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A New Method of SARS-CoV-2 Screening for Pregnant Women Based on Non-Invasive Prenatal Test

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Abstract

Aims: Developing a new system to detect SARS-CoV-2 nucleic acid and fetal aneuploidy at the same time base on maternal plasma, to achieve the purpose of SARS-CoV-2 screening for pregnant women.

Methods: We optimized the Non-Invasive Prenatal Testing (NIPT) library by mixing the cell-free DNA (cfDNA) and SARS-CoV-2 cDNA as the input DNA to make the NIPT library. Three groups of libraries were set up, including negative group, trisomy positive group, and simulated SARS-CoV-2 group to detect SARS-CoV-2 and NIPT, and three different SARS-CoV-2 concentration gradients were set up in the simulated SARS-CoV-2 group.

Results: For NIPT analysis, negative NIPT result was confirmed in all cases in the negative group and simulated SARS-CoV-2 group, while positive NIPT result was confirmed in all cases in the trisomy positive group. For SARS-CoV-2 analysis, SARS-CoV-2 reads were detected in all cases in simulated SARS-CoV-2 group, and none SARS-CoV-2 reads were detected in the negative group and trisomy positive group. All the results were consistent with the actual situation. In addition, the detected viral reads had significant positive correlation with the proportion of SARS-CoV-2 reads in 1 million total reads.

Conclusion: The new technology reported in this study can achieve the purpose of NIPT detection and SARS-CoV-2 detection for pregnant women by drawing only one tube of peripheral blood. In this way, SARS-CoV-2 screening can be performed for pregnant women in the progress of routine prenatal testing, which can find asymptomatic infections and supply optimal management and treatment.

Keywords: Asymptomatic infections; COVID-19; Non-invasive prenatal testing; Pregnant women; SARS-CoV-2

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Introduction

Coronavirus Disease 19 (COVID-19) caused by Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) in Wuhan, China in December 2019 has become a serious global public health problem. SARS-CoV-2 is a positive stranded RNA virus with envelope belongs to the genus of β-coronavirus. It is the seventh kind of human coronavirus infections, closely related to Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV) and Middle East Respiratory Syndrome Coronavirus (MERS-CoV), characterized by lower respiratory infection. In the past 20 years, more than 10,000 cases of SARS-CoV and MERS-CoV infection have been reported in the world [1,2]. Compared with SARS-CoV and MERS-CoV, SARS-CoV-2 has stronger infectivity. As of September 2021, more than 200 million confirmed cases and more than 4 million deaths have been reported in the world [3]. Considering there are many infections of COVID-19 are asymptomatic, the total number of infections may larger [4]. As we known, pregnant women are a vulnerable population who were more susceptible to viral infections. SARS-CoV or MERS-CoV infection during pregnancy could lead severe complications such as maternal death, stillbirth, spontaneous miscarriage, and so on. It has been reported that the mortality rate of SARS-CoV infection in pregnant women was

25% (10% of the general population), while the mortality rate of pregnant women infected with MERS-CoV was as high as 37% [1,2,5-7]. Therefore, a SARS-CoV-2 screening method for pregnant women is required, especially to differentiate the asymptomatic from health pregnant women. The main mode of SARS-CoV-2 spread from person-to-person is through respiratory droplets, close contact and high concentration aerosol transmission. Vertical transmission is also reported occasionally. Some studies showed that a low proportion of newborns delivered from pregnant women infected with SARS-CoV-2 were infected with SARS-CoV-2, but most of them had no evidence of placental infection [8]. A Sweden team reported a confirmed intrauterine vertical SARS-CoV-2 case with high viral load in placenta [9]. Therefore, the possibility of intrauterine transmission in pregnant women infected with SARS-CoV-2 cannot be ignored. Non-Invasive Prenatal Testing (NIPT) based on cell-free DNA (cfDNA) which is present in maternal plasma has been introduced in routine pregnant examination [10]. Pregnant women in Changsha, Hunan could get free NIPT, benefitting from the financial support provided by the government's preferential policies. In this study, we present an approach of noninvasive prenatal testing of SARS-CoV-2 (NIPT-SARS-CoV-2) to achieve the screening of SARS-CoV-2 in pregnant women during routine prenatal examination.

Materials and Methods

Study design

This study was approved by the Ethics Committee of the Changsha Hospital for Maternal and Child Health Care, Hunan, China. A total of 50 pregnant women were recruited from Changsha Hospital for Maternal and Child Health Care between July and September 2020 for this study, in which 40 were NIPT negative and 10 were NIPT positive. The SARS-CoV-2 RNA sample used in this study was from COVID-19 patients visited in the First Hospital of Changsha, Hunan, China. Written consent was obtained from all participants. An optimized NIPT was designed to detect fetal aneuploidy and SARS-CoV-2 at the same time.

Plasma separating and processing

First, peripheral blood of pregnant women was centrifuged at 1,600 g for 10 min at 4°C. Second, the supernatant was transferred to 2.0 ml EP tubes, centrifuged again at 16,000 g for 10 min at 4°C. Finally, the plasma was transferred to fresh tubes and immediately stored in -80°C, with 0.6 ml each tube. The separated plasma was divided equally into two parts, one was for extracting cfDNA, and the other was for extracting SARS-CoV-2 RNA. The plasma cfDNA was extracted by QIAamp Circulating Nucleic Acid kit (Qiagen, Germany). The plasma RNA was extracted by QIAamp® Viral RNA Mini Kit (Qiagen, Germany), and then was reverse transcripted to cDNA immediately by Fapon RNA-sep cDNA Synthesis Kit (Feipeng, China).

Library constructing and sequencing

The cfDNA and SARS-CoV-2 cDNA were mixed in equal proportion and were used as the input DNA to make the NIPT library. Three groups of libraries were set up, including negative group, trisomy positive group, and simulated SARS-CoV-2 group. The negative group library was prepared by cfDNA and SARS-CoV-2 cDNA from NIPT negative pregnant women. The trisomy positive group library was prepared by cfDNA and SARS-CoV-2 cDNA from NIPT positive pregnant women. The simulated SARS-CoV-2 group library was prepared by cfDNA from NIPT negative pregnant women and SARS-CoV-2 cDNA from COVID-19 patients. Three different SARS-CoV-2 concentration gradients were set up in the simulated SARS-CoV-2 group, including 1,400 copies/ml, 14,000 copies/ml, and 140,000 copies/ml. Through end repairing, adding adapter, and PCR amplification to prepare the NIPT library, after quality control, the products were sequenced on the Ion Proton platform (Life Technologies, USA).

Data analysis

The Quality Control (QC) analysis was used to filtered the low-quality reads from raw sequencing data. The clean data were mapped to human genome 19 (hg19). The BAM files obtained were sorted and the PCR repeat reads were filtered. NIPT analysis software (Darui Biotechnology Co., Ltd., Guangzhou, China) was used to preform GC-bias correcting and Z-score calculating to determine the risk of fetal chromosome aneuploidy. When the Z-score >3 or <-3 of any chromosome showed that the fetal with high risk of fetal chromosome aneuploidy. At the same time, the clean data were mapped to the SARS-CoV-2 reference genome (NC_045512.2), and the low-quality reads (mapped length less than 60) were filtered. Then through calculating the number of mapped SARS-CoV-2 reads to determine whether there was SARS-CoV-2 infection.

Statistical analysis

Paired t-test was used to compare the amount of data that generated by different methods, and Pearson correlation coefficient was used for data correlation analysis. All statistical analysis were performed using R3.5.1.

Result

Detection of fetal chromosome aneuploidy

NIPT analysis and SARS-CoV-2 analysis were performed for all samples. Negative NIPT result was confirmed in all cases in the negative group and simulated SARS-CoV-2 group, while positive NIPT result was confirmed in all cases in the trisomy positive group, including 4 cases for trisomy 21, 4 cases for trisomy 18, 1 case for trisomy 13, and 1 case for trisomy 21 combined with trisomy 13. 20 clinical routine NIPT samples from Changsha Hospital for Maternal and Child Health Care were selected randomly to evaluate the sequencing data of the new technology of NIPT-SARS-CoV-2. The results demonstrated that there was no significant difference of total sequencing data in the two technologies', which indicated the new NIPT-SARS-CoV-2 could accurately analyze fetal chromosome aneuploidy without increasing the sequencing data.

Detection of SARS-CoV-2

SARS-CoV-2 reads were detected in all cases in simulated SARS-CoV-2 group, with average 35 reads in 1400 copies group (range 4-64), average 337 reads in 14,000 copies group (range 252-464), and average 2,759 reads in 140,000 copies group (range 2392-3220). None SARS-CoV-2 reads were detected in the negative group and trisomy positive group. Combined the NIPT result indicated that the new NIPT-SARS-CoV-2 could detect SARS-CoV-2 in the three concentration gradients without affecting the fetal chromosome aneuploidy analysis. The fewest number of SARS-CoV-2 reads only 4 in 1,400 copies group, suggesting that this method could accurately detect SARS-CoV-2 with a viral load of 1,400 copies/ml. The proportion of SARS-CoV-2 reads in 1 million total reads was calculated to analysis the relationship between SARS-CoV-2 reads proportion and detected viral reads. The correlation coefficient

between the two up to 0.977, suggesting that the new NIPT-SARS-CoV-2 has potential for quantitative or semi-quantitative analysis of SARS-CoV-2.

Discussion

Since the outbreak of COVID-19, asymptomatic infections have been reported in many studies [11-13]. Asymptomatic infections mean who have no clinical symptoms and abnormalities in images but with positive detection of SARS-CoV-2. A study focuses on asymptomatic ratio showed that the incidence of asymptomatic was estimated to be 30.8% among 565 Japanese citizens evacuated from Wuhan [13]. Asymptomatic infection is also existed in pregnant women. Another study investigated 675 pregnant women for delivery in three New York City hospitals found that 10.4% were positive for SARS-CoV-2 and up to 78.6% of them were asymptomatic [14]. Some studies indicated that the viral load in asymptomatic infections is similar to COVID-19 patients, and the infectivity between the two has no significant difference, which signified asymptomatic infections may accelerate the spread of COVID-19 [15-18]. But it has great challenge to identify and control asymptomatic infections due to no obvious clinical manifestations with them. Screening high-risk populations is a possible effective measure to find asymptomatic. The new method NIPT-SARS-CoV-2 reported in this study can accurately analyze fetal chromosome aneuploidy and SARS-CoV-2 without increasing the sequencing data, and the virus load could low to 1,400 copies/ml. So that through this method SARS-CoV-2 can be detected for all pregnant women when they carry out the routine prenatal testing. In addition, detecting viral nucleic acid by peripheral blood can avoid the drawbacks of collecting nasopharyngeal swabs collection, such as the discomfort and psychological pressure to pregnant women, the exposure of healthcare staff. In this way, pregnant women with asymptomatic infection can be early recognized and controlled, which will be conducive to get the optimal management and treatment for them. The collection of upper respiratory specimens, especially nasopharyngeal swabs, has been widely used to confirm the diagnosis of COVID-19. However, in addition to nasopharyngeal swabs, some researches show that SARS-CoV-2 nucleic acid can also be detected in other sites including sputum, feces, blood and other types of samples [19,20]. SARS-CoV-2 nucleic acid can be detected in the blood of COVID-19 patients 2~3 days after onset, and there is even report shown that the same one COVID-19 patient with positive SARS-CoV-2 detection in blood samples, but no SARS-CoV-2 is detected in throat swabs, which suggesting that it is feasible and necessary to detect SARS-CoV-2 nucleic acid by blood samples. Once virus infection occurs, although it takes a period of time to form toxemia or show obvious clinical symptoms, the nucleic acid fragments of virus degraded in the process of metabolism will enter the peripheral blood at an early stage. Therefore, the new technology NIPT-SARS-CoV-2 has the advantage of screening pregnant women carrying the virus in the early stage of infection. The diagnosis of COVID-19 mainly depends on clinical manifestations, epidemic history, CT imaging screening and etiology detection, among which etiology detection is the most accurate diagnosis scheme [21]. Etiology detection includes viral nucleic acid detection, antibody and antigen detection, and the viral gene detection by quantitative Reverse Transcription Polymerase Chain Reaction (RT-qPCR) is a priority test recommended by National Health Commission of the People's Republic of China [22]. RT-qPCR can detect viral nucleic acids efficiently and rapidly, with high sensitivity and high specificity. But this method only can detect some specific viral gene regions, such as Open reading frame 1 ab (*ORF1ab*), Nucleocapsid protein (*N*) and Envelope (*E*), which may cause false negative due to the virus variant. Unlike RT-qPCR, the NIPT-SARS-CoV-2 can detect whole genome of the virus with low-depth sequencing, which can identify virus variants and trace the cryptic origins of the virus in theory. A recently study reported an intrauterine infection case of COVID-19, the puerperal and the newborn were infected with the same virus, but a variant of the virus occurred in the newborn a few days later [9]. The researchers thought it might be caused by the contact with the external environment after birth, and the virus mutated surprisingly fast, which also suggested the importance of virus gene sequencing. In conclusion, the technology of NIPT-SARS-CoV-2 reported in this study can achieve the purpose of NIPT detection and SARS-CoV-2 detection for pregnant women in the progress of routine prenatal testing by drawing only one tube of peripheral blood. In this way, SARS-CoV-2 screening can be performed for pregnant women, so that asymptomatic infections can be early recognized and controlled, which ensure the optimal management and treatment for pregnant women and newborns. In addition, it has the potential for monitoring virus variant and tracking virus transmission, which will be helpful for control the spread of this serious epidemic.

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