EARLY DIAGNOSIS AND CLINICAL CHARACTERISTICS OF NEONATAL PURULENT MENINGITIS

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ABSTRACT

Purpose: *This study aims to improve early diagnosis of neonatal purulent meningitis (neonatal brain).*
Methods: *Universal primers, Gram-positive bacteria, and Gram-negative bacteria dual fluorescent probes are designed using bacterial 16S-r RNA genes. This study tests the cerebrospinal fluid specimens of patients with suspected purulent meningitis admitted in the department of neonatology of the provincial women and children health hospital in 2014, and detects the blood routine, CRP, blood culture, cerebrospinal fluid routine, cerebrospinal fluid biochemistry, cerebrospinal fluid bacterial culture and cerebrospinal fluid bacterial smear using double-fluorescent quantitative PCR.*
Results: *Among the 125 patients, 1 case is diagnosed with meningitis, and the Streptococcus agalactiae is cultured from its cerebrospinal fluid (CSF), which is confirmed to be Gram-positive bacterium by double fluorescent quantitative PCR.*
Conclusions: *If the full-term infant has fever, the blood CRP is obviously increased. Premature infants that have a history of intrauterine infection and show poor response, shortness of breath, and abnormal muscle tension, shall be wary of the possibility of meningitis.*

Keywords: *Purulent meningitis, Early diagnosis, Clinical characteristics.*

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Introduction

Neonatal purulent meningitis (abbreviated as neonatal meningitis) refers to the meningitis caused by pyogenic bacterial infection within 4 weeks after birth. Neonatal meningitis is an important infectious disease that causes neonatal death and severe neurological sequelae. In the past few years, due to the development of neonatal nursing techniques and the use of antibiotics, the mortality rate of neonatal meningitis in developed countries has dropped from 29% to 10%. In developing countries, however, neonatal meningitis mortality is still between 40% and 58%. Although the mortality rate of neonatal meningitis has decreased, its incidence rate has not obviously decreased. Neonate meningitis is found in 25% of neonates with neonatal sepsis. In a study on neonatal infectious diseases, it is found that approximately 0.8-1.6 neonates are infected with purulent meningitis in 1,000 live births(1).

Furthermore, the incidence of neurologic sequelae of long-term neonatal meningitis, such as hydrocephalus, abnormal hearing and visual acuity, and mental retardation is still high. A follow-up study on meningitis finds that 24% of patients have severe neurological sequelae after 5 years of the disease and 26% have neurological sequelae after 10 years. Due to the atypical clinical symptoms, blurred normal limits of leukocyte counts and protein quantification in cerebrospinal fluid, low positive rate of bacterial culture in cerebrospinal fluid, and the application of early-stage broad-spectrum antibiotics, atypical changes occur in cerebrospinal fluid, resulting in difficult early diagnosis of neonatal meningitis, regardless of its high incidence, mortality and disability rates(2). The polymerase chain reaction (PCR) technique developed in recent years has provided a good technical support for the early diagnosis of neonatal meningitis by virtue of its high sensitivity, high specificity, short detection time and being unaffected by antibiotics.

The disease is often part of or secondary to sepsis, and it is generally believed that the pathogenic bacteria of purulent meningitis are consistent with that of sepsis. But this is not exactly the case, because some meningitis may be sepsis-free, but is caused by pathogenic bacteria directly invading the meninges or is only combined with transient bacteraemia. There is group B haemolytic streptococcus, Escherichia coli, Listeria monocytogenes, Klebsiella spp., Salmonella, degenerative bacilli, and others pathogenic bacteria outside of China. In China, there are different pathogenic bacteria s, such as Escherichia coli, Staphylococcus, Acinetobacter, Proteus, etc.(3). The ways of infection of this disease are as follows.

• *Infection before birth*

It is very rare. When maternal Listeria monocytogenes infection is associated with bacteraemia, the bacteria can cause miscarriage, stillbirth, and premature birth through the placenta. Purulent meningitis can occasionally become part of the foetal systemic infection.

• *Infection at birth*.

Many infected children have premature rupture of membranes, prolonged labor, dystocia and other production history. In this case, the pathogenic bacteria induce the disease by contaminating amniotic fluid from the mother's rectum or vagina or by being inhaled or swallowed by foetus when it goes through the birth canal(4). Infection after birth. The pathogenic bacteria can invade the blood circulation from the respiratory tract, umbilicus, damaged skin and mucous membrane, digestive tract and conjunctiva, and then reach the meninges. If the neonate has otitis media, infectious head hematoma, cranioschisis, spina bifida, meningocele, skin sinus (few are connected with subarachnoid space), that pathogenic bacteria will invade the meninges directly through these diseases to cause meningitis(5).

In view of the conserved regions of the 16S-r RNA gene in bacteria, this study designs universal primers, Gram-positive bacteria, and Gram-negative bacteria dual fluorescent probes and tests the cerebrospinal fluid specimens of patients with suspected purulent meningitis admitted in the department of neonatology of the provincial women and children health hospital in 2014 using double-fluorescent quantitative PCR.

Finally, this study assesses the value of double-fluorescent quantitative PCR in the diagnosis of neonatal meningitis by comparing the difference between double-fluorescent quantitative PCR and cerebrospinal fluid bacterial culture and smear in diagnosing the positive rate of neonatal meningitis.

Research Principles and Methods

The neonatal patients admitted in the department of neonatology of the provincial women and children health hospital in January 1, 2014 to December 31, 2014 are selected as subjects. Inclusion criteria:

• Patients with infectious focus and abnormal neurological signs in physical examination.

• Patients with blood culture positive sepsis.

• Patients with convulsions that cannot be explained by ischemia or hypoxia or intracranial hemorrhage.

• Patients with unexplained hydrocephalus, subdural effusions, brain abscesses found by brain imaging examination.

• Patients with severe infection and poor drug treatment or recurrent infection with unclear infection focus.

Exclusion criteria:

• Patients who fail to perform lumbar puncture due to critical condition, abnormal coagulation function or family refusal.

• Children with congenital brain development malformations.

• Patients with chromosome abnormality, severe congenital malformation, or genetic metabolic disease.

The study subjects are examined for blood routine, CRP and blood culture before using antibiotics after admission. Brain imaging examination is performed 3-4 days after admission. Once the inclusion criteria are met and the family members agree to undergo lumbar puncture, the examinations of cerebrospinal fluid routine, cerebrospinal fluid biochemistry, cerebrospinal fluid bacterial culture, and cerebrospinal fluid bacterial smears are performed immediately. Approximately 1 ml of cerebrospinal fluid specimen is also taken for PCR detection. Spinal fluid samples shall be packed using sterile test tubes and stored in a refrigerator at -80 °C(6). Take 1m of cerebrospinal fluid specimen, centrifuge at 12000r/min for 10 minutes, and remove the supernatant. Add 200ul of Insta Gene Matrix to the precipitate, take a water bath at 56°C for 30 minutes, oscillate the mixture at a high speed for 10s, boil it at 100°C for 8 minutes, and then oscillate it at a high speed for 10s. After centrifuging at 12000r/min for 5 minutes, take the supernatant

as a template[7]. Gram-positive bacteria causing neonatal purulent meningitis mainly include: Streptococcus agalactiae, Staphylococcus epidermidis, Staphylococcus aureus, Staphylococcus haemolyticus, Streptococcus pneumoniae, Streptococcus pyogenes, Enterococcus faecalis, and Enterococcus faecium. Common Gram-negative bacteria causing purulent meningitis mainly include Escherichia coli, Pseudomonas aeruginosa, Neisseria meningitidis, Proteus vulgaris, Proteus mirabilis, Enterobacter cloacae, Klebsiella pneumoniae, and Haemophilus influenzae. The 16S-r RNA gene sequences of these bacteria queried in Genebank are input to Clu Star X software for gene sequence analysis[8]. At the same time, according to the principles of primer and probe design, a pair of universal primers is selected in the conserved region of 16S-r RNA gene, and Gram-positive bacteria and Gram-negative bacteria probes were selected in the variable region. Universal primers, Gram-positive probes and Gram-negative probe sequences are detailed in Table 1.

Name	Sequence	Position
Upstream primers	GCAACGCGAAGAACCTTACC	946-965
Downstream primers	ACGTCATCCCCACCTTCCT	1156-1174
Gram-positive probe	FAM-ACGACAACCATGCACCACCTG-TAM-RA	1029-1048
Gram-negative probe	HEX-CTGACGACAGCCATGCAGCA-TAMRA	1056-1076

Table. 1: Universal primers, Gram-positive probes, and Gram-negative probe sequences.

Due to the high sensitivity of PCR technique, in order to avoid the accidental contamination, the experimental specimens are operated in batches. In addition, "blank control", "Gram-positive control" and "Gram-negative control" are set up in each batch. That is, the sterilization injection water is added to each batch as "blank control", the sterilization injection water contaminated by Escherichia coli is used as "G-bacteria control", and the sterilization injection water contaminated by Streptococcus agalactiae is used as "G + bacteria control".

The PCR reaction system is 20ul, the concentration of primer and fluorescent probe is 0.2umol, ROX internal reference dye is 0.4ul, DNA template is 1ul, and 20ul is supplemented with sterile deionized water. All reaction solutions are filtered by 0.22 um filter before adding the template.

All instruments and test tubes are irradiated with ultraviolet light for 30 minutes before the experiment[9]. The fluorescence quantitative reaction is performed on ABI7500. Amplification conditions: pre-denaturation at 95°C for 30s, 95°C for 5s, and 60°C for 34s. Read the results after 35 cycles. If the fluorescent signal is found before 35 cycles, and then the result is positive. The signal marker of Gram-positive bacteria probe is G +, and the signal marker of Gram-negative bacteria probe is G -. If no fluorescent signal is found, then the result is negative[10].

SPSS 18.0 software is used for statistical processing. The count data are expressed using the composition ratio and the comparison between groups is analyzed using the χ^2 test. $P<0.05$ is considered statistically significant[11].

Results and Analysis

Of the 4,103 subjects, 125 specimens are included in this study. According to the diagnostic criteria of neonatal meningitis, 42 cases are diagnosed as neonatal purulent meningitis and 83 cases non- purulent meningitis. The double-fluorescent quantitative PCR results are shown in Figure 1.

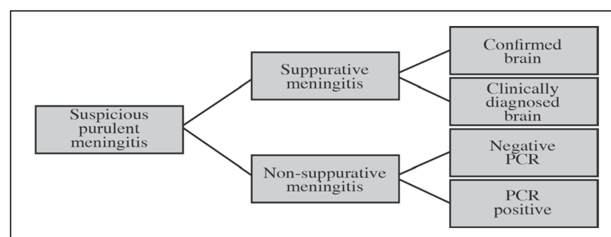

Figure 1: Schematic diagram of double-fluorescent quantitative PCR experiments.

The cerebrospinal fluid bacterial culture is used as a diagnostic "golden criteria". The sensitivity and specificity of the double fluorescent quantitative PCR assay are 100% and 84.6% respectively. The positive rate of double fluorescent quantitative PCR is 16% (20/125), which is significantly higher than that of bacterial culture and smear in cerebrospinal fluid (0.8%, 1/125). There is a statistically significant difference between the two ($P<0.01$) (See Table 2).

The clinical characteristics of 20 cases of double-fluorescent quantitative PCR positive meningitis patients are divided into Gram-positive meningitis and Gram-negative meningitis (See Table 3) for comparison. The laboratory tests (see Table 4) show that there are no significant differences in

PCR test	Cerebrospinal fluid bacterial culture	
	Positive	Negative
Positive	1	19
Negative	0	105
χ^2	17.052	
P	<0.0001	

Table. 2: Comparison of double-fluorescent quantitative PCR and cerebrospinal fluid bacterial culture results.

Clinical manifestations	Gram-positive meningitis	Gram-negative meningitis	P
Fever	7 (77.7)	6 (54.5)	0.374
Shortness of breath	3(33.3)	6(54.5)	0.342
Less	6(66.6)	5(45.4)	0.405
Jaundice	3(33.3)	6(54.5)	0.342
Hairpin	5(55.5)	2(18.1)	0.081

Table. 3: Comparison of clinical characteristics of Gram-positive meningitis and Gram-negative meningitis.

Project	G+ meningitis	G-meningitis	P
PLT($\times 10^9$/L)			
<150	1(11.1)	1(9.0)	0.521
≥150	8(88.9)	10(91.0)	0.521
CRP(mg/L)			
<2	2(22.2)	2(18.1)	0.408
2-10	0(0)	2(18.1)	0.289
10-20	0(0)	2(18.1)	0.289
≥20	7(77.8)	5(45.5)	0.132

Table. 4: Comparison of laboratory tests of Gram-positive meningitis and Gram-negative meningitis.

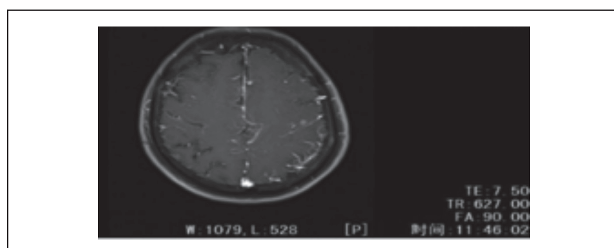

Figure 2: Image of purulent meningitis.

clinical characteristics and laboratory test results between the two types. The image of a patient with purulent meningitis is shown in Figure 2[12-15].

Conclusions

Neonatal meningitis is an important infectious disease leading to neonatal death and severe neurological sequelae. However, due to the atypical and lack of specificity of the clinical symptom of neonatal meningitis, the bacterial culture of cerebrospinal fluid, currently the diagnostic golden criteria of neonatal meningitis, has a low positive rate and is time-consuming (at least 48 hours), which make it difficult for the early diagnosis of neonatal meningitis. Routine and biochemical cerebrospinal fluid are important indicators of clinical diagnosis of neonatal meningitis. Due to their large range of normal values, there is an overlap between their normal and abnormal values. Even if the leucocyte count, protein quantification, and glucose concentration in the cerebrospinal fluid are normal, there may still be purulent meningitis.

In the United States, a study on 9,111 newborns undergoing lumbar puncture finds that 95 of them are positive for bacterial cultures of cerebrospinal fluid and proved to be purulent meningitis. Among these 95 cases, 12 cases (13%) have normal leucocyte count, protein quantification, and glucose concentration in the cerebrospinal fluid. In this study, 1 case with meningitis is confirmed by cerebrospinal fluid culture positive, and its cerebrospinal fluid protein concentration and glucose concentration are in the normal range, and cerebrospinal fluid leucocyte count is slightly higher than normal. It indicates that it is difficult to make accurate diagnosis of neonatal meningitis in the early stage only by routine and biochemical examination of cerebrospinal fluid, which may lead to missed diagnosis or excessive medical treatment in a large number of cases, causing pain to patients and increasing social burden.

Compared with the bacterial culture of cerebrospinal fluid, double fluorescent quantitative PCR has the advantage of small time-consumption (3-4 hours), low cost (100 yuan/person/time), high sensitivity and being unaffected by antibiotics. In addition, when the test is positive, it can also further distinguish between purulent meningitis caused Gram-positive bacteria and that by Gram-negative bacteria, which provides assistance for selecting suitable antibiotics in the early stage of clinic. It

can also be combined with routine, biochemical and bacterial cultures of cerebrospinal fluid to improve the early diagnosis of neonatal meningitis.

References

1) Bentlin MR, Ferreira GL, Rugolo LM, Silva GH, Mondelli AL, Rugolo Júnior A. Neonatal meningitis according to the microbiological diagnosis: a decade of experience in a tertiary center. Arquivos de Neuro-Psiquiatria 2010; 68(6): 882-887.
2) Ashraf MA, Maah MJ, Yusoff I, Mahmood K, Wajid A. Study of Antioxidant Potential of Tropical Fruit. International Journal of Bioscience, Biochemistry and Bioinformatics 2011; 1(1): 53-57.
3) Abhilash KP, Mitra S, Arul JJ, Raj PM, Balaji V, Kannangai R, Thomas SA, Abraham OC. Changing paradigm of Cryptococcal meningitis: An eight-year experience from a tertiary hospital in South India. Indian Journal of Medical Microbiology 2015; 33(1): 25-29.
4) Anila K, Bilal A, Atifa M, Hira A, Hafiz MA. Gastroprotective Effect of Avena Sativa (Oat) Seed Grains Against Gastric Ulcer Induced by Indomethacin in Healthy Adult Male Albino Rabbits. Matrix Science Pharma 2018; 2(1): 14-18.
5) Gast RA, Soriano AR, Teshome G. 303: Predictors of Meningitis In 0-3-Month-Old Infants with Urinary Tract Infection. Annals of Emergency Medicine 2010; 56(3): S99-S99.
6) Mehar V, Yadav D, Somani P, Bhatambare G, Mulye S, Singh K. Neonatal sepsis in a tertiary care center in central India: microbiological profile, antimicrobial sensitivity pattern and outcome. Journal of Neonatal-perinatal Medicine 2013; 6(2): 165-72.
7) Maryam R, Ghulam M. Copper Deficiency in Ruminants in Pakistan. Matrix Science Medica, 2(1): 18-21.
8) Zinayyera S, Muhammad S, Muhammad SS, Muhammad N, Hira M. Adverse Effect of Oxalis Corniculataon Growth Performance of Broiler Chicks during aflatoxicosis. Matrix Science Pharma 2018; 2(1): 10-13.
9) Slade MS, Simmons RL, Yunis E, Greenberg LJ. Immunodepression after major surgery in normal patients. Surgery 1975; 78(3): 363-372.
10) Mcbride WT, Armstrong MA, Mcbride SJ. Immunomodulation: an important concept in modern anesthesia. Anesthesia 1996; 51(5): 465-473.
11) Loze GM, Collins D, Holmes PS. Pre-shot EEG alpha-power reactivity during expert air-pistol shooting: a comparison of best and worst shots. Journal of Sports Sciences 2001; 19(9): 727-733.
12) Di-Russo F, Martinez A, Sereno M, Pitzalis S, Hillyard S. Cortical sources of the early components of the visual evoked potential. Human Brain Mapping 2002; 15(2): 95–111.
13) Saira M, Sajjad UR. Optimization of Countercurrent Immuno electrophoresis And Agar Gel Immunodiffusion Tests for The Comparative Detection of Horse and Donkey Meat. Matrix Science Medica 2018; 2(1): 14-17.
14) Samuel JV, Mark RW. Quiet Eye Training: Effects on Learning and Performance under Pressure. Journal of Applied Sport Psychology 2010; 22(4): 361-376.
15) Gunawardhana SA, Somaratne SC, Fernando MA, Gunaratne PS. Tuberculous meningitis in adults: a prospective study at a tertiary referral center in Sri Lanka. Ceylon Medical Journal 2013; 58(1): 21-25.

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