Cytokine levels in amniotic fluid: a marker of preterm labor?

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Summary

The aim of this study was to determine levels of Interleukin 6 (IL-6) in amniotic fluid at the beginning of the second trimester and to establish whether IL-6 can be used as a marker for premature birth as it would appear to be an important prenatal marker of chorionic inflammation.

Thirty-three patients, between 16 and 19 weeks of gestation, who were undergoing amniocentesis to establish the presence or not of fetal genetic pathologies were enrolled into the study. Amniotic fluid (3 ml) was taken from each patient and used to perform enzyme-linked immunosorbent assays (ELISAs). The results were analyzed using the Mann-Whitney test and Pearson and Spearman coefficient.

The patients were divided into three groups on the basis of the levels of IL-6 found:

a) up to 450 pg/ml;

b) between 450 and 900 pg/ml;

c) over 900 pg/ml;

These data were then evaluated alongside the date of parturition and the presence of any maternal or fetal pathologies. The results of our analyses, however, were inconclusive: levels of IL-6 were normal in patients presenting pathologies while obstetric pathologies were absent in patients with high levels of IL-6.

In conclusion, this data would indicate that a different method or approach is required for the identification of a marker for premature birth.

Key word: Interleukin 6; Amniotic fluid; Premature birth.

Introduction

It is well known that premature birth is closely linked to infections of the genital tract and, the more serious the infection, the more premature the birth [1]. A possible explanation that has been put forward for this is that inflammation of the chorion and amnion stimulates the production of prostaglandins, which in turn generate a series of events leading to changes in the cervix and uterine contractions [2].

In addition, numerous studies have demonstrated levels of cytokines and IL-6 in amniotic fluid during colonization of the chorion and amnion [3, 4].

IL-6, in particular, appears to be an important prenatal marker of chorion inflammation [5, 6].

On the basis of these findings, we decided to evaluate whether measuring IL-6 levels at the beginning of the second trimester could be of use in predicting premature birth.

Patients and Methods

Thirty-three patients who had been admitted to the Prenatal Diagnostic Clinic of Avezzano Hospital for an amniocentesis to establish the presence of any fetal genetic pathologies between January and June 2004 were enrolled into the study. At the time of the test, none of the women presented obstetric or systemic pathologies. Their ages ranged from 32.5 to 43.6 years (average age 36 ± 4) and the gestational period ranged from 16 to 19 weeks. Seventeen women were primiparae, nine secundiparae and seven multiparae.

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Amniotic fluid (3 ml) was taken from each patient and the samples were centrifuged for ten minutes at 400 rpm to remove insoluble material; the supernatants were frozen within an hour and stored in aliquots at –70°C until used.

Highly sensitive (0.1pg/ml) ELISAs (enzyme-linked immunosorbent assays) were used to determine IL-6 levels (Biosource International, CA, USA). Standards and samples were placed in wells coated with a monoclonal antibody specific for IL-6. After incubation and washing to remove any unbound protein, a biotinylated polyclonal antibody specific for a different IL-6 epitope was added; after incubation and washing, the enzyme streptavidin peroxidase was added to bind to the biotinylated antibody. After a third period of incubation and further washing, a substrate solution for the enzyme was added and the intensity of the resulting coloration was used to determine the concentration of IL-6 present in the samples. The color intensity was measured by spectrophotometry at an optical density of 450 nm and on the basis of the optical density of the standards, a curve was established allowing us to determine, using specific software, the concentration of the samples. The test is specific for IL-6 and does not produce cross reactions with other cytokines.

Statistical analysis

The results were expressed as mean ± standard deviation. The differences between groups were analyzed with the Mann-Whitney test while the Pearson and Spearman coefficients were used to calculate the correlations between variables. The level of significance was set at 0.05 and all tests were two-tailed.

Results

The levels of IL-6 in the amniotic fluid taken ranged from a minimum of 48.6 pg/ml to a maximum of over

900 pg/ml. The patients were divided into three groups on the basis of the levels of IL-6 found, which differed quite significantly:

Group 1: up to 450 pg/ml (no. of patients = 28; IL-6: 185,54 ± 107,50 pg/ml);

Group 2: from 450 to 900 pg/ml (no. of patients = 3; IL-6: 708,40 ± 107,35 pg/ml);

Group 3: over 900 pg/ml (no. of patients = 2; IL-6: > 900 pg/ml).

The group results were analyzed retrospectively in light of the date of parturition and the presence of any maternal and/or fetal pathologies. The two patients in group 3 did not present any maternal/fetal pathologies; one patient in group 2 suffered from preeclampsia and two premature births were recorded in group 1.

Discussion

It has not been possible to draw any significant conclusions from this study. High levels of IL-6 were only found in two patients while the IL-6 levels in the rest of the groups, even in those patients who did present pathologies (premature birth and preeclampsia), were not significant enough to be considered of use in predicting premature birth.

These data therefore lead us to think that either the timing of our evaluation was too early and that useful insights might be gained by repeating the evaluation at a later stage of pregnancy or that a different approach is required involving the storage of amniotic fluid, and evaluation only in those cases that present pathologies in the third trimester.

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