Value of amniotic fluid interleukin-8 for the prediction of histological chorioamnionitis in preterm premature rupture of membranes

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Abstract

OBJECTIVE: To determine whether amniotic fluid levels of interleukin-8 (IL-8) are of value in the antenatal diagnosis of acute histological chorioamnionitis (HCA) in preterm premature rupture of membranes (PPROM).
SETTING: Department of Obstetrics and Gynaecology, Charles University, Medical School and University Hradec Kralove, Czech Republic.
METHODS: We compared amniotic fluid IL-8 levels in twenty-nine pregnant women with preterm premature rupture of membranes between 24th and 36th gestational weeks with presence and absence acute histological chorioamnionitis or/and microbial invasion in the amniotic cavity using nonparametric tests (Mann-Whitney test), given the non-normal distribution of analyte. Comparisons of proportions were performed with Shapiro-Wilk normality test.
RESULTS: Patients with HCA had a significantly higher median amniotic fluid IL-8 concentration than patients without the histological signs of chorioamnionitis (1867 pg/mL, 826–5577 versus 1045 pg/mL, 60–4133, p=0.013). Patients with MIAC had a significantly higher median amniotic fluid level than patients without invasion (1888 pg/mL, 519–5577 versus 1225 pg/mL, 60–2766, p= 0.017). Women with HCA and MIAC had a significantly higher median amniotic fluid IL-8 level than women without histological signs of chorioamnionitis and microbial invasion (3117 pg/mL, 826–5577 versus 1468 pg/mL, 394–2766, p=0.034).
CONCLUSIONS: HCA or/and MIAC are associated with a significant increase of amniotic fluid interleukin-8 levels. Amniotic fluid IL-8 seems to be a marker of intraamniotic inflammation.

ORIGINAL ARTICLE

Abbreviations :

IL-8	- interleukin-8
HCA	- acute histological chorioamnionitis
PPROM	- preterm premature rupture of the membranes
MIAC	- microbial invasion of the amniotic cavity
PCR	- polymerase chain reaction

INTRODUCTION

Preterm delivery is one principal cause of perinatal mortality and morbidity worldwide. It is an escalating problem in the Czech Republic and has increased to 8%. Premature rupture of the membranes is defined as fetal membranes rupture with leakage of amniotic fluid that precedes the onset of uterine contraction by at least 2 hours. It complicates 4–7% of all births and is significantly associated with a shorter length of gestation and an increased perinatal morbidity and mortality. There are predisposing conditions connected with the occurrence of premature rupture of the membranes, such as, local infections, cervical incompetence and low socio-economic condition (Gray *et al.* 1992, Watts *et al.* 1992). Moreover, the aetiology remains unknown in the majority of cases. Increasing biosynthesis of prostaglandins by intrauterine tissue is widely accepted as a key event in the initiation of parturition (Li *et al.* 2000). Histological chorioamnionitis (HCA) is a measure of intrauterine infection, which correlates to the presence of microbes in amniotic fluid (Yoon *et al.* 1998). HCA is accompanied with high concentration of inflammatory mediators in amniotic fluid, including proinflammatory cytokines (Dollner *et al.* 2002). Furthermore, HCA is associated with neonatal morbidity in preterm born infants (De Felice *et al.* 2001), emphasizing that HCA represents a clinically important outcome. The problem is that diagnosis is not known to the obstetricians until, after delivery and, therefore, cannot be used for clinical management. Low grade leukocyte infiltration in placental tissue is a common finding in normal term deliveries (Salafia *et al.* 1989), and only HCA with high grade infiltration usually indicates intrauterine infection. Interleukin-8 (IL-8), a 72 amino acid peptide is produced by many cells including macrophages and neutrophils, and cells of the decidua, amnion and chorion. IL-8 belongs to the CXC chemokine family in which two of the four cysteines in the peptide chain are separated by one amino acid. Unlike other interleukins IL-8 appears to be involved in a positive feedback cascade in the context of inflammation. Members of this family have chemotactic properties for neutrophils. Thus IL-8, produced in intraamniotic infection or/and inflammation, causes an influx of neutrophils (Stiemer *et al.* 1997).

The purpose of this study was to evaluate amniotic fluid concentration of IL-8 in patients with preterm premature rupture of the membranes and to determine whether amniotic fluid IL-8 concentrations are of value in the identification of patients with acute histological chorioamnionitis.

MATERIALS AND METHOD

A prospective cohort study was performed. The study population involved 29 pregnant women between 24th and 36th gestation weeks who were admitted to the Department of Obstetrics and Gynaecology in Hradec Kralove between June 2008 and February 2009 with a diagnosis of preterm premature rupture of the membranes (PPROM). The only women who fulfil following criteria: singleton pregnancy, certain gestational age, an ultrasound estimated weight of fetus between 10th and 90th percentile for gestational age and absence of fetal structural malformations or chromosomal anomalies, respectively, were enrolled to this study. Gestational age was established by last menstrual period and confirmed by ultrasound measurement of a crown-rump length (CRL) during the first trimester. Clinical management in the study group was performed according to standard protocols at our department. Rupture of membranes was diagnosed by an examination with a sterile speculum and a combination of vaginal pooling of amniotic fluid and the nitrazine test or the presence of the insulin-like growth factor binding protein – 1 (Actim PROM test, Medix Biochemica OY AB, Kauniainen, Finland) in the vaginal fluid. Clinical chorioamnionitis was defined according to the criteria proposed by Gibbs (Gibbs *et al.* 1982). The diagnosis required a temperature elevation to 37.8 °C and two or more of the following criteria: uterine tenderness, malodorous vaginal discharge, maternal tachycardia, fetal tachycardia, and leukocytosis. Leukocytosis was defined as a white blood cell count of more 15×10^9 cells/L. The staging and grading of inflammation in the placenta was performed according to standard published protocol (Salafia *et al.* 1989). The degree of polymorfonuclear leukocyte infiltration was assessed separately in the free membranes (amnion and chorion-decidua), in the chorionic plate, and in the umbilical cord according criteria given by Salafia *et al.* Diagnosis of HCA was made based on the presence histologic grades of chorion-decidua 3–4 and/or chorionic plate 3–4 and/or umbilical cord 1–4 and/or amnion 1–4. Placentas without leukocyte infiltration or with presence histologic grades of chorion-decidua 1–2 and/or chorionic plate 1–2 were classified as without presence histological chorioamnionitis.

Study has been approved by the Ethics Committee of the University Hospital in Hradec Kralove. Informed written consent was obtained from each patient.

Sampling

In the group of women with PPROM, an amniotic fluid sample was taken on admission before administration of corticosteroids, antibiotics or tocolytics. In all cases, amniotic fluid was collected by transabdominal amniocentesis under ultrasound guidance. The volume app. 5 mL was aspirated. Amniotic fluid was stored in polypropylene tubes at –20 °C until testing. A sample of amniotic fluid was immediately transported for polymerase

chain reaction (PCR) analysis for Ureaplasma spp. and Mycoplasma hominis and for aerobic and anaerobic culture. Microbial invasion of the amniotic cavity (MIAC) was defined as a positive PCR and/or growth of any bacteria in the amniotic fluid except for coagulase – negative Staphylococcus, which was considered to be a skin contaminant. At delivery the placenta was fixed in 10% neutral buffered formalin. Tissue samples were obtained from placenta (at least 2 samples), umbilical cord (usually 1 samples) and placental membranes (at least 2 samples) routinely processed and embedded in paraffin. Sections of tissue blocks were stained with haematoxylin and eosin. Histopathologic examination was performed by a single pathologist (H.H.) who was blinded to the clinical status of patients.

Concentration of IL-8 in amniotic fluid were determined using sandwich enzyme immunoassay technique (ELISA) with commercial kits Human CXCL8/IL-8 Immunoassay manufactured by R&D Systems Inc., Minneapolis, USA, according to the instructions of manufacturer. Absorbance values were read at 450 nm in an automatic ELISA reader (Multiskan RC, Thermo Fisher Scientific, USA). The sensitivity of this kit is 3.5 pg/mL.

Statistical methods

The demographic characteristics were compared by using t tests for continuous variables. IL-8 concentrations were compared between the study group and controls using nonparametric tests (Mann-Whitney test), given the non-normal distribution of analyte. Comparisons of proportions were performed with Shapiro – Wilk normality test. Differences were considered statistically significant at $p<0.05$. A receiver- operator characteristic curve was constructed to describe the relationship between the sensitivity and the false positive rate for different values of amniotic fluid IL-8 in the identification of acute histological chorioamnionitis. Analysis was performed with GraphPad Prism 5 (GraphPad Software, Inc., La Jolla, USA).

RESULTS

Twenty nine women with preterm premature rupture of membranes between 24th and 36th gestational weeks were enrolled to the study. All probands were Caucasian. Clinical background information of the study population is presented in Table 1. None of the 29 patients with PPROM developed clinical chorioamnionitis. Histological chorioamnionitis was found in 9 women (31%), microbial invasion in the amniotic cavity was identified in 10 patients (34%) (Table 2), MIAC and HCA had 6 (21%) women. Patients with HCA had a significantly higher median amniotic fluid IL-8 concentration than patients without HCA (1867 pg/mL, 826–5577 versus 1045 pg/mL, 60–4133, $p=0.023$; see Figure 1). The histologic grades of HCA and corresponding levels of IL-8 are presented in Table 2.

Tab. 1. Clinical backgrounds variables in women with and without histological chorioamnionitis.

Parameter	Presence HCA (n=9)	Absence HCA (n=20)	*p*-value
Gestational age	32.4±3.4	30.6±4.2	0.15
Maternal Age	30.8±5.8	30.4±4.4	0.8
Nulliparous	3 (33%)	9 (45%)	0.64
Multiparous	6 (67%)	11 (55%)	0.64
Birth weight (g)	1946±665	1526±713	0.06
Corticosteroid therapy	3 (33%)	10 (50%)	0.52
Antibiotic therapy	9 (100%)	20 (100%)	1
MIAC	6	4	0.013

Tab. 2. Presence of HCA (histologic grades of chorion/decidua, chorionic plate, umbilical cord and amnion) and the corresponding levels of interleukin-8.

Chorion / Decidua	Chorionic plate	Umbilical cord	Amnion	Interleukin-8 (pg/mL)
3	1	0	0	1227
3	2	0	0	2800
3	2	0	0	1526
4	2	0	0	3433
4	3	2	2	5577
3	3	0	2	1867
3	4	3	2	826
2	3	1	0	1292
3	3	0	0	4259

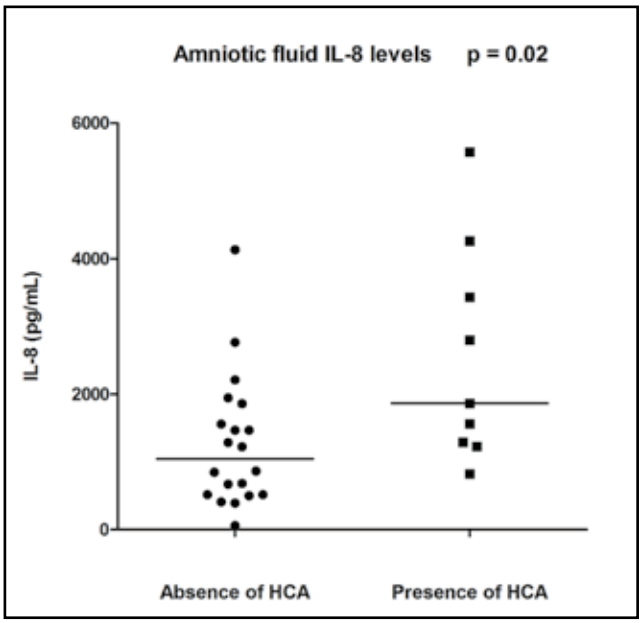

Fig. 1. Significant difference between amniotic fluid IL-8 concentrations in PPROM women with absence (n=20) and presence (n=9) histological chorioamnionitis (HCA).

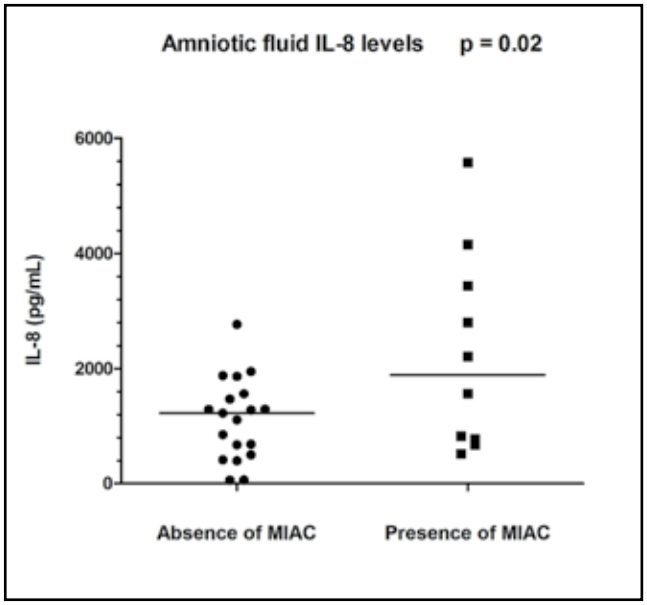

Fig. 2. Significant difference between amniotic fluid IL-8 concentrations in PPROM women with absence (n=19) and presence (n=10) microbial invasion in the amniotic cavity (MIAC).

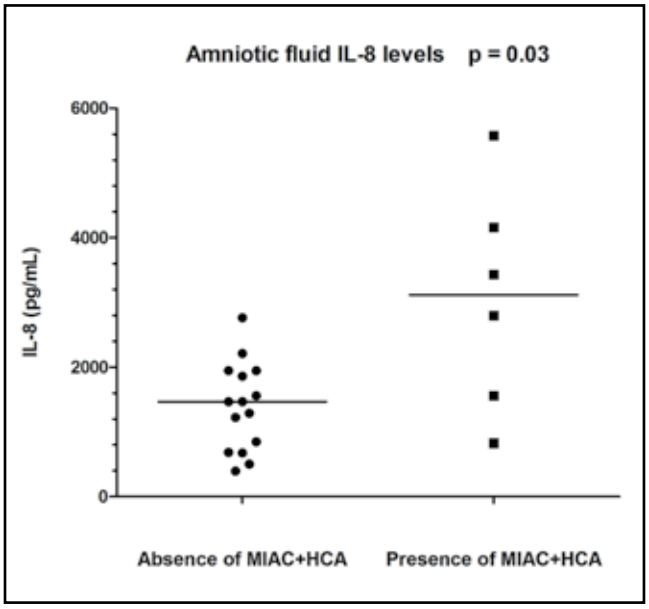

Fig. 3. Significant difference between amniotic fluid IL-6 concentrations in PPROM women without HCA+MIAC (n=15) and with HCA+MIAC (n=6).

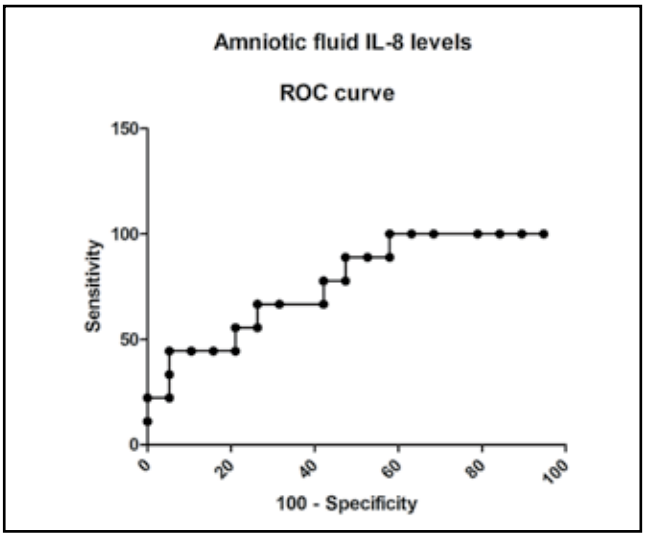

Fig. 4. Receiver operator characteristic curve for amniotic fluid IL-8 levels.

Tab. 3. Microbes isolated form amniotic fluid and the corresponding levels of interleukin-8.

Organism	Interleukin-8 (pg/mL)
Ureaplasma urealyticum	778
Ureaplasma urealyticum	2800
Ureaplasma parvum	1526
Fusobacterium species	3433
Ureaplasma parvum + Mycoplasma hominis	5577
Streptococcus agalactie	519
Ureaplasma urealyticum	4259
Ureaplasma urealyticum + Chlamydia trachomatis	674
Enterococcus species	826
Ureaplasma parvum	2213

Patients with MIAC in amniotic fluid had significantly higher levels of IL-8 than those without microbial invasion (1888 pg/mL, 519–5577 versus 1225 pg/ml, 60–2766, p=0.017; see Figure 2). The bacterial species and corresponding levels of IL-8 are presented in Table 3.

Moreover, patients with the presence of HCA and MIAC had significantly higher levels of IL-8 than those without HCA and MIAC (3117 pg/mL, 826–5577 versus 1468 pg/mL, 394–2766, p=0.034 ; see Figure 3).

An ROC curve analysis of histologic chorioamnionitis for various cut-off levels of IL-8 is shown in Figure 4. An IL-8 level of 1561 ng/mL was found to be best cut-off point (sensitivity 67%, specificity 74%).

DISCUSSION

Our data clearly show that woman with preterm premature rupture of membrane with intraamniotic inflammation (HCA) and/or infection (MIAC) had significantly higher amniotic fluid IL-8 concentrations. The results of this study demonstrate that in this PPROM population (median gestation 31 weeks) 34% had detectable levels of microorganism in the amniotic fluid and approximately 31% had intraamniotic inflammation (indicated by histologic chorioamnionitis).

Our results are in agreement with other authors who found that IL-8 in amniotic fluid were associated with

HCA (Arntzen *et al.* 1998, Cherouny *et al.* 1993, Saji *et al.* 2000, Holst *et al.* 2007). Neutrophils play an important role in the inflammatory processes because these neutrophils can migrate to the inflammatory site. Chemokines have been suggested playing an important role in the pathogenesis of leukocyte recruitment. There are two subfamilies of chemokines, which are classified on the basis of whether the first two conserved cysteines are separated by one amino acid (CXC or α chemokines) or adjacent to each other (CC or β chemokines) (Miller, Krangel, 1992). Both subfamilies of chemokines can attract and activate leukocytes; however, the CXC subfamily of chemokines mainly acts on neutrophils. IL-8, the CXC subfamily of chemokines is potent neutrophil chemoattractant and activator. Because one of the important biologic functions of chemokines is to activate and chemoattract inflammatory leukocytes, elevated amniotic fluid IL-8 in PPROM patients with intraamniotic inflammation and/or infection could result in increased activated amniotic fluid leukocytes through their chemotactic effects (Hsu *et al.* 1998).

Assessment of the number of polymorfonuclears forms the basis of the diagnosis of HCA in all studies, but the degree of polymorfonuclears infiltration as well as the location of these cells varies considerably. Therefore, the definition of HCA in most previous studies would (Hillier *et al.* 1993, Greig *et al.* 1993) correspond to the absence HCA group in our study. In only one study (Yoon *et al.* 1995) was the relationship between amniotic fluid interleukin levels and signs of HCA in any of the different locations (amniotic fluid, chorion-decidual membrane, chorionic plate, and umbilical cord) compared. Holst *et al*. require in their study funisitis, chorioamnionitis in extraplacental membrane, fetal vessel vasculitis and subchorionic fibrin polymorfonuclear infiltration in the form of diffuse infiltration with polymorfonuclears in all sites for definition of HCA. In addition, they included a separate group with a polymorfonuclear infiltration in one or several of these sites, but not all – inflammatory sings group (Holst *et al.* 2007)

The prevalence of MIAC found currently (34%) in women with PPROM using PCR for the genital mycoplasmas combined with aerobic and anaerobic cultures, is within the wide range (15–57%; median 34%) of earlier reports. Comparisons between different reports are difficult because of differences in the definition of PPROM with respect to detection method of rupture of membrane, gestational age and microbiological technique used (Jacobsson *et al.* 2003).

In conclusion, amniotic fluid concentration of IL-8 was significantly elevated and correlated in premature rupture of the membranes patients with intraamniotic inflammation (HCA) and infection (MIAC).

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