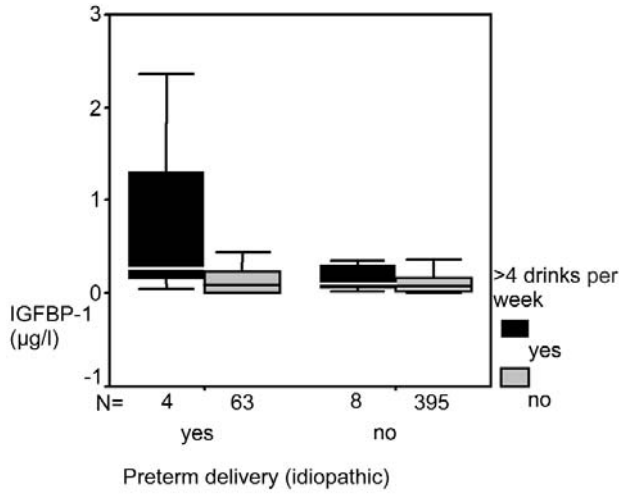

Figure 2. *npIGFBP-1 in vaginal fluids in early pregnancy was associated with the number of drinks consumed per week during pregnancy (Spearman p<0.05). The figure shows the distribution of npIGFBP-1 (µg/l) by alcohol consumption (> 4 drinks per week). Boxes indicated median, 5, 25, 75 and 95 percentiles.*

The npIGFBP-1 level in vaginal fluid obtained in the 17th week of pregnancy was weakly associated with gestational age at delivery (Cox regression, r=0.031, $p<0.05$). Figure 1 depicts a ROC curve demonstrating the predictive abilities of vaginal npIGFBP-1 as a single early predictor of preterm delivery (< 37 weeks gestation). Area under the curve (0.54 [95%CI: 0.46-0.62]) was not significantly different from 0.5 (N=473). Using the ROC-curve the optimal cut-off of IGFBP1 was found to be at 1.14 µg/l. Elevated npIGFBP-1 (>1.14 µg/l) gave a significantly increased risk of preterm delivery (Cox-regression, crude HR 2.8 [95% CI: 1.4-5.7]). Elevated IGFBP-1 provided a sensitivity of 13% at a false-positive rate (1-specificity) of 4.4%. The positive predictive value was thus 33%.

Thus, the association between npIGFBP-1 and gestational age was evaluated on the base of all other co-variates. npIGFBP-1 in vaginal fluid obtained in the 17th week of pregnancy was not significantly different in women where delivery commenced with rupture of membranes compared to contractions (0.084 *vs.* 0.082 µg/l, NS). Vaginal npIGFBP-1 was not associated with any examined microorganisms or microbiological conditions (anaerobe bacteria, bacterial vaginosis, *Bacteroides spp., Candida albicans, Chlamydia trachomatis, Eschericia coli, Enterobacteria faecalis, Gardnerella vaginalis, Group B streptococci, Lactobacillus spp., Micrococci spp., Non-hemolytic streptococci, Staphylococcus aureus, Ureaplasma urealyticum or Corynebacteria spp*). npIGFBP-1 was only correlated with alcohol consumption (Spearman r=0.089, $p<0.05$) besides gestational age.

The following co-variates were all individually associated with gestational age at delivery using Cox-regression; previous preterm delivery [HR 3, 95% CI 1.6-6], high alcohol intake (> 4 drinks per week [HR 2.8, 95% CI 1.04-7.8]), high sexual activity (> twice per week in early pregnancy [HR 2, 95% CI 1.2-3.1]), socio-economic status [HR 1.2, 95% CI 1-1.5] and *Corynebacteria spp.* in vaginal fluids [HR 0.3, 95% CI 0.1-0.95].

When combining these factors in one model using Cox-regression, npIGFBP-1 was reduced to a non-significant variable, due to a significant interaction with high alcohol intake. Other interactions among these variables were not statistically significant. Figure 2 shows the distribution of npIGFBP-1 according to the womens' alcohol consumption.

Discussion

In the present study, we found npIGFBP-1 in vaginal fluids in early pregnancy to be weakly but statistically significantly associated with gestational age at delivery. There was no association between npIGFBP-1 and vaginal microorganisms or between npIGFBP-1 and PPROM.

npIGFBP-1 is found in high concentrations in amniotic fluid, and npIGFBP-1 in vaginal fluids can be used to verify equivocal Preterm Prelabor rupture of Membranes (PPROM). Fetal fibronectin, which is also a predictor of preterm delivery, is also found in high concentrations in amniotic fluid (11). The presence of fetal fibronectin in the vaginal environment has been hypothesized to be because of a leakage of amniotic fluid through micro-ruptures in the fetal membranes. Accordingly, we hypothesized that elevated npIGFBP-1 would be a marker of early leakage of amniotic fluid. If npIGFBP-1 in vaginal fluids was caused by micro-ruptures in the fetal membranes, one would expect npIGFBP-1 to be associated with an increased risk of PPROM. However, no association was found.

Elevated *phosphorylated* IGFBP-1 (pIGFBP-1) in cervical secretions has been described as a predictor of preterm delivery among women in labor (4,5). Further, increased levels of pIGFBP-1 in cervical secretions have been found to predict cervical ripening at term (6). pIGFBP-1 is produced by decidualized endometrium.

The assay used in the current study predominantly recognizes the less and non-phosphorylated forms of IGFPB-1 present in amniotic fluid, but not the entirely phosphorylated IGFBP-1 found in vaginal fluids (data from Medix Biochemica, Kauniainen, Finland). Vaginal npIGFBP-1 obtained in early pregnancy was, in the current study, found to be weakly associated with preterm delivery in a low-risk population. However, when examining the predictive abilities of npIGFBP-1 using a ROC-curve, npIGFBP-1 was not useful for clinical use as a single predictor in low-risk pregnancies as the sensitivity was only

13% at a false-positive rate of 4.4%. Further, the area under the ROC-curve was not significantly different to 0.5. It still remains to be examined if npIGFBP-1 has predictive abilities in women in preterm labor as pIGFBP-1. npIGFBP-1 was found only to be associated with alcohol consumption besides gestational age.

High alcohol intake is a well-known cause of preterm delivery (12) and low birth weight (13). In the current study the alcohol intake was estimated using a questionnaire. The validity of self reported alcohol intake has previously been examined and found useful in a Danish population (14). The current study confirms the association between high alcohol intake (>4 drinks a week) and the risk of preterm delivery. When adjusting for alcohol consumption, the association between npIGFBP-1 and gestational age at delivery disappeared. npIGFBP-1 was significantly correlated to the number of drinks consumed per week. The high alcohol consumption of more than four drinks per week was only found in a very limited group of the study population (N=12 total, of these 4 preterm). The association between alcohol and high npIGFBP-1 has also been found in other studies (15-17). One may hypothesize that elevated npIGFBP-1 levels are a consequence of the intrauterine growth restriction found in fetuses exposed to high alcohol consumptions.

IGF-I is a growth hormone-dependent anabolic hormone stimulating longitudinal growth and protein synthesis after birth. IGFBP-1 is an important IGFBP, which is believed to be the main regulator of free IGF-I in serum; thus high IGFBP-1 levels will result in a low bio-availability of IGF-I (2). The exact role of IGF-I in fetal and placental development remains poorly understood. In pregnancy, maternal serum concentrations of both IGF-I and IGFBP-1 increase (18). In human cord blood, IGF-I is positively correlated with birth weight (19), whereas low IGF-I and elevated IGFBP-1 are associated with intrauterine growth restriction (20). IGF-I in human cord blood is affected by fetal nutritional status (21), and in transgenic mice overexpression of IGFBP-1 causes growth restriction (22).

Kekki *et al.* found pIGFBP-1 and bacterial vaginosis to be independent and additive predictors of preterm delivery (23). The current study only had nine women with bacterial vaginosis who delivered preterm and among these only four had elevated npIGFBP-1, which did not provide us with enough data for a reliable analysis (OR=1.2 [0.5-2.9]).

In the current study it was possible to examine the association between npIGFBP-1, infectious parameters and different variables known to be associated with preterm delivery. One major limitation of the study was the variability of sample dilution due to sampling procedure, where a swap was soaked in vaginal fluids at fornix posterior, and thereafter diluted in a constant volume (1 ml). A fifth of the samples had immeasurable npIGFBP-1 levels and, thus, it cannot be excluded that undetectable levels were not due to dilution of samples below the detection limit. However, albumin levels showed an equal distribution in samples with undetectable npIGFBP-1 compared to samples with detectable npIGFBP-1.

In conclusion, in the current study, vaginal npIGFBP-1 obtained in 17th gestational week was weakly associated with gestational age. The predictive abilities of npIGFBP-1 as a single early predictor of preterm delivery in low-risk pregnancies were, however, not good enough for clinical use. npIGFBP-1 was elevated in samples from women with a high alcohol intake. The association of a major growth hormone-binding protein and alcohol consumption found in this study deserves further investigation due to severe complications of high alcohol exposure in pregnancy such as stillbirth, preterm delivery and intrauterine growth retardation.

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