Subclinical Intrauterine Infection

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Abstract

Intrauterine (intra-amniotic) infection is recognized as the leading cause of preterm delivery, and is a major cause of a poor neonatal prognosis worldwide.

Maternal clinical chorioamnionitis (CAM), characterized by maternal fever, tachycardia, uterine tenderness, and leukocytosis, has been implicated in extremely and very preterm deliveries as well as a poor neonatal prognosis such as early-onset neonatal sepsis and necrotizing enterocolitis. Many cases of preterm birth with or without clinical CAM show intrauterine inflammation (histological CAM). Further clarification of the relationship between intrauterine infection and inflammation may contribute to the development of novel therapeutic strategies.

Approximately 30% of idiopathic preterm labor cases have been linked to subclinical intrauterine infection; however, inflammation associated with subclinical infection is difficult to detect using a peripheral blood analysis. Recent studies showed that amniotic fluid (AF) is very useful material for evaluating intra-amniotic infection and inflammation. The detection rate of microorganisms in AF using a polymerase chain reaction (PCR)-based methodology is higher

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H. Sameshima (ed.), *Preterm Labor and Delivery*, Comprehensive Gynecology and Obstetrics, https://doi.org/10.1007/978-981-13-9875-9_4

than that with a standard microbial culture system. By using highly sensitive and reliable PCR, polymicrobial infections with *Mycoplasma*/*Ureaplasma* and other bacteria were shown to induce severe intrauterine inflammation associated with a poor perinatal prognosis in preterm labor. Appropriate antibiotic therapy for PCR-positive preterm labor cases may prolong the gestational period. Furthermore, a PCR analysis showed that AF sludge reflects intra-amniotic inflammation with or without microorganisms.

In this section, we discussed subclinical intrauterine infections detected using highly sensitive and reliable PCR as well as an IL-8 analysis of AF.

Keywords

Amniotic fluid "sludge" · Interleukin-8 (IL-8) · Intrauterine infection · Intrauterine inflammation · Polymerase chain reaction (PCR)

4.1 Introduction

Microbial invasion of the amniotic cavity is detected in 20–60% of cases of preterm labor (PTL) at <28 weeks' gestation and 10–25% of those at 28–32 weeks' gestation [1–3]. Intrauterine (intra-amniotic) infection and inflammation are the main causes of PTL with intact fetal membranes, particularly in extremely and very preterm deliveries [4].

Chorioamnionitis (CAM) is a common cause of preterm births and is often associated with fetal inflammatory response syndrome (FIRS). FIRS is characterized by increased systemic inflammatory cytokine concentrations, fetal vasculitis, and funisitis [5].

The presence of histological CAM and maternal and/or neonatal infection after a preterm birth is considered to be evidence of subclinical intrauterine infection [6]. However, difficulties are associated with detecting subclinical intrauterine infection in PTL cases using a peripheral blood analysis [7].

Regarding the detection of subclinical intrauterine infection, amniotic fluid (AF) is very useful for evaluating intra-amniotic inflammation and infection. A bacterial culture of AF remains the "gold standard" and most specific test for the documentation of intra-amniotic inflammation, but is limited because it may take a few (3–7) days to obtain definitive results, which is too long to be clinically useful [8]. Although molecular microbiology techniques, such as a polymerase chain reaction (PCR), are rapid and highly sensitive, false positives have been reported due to contaminating bacterial DNA in *Taq* DNA polymerase being amplified by PCR (Fig. 4.1). Eukaryote-made thermostable DNA polymerase has been established in order to overcome this issue (Fig. 4.1) [9]. In the following section, we described intrauterine infection and sterilized inflammation in cases of PTL using this highly sensitive and reliable PCR system.

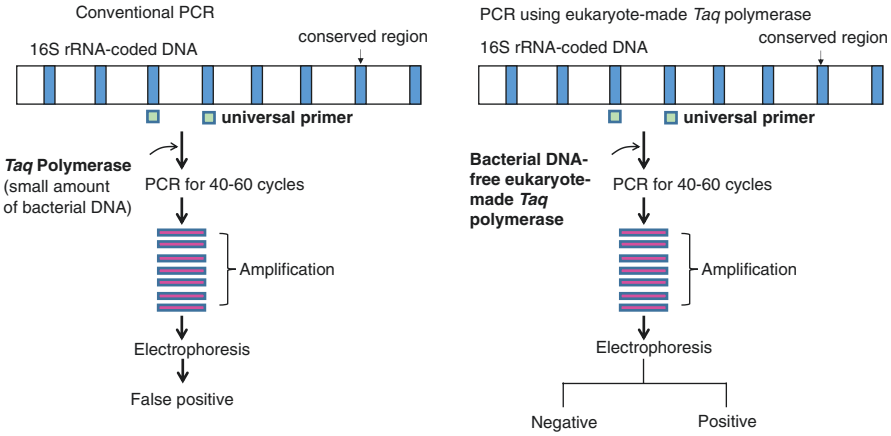

Fig. 4.1 Conventional PCR and PCR using eukaryote-made *Taq* polymerase

4.2 Detection of Microorganisms in AF by a Culture Method and PCR

The microbial cultivation method of AF is recognized as the "gold standard" for the detection of intrauterine infection; however, 1 week is needed to obtain results on possible infections by *Mycoplasma*, *Ureaplasma*, and other bacteria. Furthermore, difficulties are associated with detecting *Mycoplasma* and *Ureaplasma* using conventional cultivation systems. Molecular microbiology techniques, such as PCR, have emerged as rapid and highly sensitive methods for the detection of microorganisms including *Mycoplasma* and *Ureaplasma* in AF samples [10–18]. The detection rate of microorganisms in AF using a PCR-based methodology was previously reported to be higher than that by a standard microbial cultivation system [10, 11, 19–23]. The amniocentesis-to-delivery interval of PCR-positive culture-negative PTL cases was found to be significantly shorter than that of PCR-negative culture-negative cases, suggesting that PCR-positive culture-negative cases ($P = 0.03$) are pathogenic [24].

A quantitative real-time PCR assay for detecting bacteria is also important for assessing the load of microorganisms. High copy numbers of bacterial DNA have been reported in CAM stage III [25, 26], and the following 11 bacterial species are the dominant species of CAM stage III: *Ureaplasma parvum*, *Streptococcus agalactiae*, *Gardnerella vaginalis*, *S. anginosus*, *Sneathia sanguinegens*, *Eikenella corrodens*, *Prevotella bivia*, *Lactobacillus jensenii*, *Bacteroides fragilis*, *Porphyromonas endodontalis*, and *Mycoplasma hominis* [25]. Therefore, it is important to detect pathogenic microbes in AF.

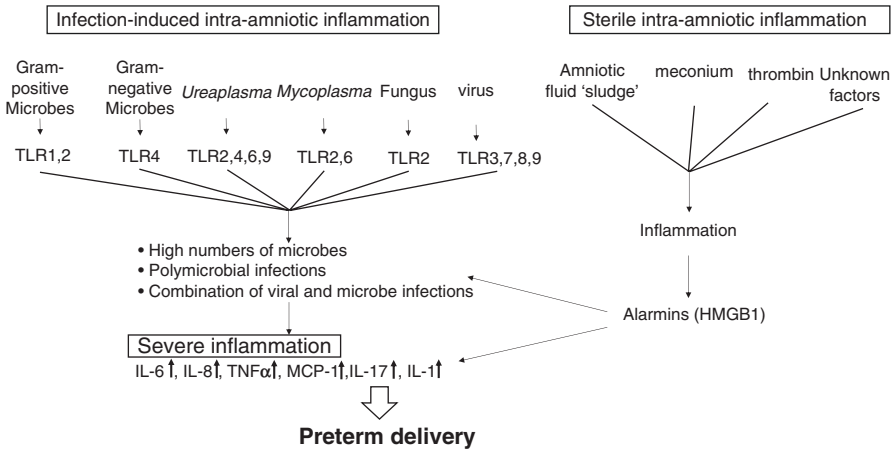

Fig. 4.2 Infection induced inflammation and sterile intra-amniotic inflammation in preterm delivery cases

4.3 Sterile Intra-Amniotic Inflammation and Micobial-associated Intra-Amniotic Inflammation in Preterm Delivery

Most intra-amniotic infections are subclinical in nature, and, thus, occur in the absence of clinical CAM. The most frequent microorganisms detected in AF are genital *Mycoplasma* [27–30], *Ureaplasma* species [31, 32], *Gardnerella vaginalis*, and *Fusobacteria* species. Polymicrobial invasion of the amniotic cavity is detected in approximately 30% of cases [23, 33].

Romero et al. previously proposed that intra-amniotic inflammation without detectable microbes is a mechanism of disease in PTL [2, 34, 35] based on elevations in the amniotic inflammatory mediators (interleukin (IL)-6 [2, 34, 35], IL-8 [36], matrix metalloproteinase 8 (MMP-8) [37], monocyte chemotactic protein-C (MCP-1) [38]), and other inflammatory markers (Fig. 4.2) [39]. Sterile intra-amniotic inflammation is more frequent than microbial-associated intra-amniotic inflammation [40], and we also showed that sterile intra-amniotic inflammation was common in PTL cases [41]. Therefore, infection and inflammation both need to be analyzed.

Damage-associated molecular patterns (DAMPs) are host biomolecules that initiate non-infectious inflammatory responses. Some alarmins, such as IL-1α [42, 43] and high mobility group box-1 (HMGB1) [44, 45], are elevated in the AF of patients with intra-amniotic inflammation (Fig. 4.2). Romero et al. reported an inflammatory-related protein network in spontaneous PTL. PTL cases with microbial-associated intra-amniotic inflammation had more coordinated AF inflammatory-related proteins than those with or without sterile intra-amniotic inflammation. These relationships were also stronger in patients with than in those without sterile intra-amniotic inflammation [46]. Therefore, sterile intra-amniotic inflammation is an important factor that induces preterm delivery. We previously reported that AF "sludge" is related to intra-amniotic inflammation with or without

microorganisms. AF "sludge" may be one of the factors that induces sterile intra-amniotic inflammation (Fig. 4.2) [41]. Meconium and thrombin also induce inflammation (Fig. 4.2).

4.4 Highly Sensitive and Reliable PCR Analysis of AF

PCR is useful for detecting microorganisms in AF; however, commercially available thermostable DNA polymerase (e.g., recombinant Taq polymerase) contains trace amounts of host (prokaryote)-derived bacterial DNA, which results in false positives when PCR cycles increase to improve sensitivity (Fig. 4.1) [22].

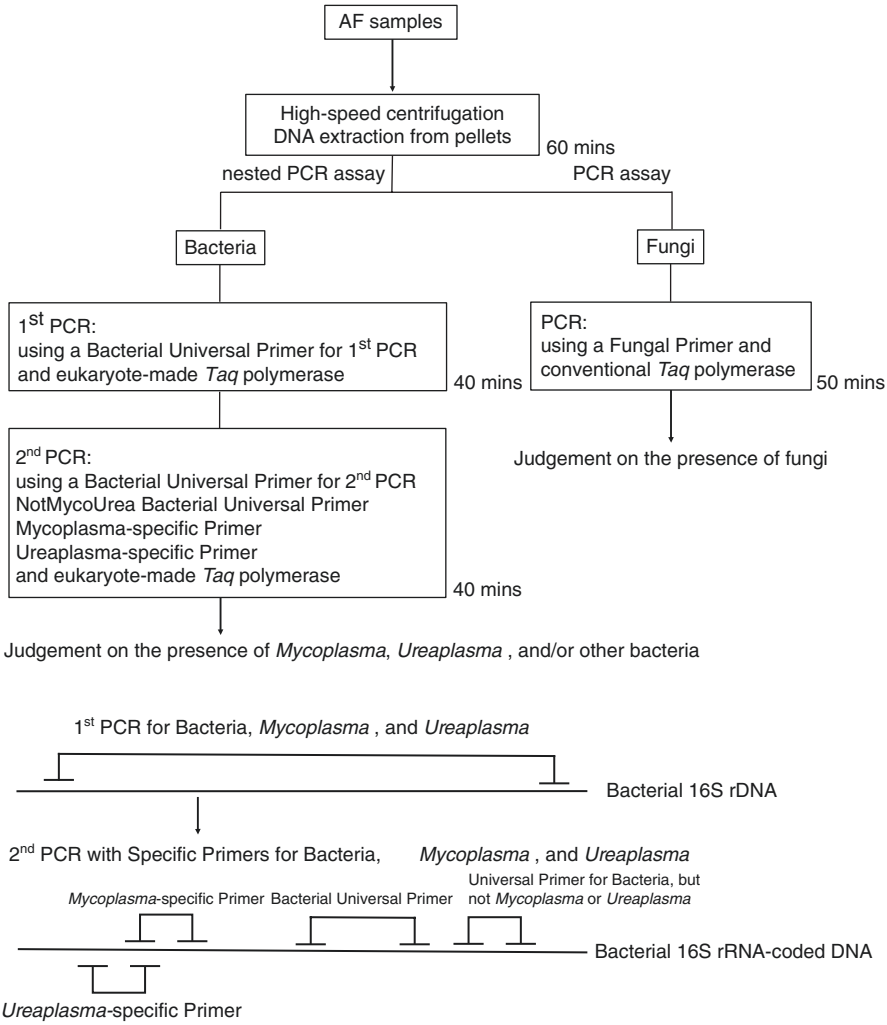

Fig. 4.3 Highly sensitive and reliable PCR method to detect microorganisms in amniotic fluid (AF) samples at the University of Toyama

In order to overcome this issue, a recent study reported a nested PCR-based method using eukaryote-made thermostable DNA polymerase, which is free of bacterial DNA contamination (Fig. 4.3) [9]. By using this eukaryote-made thermostable DNA polymerase, the highly sensitive and reliable detection of bacteria has become practicable. Regarding the detection of microorganisms in the AF of PTL cases, a method for detecting *Mycoplasma*, *Ureaplasma*, bacteria, and fungi using devised primer sets has been established (Fig. 4.3) [47].

This highly sensitive and reliable PCR method is useful for detecting intrauterine microorganisms. We previously reported that microorganisms were detected in the AF of 7.6% (9/118) of PTL cases using culture tests, and in 33% (39/118) of cases using the PCR method [24]. The PCR method is useful for detecting microorganisms that are difficult to culture and separate on conventional culture medium.

We also demonstrated that the amniocentesis-to-delivery interval of the culture (−) -PCR (+) group was significantly shorter than that of the culture (−) -PCR (−) group ($P = 0.03$) [24].

This finding suggested that viable but non-culturable microbes are pathogenic for PTD.

Previous studies reported that AF cytokines levels, such as IL-6 and IL-8, increased in PCR-positive culture-negative preterm delivery cases [10, 20, 23, 48]. Our findings also confirmed intrauterine inflammation in PCR-positive culture-negative cases.

Highly sensitive and reliable PCR showed that polymicrobial infections with *Mycoplasma*/*Ureaplasma* and other bacteria induced severe intrauterine inflammation associated with a poor perinatal prognosis in PTL (Fig. 4.1) [24]. In this case, multiple pathways of toll-like receptors (TLR) are activated, resulting in severe inflammation (Fig. 4.2). Severe inflammation is commonly observed in extremely preterm births.

4.5 AF Cytokine Levels

Previous studies described the evaluation of histological CAM using biological markers, such as maternal body temperature [49, 50], maternal white blood cell count (WBC) [49, 50], maternal C-reactive protein (CRP) [51–53], maternal or amniotic IL-6 [54–57], and amniotic IL-8 [58, 63].

IL-8 is a chemokine that is produced by a number of cell types, and proinflammatory markers have been reported in various diseases [59–61]. We demonstrated that IL-8 was an accurate marker for detecting the early stage of inflammation in the amnion. Our previous findings revealed that among amniotic IL-8, TNF-α, and IL-17 levels, those of IL-8 more clearly reflected each stage of h-CAM [62].

The cut-off value for amniotic IL-8 levels to predict the stage of h-CAM before delivery was assessed. Cut-off values for h-CAM were ≥9.9 ng/mL for stage I or higher, ≥17.3 ng/mL for stage II or higher, and ≥55.9 ng/mL for stage III. The sensitivities of predicting h-CAM of stage I or higher, stage II or higher, and stage III were 57.7, 77.4, and 91.2%, with specificities of 88.9, 85.3, and 91.4%, respectively [63]. Therefore, the detection of AF infection by PCR and inflammation using cytokine levels is considered to be important for assessing the status of PTL.

4.6 Clinical Benefits of Antibiotics for Subclinical Intrauterine Infection in Cases of PTL

A Cochrane review reported that the current usage of antibiotics is associated with adverse effects in neonates, such as cerebral palsy, but is also beneficial for mothers [64]. Preterm birth may be caused not only by intrauterine infection, but also by sterile intrauterine inflammation. Therefore, antibiotic therapy may not prevent preterm birth with sterile intrauterine inflammation. Combs et al. reported that antibiotics may be useful in cases of PTL without severe intra-amniotic inflammation that test positive for microorganisms [65]. Therefore, the selection of patients is important for decision-making regarding antibiotic therapy.

Our highly sensitive and reliable PCR system revealed that antibiotic therapy increased the risk of preterm birth in cases of PTL without intra-amniotic microorganisms, but may prolong the gestation period in cases of PTL with microorganisms. In brief, antibiotic therapy may have detrimental effects in cases of PTL without microorganisms, but may be beneficial for those with microorganisms in the amniotic cavity [66]. As a possible explanation, antibiotic therapy is only effective in intra-amniotic infection cases. In sterile intra-amniotic inflammation cases, antibiotic therapy has adverse effects on PTL. We previously reported that the number of intestinal *Clostridium* species was very low in preterm delivery cases [67]. Intestinal *Clostridium* has been shown to play an important role in the induction of regulatory T (Treg) cells, which contribute to the regulation of inflammation. Therefore, antibiotic therapy may decrease the number of *Clostridium* species that induce Treg cells. Decreased Treg cell numbers did not regulate inflammation, and resulted in preterm delivery. Therefore, antibiotic therapy needs to be selected for intra-amniotic infection cases only.

4.7 Conclusions and Future Directions

The accurate detection of microorganisms in AF by highly sensitive and reliable PCR is essential for the management of PTL. The degree of intrauterine inflammation may be evaluated by the quantification of AF cytokines such as IL-8, IL-6, and MCP-1. The management of PTL based on the presence of microorganisms and inflammation in AF will improve the outcomes of future PTL cases.

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