

Latex agglutination: Diagnose the early cryptococcus neoformans test of capsular polysaccharide antigen

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Abstract: This paper aims to discuss the early diagnosis value of latex agglutination test in Cryptococcal meningitis. The cerebrospinal fluid (CSF) of 112 patients with definite Cryptococcal meningitis and 26 patients with tubercular meningitis and virus meningitis were collected, latex agglutination test is adopted to detect Cryptococcal capsular polysaccharide antigen. Then it was compared with fungal culture and direct microscopy method for evaluating the sensitivity and specificity of the diagnosis. The sensitivity of three methods including latex agglutination test, fungal culture and direct microscopy was 91.1%, 69.6% and 73.2% respectively. The specificity of latex agglutination test was 96.0%, 100% and 100% respectively. That latex agglutination test to detect Cryptococcal capsular polysaccharide antigen could be taken as the early diagnostic method of Cryptococcus neoformans meningitis.

Keywords: Meningitis, latex agglutination test, diagnosis.

INTRODUCTOIN

Bacterial meningitis is a kind of serious infectious disease, its fatality rate and the occurrence rate of sequela are very high. This paper is based on the common Cryptococcus neoformans meningitis in the clinic for undertaking relevant research. For Cryptococcus neoformans meningitis, the traditional diagnostic methods mainly include: microscopy and culture, but the early microscopic examination of attack appears to be normal, while the cultural method is time-consuming, and its detection rate is low, thus diagnosis and treatment of the disease is delayed to a certain extent. So it is of prime importance to look for a kind of rapid, sensitive and specific method for the early detection of Cryptococcus neoformans infection. This research involves latex agglutination test (LA) to detect Cryptococcus neoformans capsular polysaccharide antigen, and discusses its early diagnostic value for cryptococcal meningitis.

Cryptococcus meningitis is an opportunistic fungal disease (caused by Cryptococcus neoformans), which mainly affect central nervous system and the lung. Central nervous system infection is the most common (Bing *et al.*, 2011). The disease has no obvious tendentiousness of age and gender. Some patients with chronic diseases, such as diabetes mellitus, leucocythemia, advanced tumor disorder, acquired immunodeficiency syndrome (AIDS), and the patients with organ transplantation are always prone to this disease.

Antigen fluorescence analysis represents that B group of capsular polysaccharide is the constituent neuraminic acid polymer as α -(2-8) is linked with 200 residues of

homopolymer in neuraminic acid. A similar structure is contained in mammal, human embryo tissues and gangliosides. Cross reaction may occur thus induce autoimmunity disease, therefore capsular polysaccharide in B group of serum is not suitable for developing vaccine (Khatami and Pollard, 2010).

In recent years, with the widespread use of clinical broad-spectrum antibiotic, immunosuppressor, glucocorticoid, tumor radiation and chemotherapy, as well as the constant growing number of AIDS cases, the patients with cryptococcal meningitis have increased significantly. Most of the patients with Cryptococcal meningitis are very serious with high fatality rate, and their clinical symptoms are not typical. Misdiagnosis often occurs in early stage of the disease. A lot of patients are mistakenly treated as inflammation and tuberculosis, part of the disease cases are diagnosed definitely after brain tumor surgery (Amulya and Shalini, 2011).

Especially the diagnostic sensibility of cryptococcal meningitis is commonly applied clinically at present is low, thus treatment opportunity is too easy to be delayed. Hence mortality and morbidity cases would increase as a result. A large number of clinical data also indicate that the efficacy and outcome of Cryptococcal meningitis depends on early diagnosis to a great extent (Fengbin *et al.*, 2010).

The hook belt phenomenon caused by high concentration of cryptococcal antigens and the masking effect of unknown non-specific proteins in the body on cryptococcal antigen, false negatives could be generated. In the meantime, according to literature report that RF positive serum and TB patients could cause false positive test of Cryptococcus capsular polysaccharide antigen (Luxia *et al.*, 2010).

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MATERIALS AND METHODS

Experiment and method

One hundred and twelve cases come from neurology patients in hospital from January 2012 to April 2013. According to medical history, clinical symptoms, physical sign, laboratory test and etiological experiment. Antibiotics like anti-tuberculosis drugs have no effect, but antifungal drugs are effective for cryptococcal meningitis. There were 70 males and 42 females, their age range was 23 ~ 71 years. All the returned specimens are before taking the antifungal drug. Twenty six patients in control group come from hospital neurology department, they were confirmed to be tubercular meningitis or virus meningitis (with 14 male cases and 12 female cases with their age range of 20 ~ 70 years).

Instrument and reagent

Method

Microscopy ink stain

Ink stain selects India ink or domestic high-quality ink, and microscopic examination is directly performed on sediment taken from cerebrospinal fluid (CSF) after centrifuging. Perfectly clear thallus and generous capsule could be seen obviously against black background. Gram's stain: after centrifuging CSF specimen, microscopic examination was performed; after sediment Gram staining, blue thallus and colorless capsule is positive (Henry *et al.*, 2013).

Cultivation and appraisal

Positive alarm culture bottle is immediately switched to sand cultivation medium, fungal identification reagents AI-20CAUX was applied for appraisal after growth and cloning.

Conventional methods of latex agglutination test

Sterile samples are collected, 1000×g centrifuge for 15min was performed to make sure to remove all the white blood cells and microsomes, cerebrospinal fluid (CSF) should be carefully absorbed, then it is added into sterile chamber (cloudy or floc specimen were abandoned), incubation of cerebrospinal fluid at 100℃ for 5min was done. A 25μl volume of cryptococcal antigen positive control, negative control and the heat treatment of cerebrospinal fluid were respectively added into separation ring of ring slides, then 25μl Cryptococcus latex is added into each ring, separated coating rods are used to mix the substance in the ring, observed result could appear after manual rotation for 5 min at room temperature.

Cryptococcal antigen detection

According to the cryptococcal antigen produced by American Immuno-Mycologics limited liability company,

the explanatory memorandum in latex agglutination test kit could be drawn to detect cerebrospinal fluid (CSF), Cryptococcus capsular polysaccharide antigen in serum, determination and dynamic observation of antigen drop degree was performed.

In the background without light, read the number and divide grades into negative to 4+. The ositive control is 2+ or higher while the positive control is lower than 1+. The detailed division standard is as follows:

Results analysis: 4+: For all of the latex agglutination, particles deposit on the edge of droplets, liquid is completely transparent; 3+: For most of the latex agglutination, particles are significant, liquid is a little cloudy; 2+: About half of latex agglutination has relatively tiny particles and cloudy liquid; 1+: There is some agglutination, the liquid is cloudy; -: Liquid drop represents original homogeneous emulsion. ≥2+ could be considered to be positive. Antigen drop degree could be denoted by highest dilution multiple of appearing positive.

Cryptococcus antibody detection

According to the explanatory memorandum on cryptococcal antigen latex agglutination test produced by American Immuno-Mycologics limited company, antibodies against Cryptococcus in serum are detected, and the measure of antibody titer is determined.

In the background without light, read the number and divide grades into negative to 4+. The positive control is 2+ or higher while the positive control is lower than 1+. The detailed division standard is as follows:

The results of interpretation: 4+: For complete agglutination, agglutination block is very big and sink on the bottom of the tube liquid clarification completely; 3+: For vast majority of aggregation, agglutination block is bigger and sink on the bottom of the tube, liquid is more cloudy; 2+: For partial agglutination, agglutinator at the bottom of tube is less than the first two, but it is still significant, liquid is of half clarification; 1+: Agglutinator is extremely few and could be found after careful observation, the liquid is cloudy; -: For no agglutination, the turbidity of the liquid is the same as the negative tube. ≥1+ could be considered as positive. Antibody agglutination titer is denoted with the highest dilution multiple of positive.

STATISTICAL ANALYSIS

SAS 9.2 statistical software was used to perform statistical treatment, each test result is denoted with mean ± standard deviation ($\mu, t \pm S$). Enumeration data adopts X2 test, inspection level takes $\alpha = 0.05$.

Table 1: The main experimental reagents and instruments

Fungal identification reagents API-20CAUX	Merry Emmanuel biotechnology Co., Ltd in French
BACTEC9120 Fully automatic blood culture instrument	Brigitte di medical equipment (Shanghai) Co., Ltd
Gram Stain	Besso company
Cryptococcal antigen latex agglutination reagents, latex cryptococcal antibody detection kit	Immuno-Mycologies limited company, USA
BACTEC9120 Fully automatic blood culture instrument	Becton Dickinson company, USA
Image analysis system of cell medicine	Thai union technology Co., Ltd, China
Optical microscope	Olympas company, Japan

Table 2: Results of three detection methods

Project	Latex agglutination test	Fungal culture	Microscopic examination
Sensitivity	91.1% (106/112)	69.9% (78/112)	73.2% (82/112)
Specificity	96.0% (25/26)	100% (26/26)	100% (26/26)

Table 3: Detection result of implicit lymphocytic antigen and antibody

Group	No. of causes	No. of improvement cases	No. of unhealed cases	No. of deaths	Fatality rate (%)
Group A	112	37	19	56	50.00%
Group B	26	18	3	6	20.00%

RESULT

CSF inspection result of patients: Eighty two cases were microscopy positive, microscopic examination includes Gram stain and ink stain. Seventy eight cases are fungal culture positive, 106 cases are positive in latex agglutination test. The positive rate of 3 methods including latex agglutination test, fungal culture and direct microscopy are 91.1%, 69.6% and 73.2% respectively. Twenty six cases of microscopic examination and propagation results in control group were negative, while there was 1 positive case in latex agglutination test, its specificity is 96%. Result of 3 detection methods are shown in table 2.

Implicit lymphocytic antigen, antibody detection (table 3, figs. 1 and 2). There were 112 cases with cryptococcal meningitis whose cerebrospinal fluid (CSF) was detected for cryptococcal antigen, the results are all positive. There were 26 cases of control group, the sensibility of one positive case reached 100%, its specificity is 98.9%. Chi-squared test suggests the detection of cryptococcal antigen in cerebrospinal fluid (CSF) is superior than cerebrospinal fluid cytology MGG dyeing ($P=0.021$), alcian blue dyeing ($P=0.012$), ink staining after centrifuging ($P=0.006$). Dynamic observation was performed on the cryptococcal antigen of cerebrospinal fluid (CSF) in 17 patients with cryptococcal meningitis, the antigen titer before treatment was 1:16 ~ 1:1024, its average titer was 1070. 02 ± 6.65 , the antigen titer after treatment was 1:8 ~ 1:1024, its average titer is 108.974 ± 3.87 , t -test suggests that the cryptococcal

antigen titer of cerebrospinal fluid (CSF) after treatment decreased significantly after treatment ($P=0.0004$). There were 15 patients with cryptococcal meningitis who were detected for the cryptococcal antigen and antibody in serum at the same time. In the detection of serum antigen, there are 14 positive case, antigen titer is 1: 8 ~ 1: 16784, the average antigen titer is 345.74 ± 7.38 . Chi-squared test and T test suggest that there is no statistical significance between the positive rate of cryptococcal antigen detection and estimated value of antigen titer in cerebrospinal fluid and serum ($P = 0.123$, $P = 0.5060$). In the detection of serum antibody, there were 8 positive cases, its sensibility is only 53.33 % . There was no positive case in control group, its specificity reaches 100 % . Chi-squared test suggests that the positive rate of cryptococcal antigen detection in serum is obviously higher than the positive rate of Cryptococcal antibody detection in serum, its difference has statistical significance ($P = 0.035$).

DISCUSSION

Cryptococcal meningitis is the relatively common type of septic meningitis. It is a kind of central nervous system infection disease caused by *Cryptococcus neoformans* and its variety. It is the most common fungal infection of central nervous system. As misdiagnosis and mistreatment is extremely easy to occur during early times, powerful drugs are in shortage during later period, its fatality rate is very high, thus it is a kind of deep fungal disease which would severely harm human's health.

In recent years, with the widespread use of broad-spectrum antibiotics, immunosuppressores, glucocorticoid, tumor radiation and chemotherapy clinically, as well as the growth of AIDS cases, patients with Cryptococcal meningitis have increased significantly. Most of the patients with Cryptococcal meningitis have serious disease and high fatality rate, and its clinical symptoms are not typical, misdiagnosis is common in early stage. A lot of patients are treated as inflammation and tuberculosis cases, part of the cases are confirmed after brain tumor surgery. Especially the sensibility of the diagnostic methods for cryptococcal meningitis is low, treatment opportunity is extremely easy to be delayed, and fatality rate and disability rate would increase as a result (Carlos *et al.*, 2009). A large number of clinical data also indicate that the effect and relapses of Cryptococcal meningitis depends on early and definite diagnosis to a great extent. Specific etiology detection means which are in short of sensitivity (another important reason for misdiagnosing Cryptococcal meningitis).

The methods of detecting Cryptococcal neoformans in most hospitals at present are ink stain, Gram stain and fungal culture. Ink staining microscopy of cerebrospinal fluid smear is easily and rapid, it is the direct and rapid diagnostic method for diagnosing Cryptococcal meningitis. In the early stages of attack, microscopic examination could represent to be normal, Cryptococcus could be found out through repeated checks. Fungal cultivation method is time consuming, and its positive rate is low, thus the treatment of Cryptococcal meningitis is delayed to a certain extent (Khatami and Pollard, 2010). Thus, it is vital to search for a kind of rapid, sensitive and specific method for early detection of deep Cryptococcal infection. Latex agglutination test is the immunological detection aiming at Cryptococcal neoformans capsular polysaccharide antigen; it is superior to traditional culture method and microscopy method for early diagnosis. The data of this research indicate that the positive rate of latex agglutination test is relatively high, and the positive rate of fungal culture and microscopy is relatively low. These experimental results indicate that the positive rate of Cryptococcus capsular polysaccharide antigen in cerebrospinal fluid (CSF) detected through applying latex agglutination test is 91.1%. It is basically consistent with that positive rate of cryptococcal antigen detection in cerebrospinal fluid (CSF) in report of Antionori, et al is 94.1%, etc. But one must pay attention to the masking effect of the hook belt phenomenon caused by high concentration of cryptococcal antigens and unknown non-specific proteins in the body on cryptococcal antigen. It is able to generate false negative. According to literature reports, RF positive serum and TB diabetic serum could make the test of cryptococcal capsular polysaccharide antigen latex agglutination be false positive.

The misdiagnosis rate of recessive meningitis is very high. According to literature reports, there are about 70 % -80 % patients with recessive meningitis who are misdiagnosed as tubercular meningitis (World Health Organization, 2010). Thus, in order to reduce misdiagnosis, one need to enhance vigilance to recessive meningitis, the patients who are suspected to have central nervous system infection should be searched for Cryptococcal from cerebrospinal fluid (CSF) as a routine check. For the patients who are suspected to have tuberculous meningitis, and the chemotherapy treatment does not improve the disease, and even the disease get worse, then the Cryptococcal should be repeatedly searched, and the existence of this disease should not be rejected as there is no Cryptococcus being found out in examination of cerebrospinal fluid. When the patients who are accompanied with underlying diseases have neurological symptoms, the possibility of recessive meningitis should be kept with consideration.

CONCLUSION

This research indicates that the method of latex agglutination test adopted to detect new Cryptococcal capsular polysaccharide antigen is a kind of simple, rapid and effective experimental approach in diagnosing Cryptococcal infection. It is significantly superior to traditional culture method and microscopy method in early rapid diagnosis, but the existence of false positives and false negatives could not be avoided. According to literature reports, the systemic lupus erythematosus, nodule disease, RF (rheumatoid factors), macroglobulin and trichosporum, capnocytophaga and acid fast bacilli have cross-reacting antigen with Cryptococcal neoformans capsular polysaccharide antigen and false positive result is easy to be caused. On the other hand, the talcum powder of latex gloves and cleanser of washing reaction plate would pollute the cerebrospinal fluid specimens during the operation process; false positive result is also easy to be caused. While the hook belt phenomenon caused by high concentrations of cryptococcal antigens and the masking effect of unknown non-specific proteins in the body on cryptococcal antigens are able to cause false-negative. That latex agglutination test applies the latex agglutination test for detecting Cryptococcal capsular polysaccharide antigens diagnosing bacterial meningitis. It is superior to the commonly used fungal culture and microscopy approach currently, which could be taken as the early diagnostic method of Cryptococcal meningitis and bacterial meningitis.

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