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The performance of nanopore sequencing method in the detection of intra-amniotic infection

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Objective

Intra-amniotic infection is the leading cause of infection in labor and delivery unit. Clinical standard methods for the detection of intra-amniotic infection are culture or 16S rDNA Sanger sequencing. However, these methods have a long sample-to-result-turnaround time. Nanopore sequencing method is a real-time long read sequencing method with fast speed. The aim of this study is to determine the diagnostic performance of 16S rDNA nanopore sequencing method for the identification of intra-amniotic infection.

Methods

We performed a prospective cohort study including 56 singleton pregnancies presenting with symptoms of preterm labor/preterm prelabor ruptured of membranes (PROM). Amniotic fluid (AF) samples were obtained for the evaluation of bacteria in the amniotic cavity using cultivation and 16S rDNA Sanger sequencing methods. Participants were classified according to the results of AF culture, 16S Sanger sequencing and AF interleukin (IL)-6 concentration into four groups: 1) no intra-amniotic inflammation (AF IL-6 <2.6 ng/mL); 2) microbial invasion of the amniotic cavity (MIAC); 3) sterile intra-amniotic inflammation (AF IL-6 ≥2.6 ng/mL without MIAC); 4) intra-amniotic infection (AF IL-6 ≥2.6 ng/mL with MIAC). Nanopore sequencing was performed. The diagnostic indices of nanopore method for the identification of intra-amniotic infection were determined.

Results

1) A positive 16S nanopore sequencing had a sensitivity of 88.89% (8/9), specificity of 80.9% (38/47), positive predictive value of 47.1% (8/17), negative predictive value of 97.4% (38/39), positive likelihood ratio 4.6 (95% CI 2.5-8.7), and negative likelihood ratio 0.14 (95% CI 0.02-0.88) for the identification of intra-amniotic infection (prevalence 16%; 9/56); 2) About 10 of 56 samples showed discordant results, of these 1 was false negative and 9 were false positive; 3) One case with false negative nanopore sequencing result, amniotic fluid culture revealed Actinotignum Schalii, however, 16S Sanger result also showed no bacteria. Placental histopathology and culture results were normal; 4) Among nine cases with false positive nanopore sequencing results, two cases were classified as MIAC by positive 16S Sanger sequencing results, 3 cases were categorized in sterile intra-amniotic inflammation group. The remaining 4 cases had no intra-amniotic inflammation; and 5) Nanopore sequencing additionally detect bacteria in 21.4% (3/14) of patients with sterile intra-amniotic inflammation; and 6) Nanopore turn-around time was about 4.5-14 hours from DNA extraction to species identification.

Conclusion

Nanopore sequencing is advantageous in detection speed with high sensitivity and negative predictive value for the identification of intra-amniotic infection. False positive nanopore cases without intra-amniotic inflammation represent contamination. Importantly, we confirmed that a subset of women with preterm/preterm PROM has sterile intra-amniotic inflammation despite deep a long read sequencing technique.