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Prediction of preterm delivery by second trimester inflammatory biomarkers in the amniotic fluid

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ABSTRACT

Objective: To search for a correlation between mid-pregnancy altered levels of inflammatory markers and preterm delivery.
Methods: A prospective cohort series included 39 patients undergoing amniocentesis one additional milliliter of amniotic fluid (AF) was stored for later dosage of interleukin-6 (Il-6), matrix metalloproteinase-9 (MMP-9), glucose and C-reactive protein (CRP). Maternal serum CRP and glucose levels were also obtained. Exclusion criteria were multiple pregnancies, chorioamnionitis, group B streptococcus colonization, bacterial vaginosis and cases with proven aneuploidy. We searched for correlation between AF and plasmatic markers and also for a difference between patients with term and preterm delivery.
Results: 33 participants were eligible and one third had preterm delivery. Levels of the plasmatic biomarkers did not correlate with the AF biomarkers except for plasmatic glucose and AF IL-6 levels ($r = 0.350$; $p = 0.016$). The levels of all AF biomarkers did not differ significantly between the pre-term and the term groups ($p > 0.05$). The optimal screening cutoffs for identifying pregnancies at risk were different than the ones initially indicated.
Conclusion: Mid-pregnancy amniotic fluid biomarker levels do not correlate with preterm delivery. Plasma CRP is not correlated with these markers. Cutoff levels suggested are sparse and heterogeneous. Larger studies are needed before advising routine measurement of these markers.

1. Introduction

Preterm delivery, defined as delivery before 37 weeks of gestation, accounts for 10% of pregnancies and leads to increased mortality and morbidity of neonates and neonatal intensive care unit admissions thus representing a social and a financial burden [1,2]. Studies established the decreased cervical length and the presence of fibronectin in the cervico-vaginal fluid as predictive biomarkers for preterm delivery [3]. One other possible underdiagnosed factor is asymptomatic intra-amniotic inflammation [4–6]. In this study, we analyze the potential role of amniotic fluid (AF) interleukin-6 (IL-6), matrix metalloproteinase-9 (MMP-9), C-reactive protein (CRP) and glucose aiming to characterize a subgroup of women at increased risk of spontaneous preterm delivery. Maternal plasma glucose and CRP levels are also considered, mainly in order to compare them to the amniotic levels. Cutoff levels were considered as used in other studies [7–10]. This series represents a pilot study in Lebanon.

2. Material and methods

A prospective cohort study took place in 2009 at the Obstetrics Department of Hotel Dieu de France University Hospital, Lebanon. It included patients who presented to our institution for mid-trimester amniocentesis. Indications for amniocentesis were advanced maternal age, increased first trimester nuchal translucency, abnormal second trimester biochemical screening results and positive ultrasound soft markers associated with maternal

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anxiety. Gestational age was established according to the patient last menstrual period in accordance to the first-trimester ultrasound scan. Cases with multiple pregnancies, clinical signs of infection or later diagnosis of fetal aneuploidies were excluded (Fig. 1). The number of patients enrolled was defined in accordance to the budget allocated to this pilot study by the research council of our institution. The study was approved by the Institutional Review Board of the Faculty of Medicine of Saint Joseph University and eligible patients signed an informed consent for the study purpose.

For the purpose of the study, one additional milliliter of AF was collected during amniocentesis for dosages of IL-6, MMP-9, CRP and glucose levels along with maternal serum CRP and glucose. The immediate results were not disclosed to both the physician and the patient. MMP-9 and Il-6 levels were measured with the corresponding Human Quantikine^{R} ELISA Kit (R and D systems) according to the instructions provided by the manufacturer. Cutoff values for AF MMP-9 and IL-6 were fixed by the kit sensitivity thresholds. Levels of AF MMP-9 > 15 ng/mL and AF IL-6 > 1430 pg/mL indicated positive results. A value of serum CRP > 9 mg/L was considered a positive result, and a value of AF CRP > 0.155 mg/L was considered a positive result suggestive of intra-amniotic fluid infection. Microbiological examination of the specimen was not done because of administrative reasons and because our patients were asymptomatic. All patients were screened for group B streptococcus colonization and bacterial vaginosis with vaginal swab and those who were positive for the test were later excluded from the study.

Studied outcome include possible biomarkers for premature delivery. Statistical analyses were performed with SPSS v20.0 software. The results are presented as mean ± standard deviation. In this study, negative results of AF and plasmatic CRP are reported as inferior to cutoffs of 0.155 mg/L and 9 mg/L respectively, resulting in a limited use of the statistical analysis of CRP. Statistical significance was determined using the Mann-Whitney and one way ANOVA test. The association between the level of the inflammatory markers and the incidence of preterm delivery was performed using a two proportions test. Correlations were measured using the Pearson's coefficient. A p value <0.05 was considered statistically significant.

3. Results

The study initially enrolled 39 women, with 33 cases meeting the inclusion criteria. Three patients were excluded because of multiple pregnancies, chorioamnionitis and aneuploidy leading to termination of pregnancy. The three other patients were excluded for colonization with group B streptococcus (GBS). All patients had a usual follow-up until delivery, whether it was a term or a preterm delivery, with or without premature rupture of membrane (Fig. 1). The mean age of our patients was 34 ± 4.5 years with 2.3 ± 1.4 gravidity. One third had preterm delivery (less than 37 weeks). Amniocentesis was done at 17.4 ± 1.4 weeks (Table 1). Socio-demographic characteristics of preterm and term delivery subgroups were not statistically different (Table 2).

Biomarker levels in the amniotic fluid revealed an IL-6 at 733.44 ± 675.78 pg/mL, MMP-9 at 5.6 ± 4.8 ng/mL, and glucose at 2.0 ± 0.5 mmol/L. Plasma glucose levels ranged 4.5 ± 0.7 mmol/L. In the term group, AF biomarkers ranged as follows: IL-6 levels at 632 ± 529 pg/mL, MMP-9 at 5.9 ± 4.8 ng/mL and glucose at 2.0 ± 0.4 mmol/L. Plasma glucose level was 4.4 ± 0.7 mmol/L. On the other hand, in the preterm group, AF biomarkers ranged as follows: IL-6 at 937 ± 895 pg/mL, MMP-9 at 5.2 ± 5.1 ng/mL, and glucose at 2.0 ± 0.7 mmol/L. Plasma glucose levels were at 4.7 ± 0.6 mmol/L. The levels of these biomarkers did not differ significantly between the two groups (p > 0.05) (Table 3). Levels of the plasmatic biomarkers did not correlate with the levels of AF biomarkers except for plasmatic glucose and AF IL-6 levels (r = 0.350; p = 0.016).

The time interval between amniocentesis and the occurrence of preterm labor showed a mean of 141.8 ± 24.9 days. Uni- and multivariate analysis did not correlate this interval between amniocentesis and preterm delivery to any of the inflammatory biomarkers measured: plasma glucose (p = 0.827), AF glucose (p = 0.494), AF IL-6 (p = 0.223), or AF MMP-9 (p = 0.806).

According to our data, the ROC curves of the measured AF biomarkers demonstrated an area under the curve (AUC) of 0.45 ± 0.11 for AF glucose, 0.55 ± 0.1 for AF MMP-9, and 0.42 ± 0.11 for AF IL-6. We used these ROC curve to elaborate cut-off points with diagnostic and screening utility (Table 4).

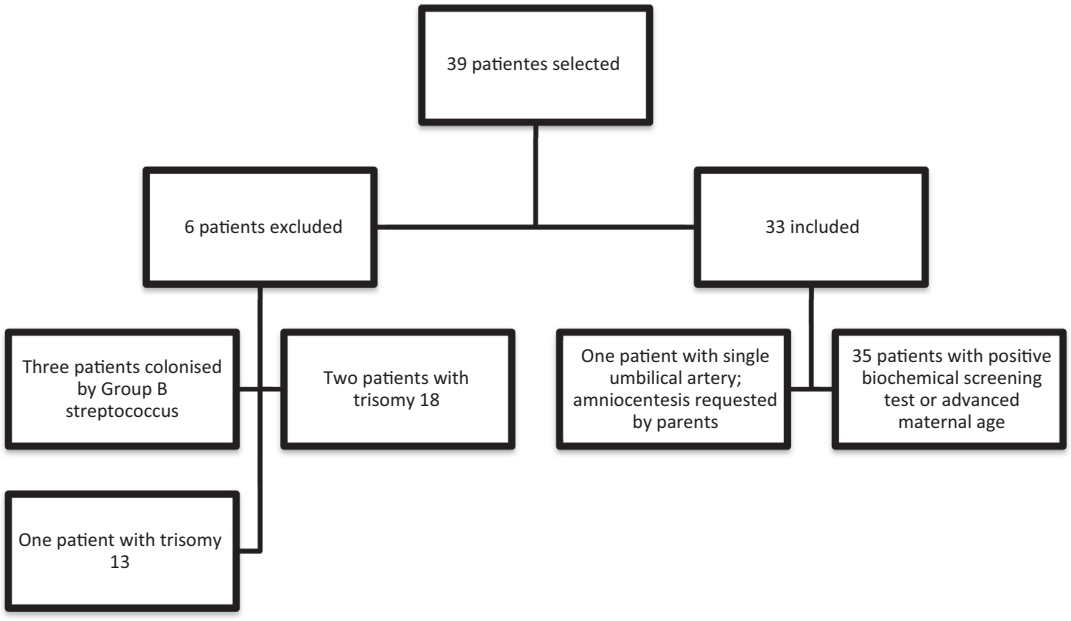

Fig. 1. Flow diagram of clinical enrollment and indication of amniocentesis.