The value of gene Xpert MTB / RIF, ADA, TB-DNA in the early diagnosis of TB meningitis

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ABSTRACT

This study was to explore the expression and correlation between gene Xpert MTB / RIF, ADA, and TB-DNA in TB meningitis. For this purpose, we selected 102 patients in the TB meningitis progression diagnosed and treated in our hospital from January 2019 to December 2020, and another 100 patients in the non-TB meningitis group were selected for the control experiment. Two sets of CSF samples were taken to analyze the gene Xpert MTB / RIF positive rate and the correlation between the expression and the progression of tuberculous meningitis by testing the levels of ADA and TB-DNA in the patient body using an automatic biochemical analyzer. Research indicated that The levels of gene Xpert MTB / RIF, ADA, and TB-DNA in the non-tuberculous meningitis group were lower than those in the tuberculous meningitis group (P<0.05; Levels of gene Xpert MTB / RIF, ADA, and TB-DNA were higher (P<0.05) in patients with group III tuberculous meningitis compared with those under grades I-II tuberculous meningitis, and levels of gene Xpert MTB / RIF, ADA, and TB-DNA were higher (P<0.05) in patients with group VI tuberculous meningitis compared with group III tuberculous meningitis; Gene Xpert MTB / RIF, ADA, TB-DNA) as factors occurring in TB meningitis progression, and all three were associated (P<0.05) with TB meningitis progression; Gene Xpert MTB / RIF, ADA showed a positive correlation (r = 0.296, P = 0.002); Gene Xpert MTB / RIF, TB-DNA showed a positive correlation (r = 0.422, P = 0.001); ADA, TB-DNA showed a positive correlation (r = 0.366, P = 0.001). It was concluded that Gene X-Pert MTB / RIF, ADA, and TB-DNA showed high levels in TB meningitis progression, and as the disease worsened, all three showed a positive association in TB meningitis progression.

Introduction

In recent years, there has been an increasing number of studies on serum factor-related indicators in cardiovascular and cerebrovascular diseases. Serum factors play an important clinical role in assessing disease conditions, taking targeted therapeutic measures and improving prognosis (1,2). Gene Xpert MTB/RIF is a new WHO-approved method for the diagnosis of tuberculosis. Gene Xpert MTB/RIF can detect the presence or absence of Mycobacterium tuberculosis as well as rifampicin resistance mutations, and the test takes only 2 h. Mycobacterium tuberculosis is the causative agent of tuberculosis and affects mainly the lungs, while it can also impact any other part of the body leading to extrapulmonary tuberculosis. However, changes in the combination of the three in patients with tuberculous meningitis have not been clinically studied. In this study, patients with tuberculous meningitis were tested to analyze the expression and correlation of Gene X-pert MTB/RIF, ADA, and TB-DNA in tuberculous meningitis.

Materials and Methods

Study population

One hundred and two patients with progressive tuberculous meningitis received in our hospital from January 2019 to December 2020 were selected and recorded as a tuberculous meningitis group, including 57 males and 45 females with an average age of (50.23 ± 10.62) years. By their condition, they were classified into 53 cases of grade I-II, 37 cases of grade III, and 12 cases of grade VI. Another 100 cases of healthy people who underwent physical examination in our hospital were randomly selected and recorded as the non-tuberculous meningitis group, which included 53 males and 47 females with an average age of (50.62 ± 10.85) years. The general conditions such as age and sex of the two groups were compared and balanced. The study subjects and their families signed the informed notice. The study was reviewed and approved by the ethics committee of our hospital.

Inclusion criteria: Diagnosis of meningitis was made by a comprehensive evaluation combining clinical manifestations, cranial imaging, cerebrospinal fluid and extracerebral tuberculosis lesions.

Exclusion criteria: ①HIV infection or severe coagulation dysfunction; ②the etiology of meningitis was unclear; ③severe infection of the skin and soft tissue at the site of lumbar puncture; ④shock or severe systemic infection, or restlessness.

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Detection method

Specimen collection

In the tuberculous meningitis group, 5.00 mL of fasting venous blood was collected at 8:00 AM on the day after admission, and in the non-tuberculous meningitis group, 5.00 mL of fasting venous blood was collected at 8:00 AM on the day of physical examination. The extracted blood was centrifuged at room temperature, and the supernatant was extracted and placed in 1.5 mL EP tubes, and then stored at -40°C for further use.

Indicator detection

The assay was performed using the Meizhou Kangli K-LITE8 automatic electrolyte analyzer, and the assay reagents were original reagents from Meizhou Kangli.

Cerebrospinal fluid Mycobacterium tuberculosis drug resistance gene detection: After excluding contraindications to lumbar puncture in all patients, 1~2 mL of cerebrospinal fluid was taken and added to an equal volume of 1 mL of Gene Xpert special digestion solution, and shaken for 15~30 s. The sample was placed at room temperature for 15 min, and 2 mL of the treated specimen was pipetted into the sample reaction kit, which was placed in the reaction chamber of the Gene X-pert instrument for fully automated detection, and the results were automatically interpreted after 2 h. Cerebrospinal fluid adenosine deaminase (ADA) levels were also measured. The Gene X-pert automatically filtered and washed the sample tissues, dissociated the DNA by ultrasonic lysis and mixed it with PCR reagents for fluorescence detection by real-time amplification. 1.5~2mL of cerebrospinal fluid was added directly into Xpert kit (cepheid, USA) and inserted into the Xpert-MTB/RIF detection platform. After inputting sample-related information, the instrument automatically filtered and washed the sample, released DNA by ultrasonic lysis and mixed it with PCR reaction reagents, and detected the fluorescence signal by semi-nested real-time amplification. The instrument automatically gave the test results after 2h. TB-DNA values in cerebrospinal fluid were measured using the ABIPrism 7500 real-time fluorescence quantitative assay system.

Statistical processing

SPSS26.0 statistical software was used for analysis and processing. The Kolmogorov-Smirnov was applied to test whether the data conformed to the normal distribution, and the measurement data conforming to normal distribution were described by mean ± standard deviation ($\bar{x}\pm s$). An independent sample t-test was performed for comparison between two groups, and F-test was carried out between multiple groups. The correlation between Gene X-pert MTB/RIF, ADA, TB-DNA and tuberculous meningitis was analyzed by logistic regression. Pearson correlation analysis was performed between the three correlations. $P<0.05$ was considered statistically significant.

Results

Comparison of Gene Xpert MTB/RIF positivity rates

As shown in Table 1, the Gene Xpert MTB/RIF positivity rates were compared between the two groups. The positive expression rate was higher in the tuberculous meningitis group compared with the non-tuberculous meningitis group, and the difference was statistically significant ($P<0.05$).

Comparison of ADA and TB-DNA in non-tuberculous meningitis group and tuberculous meningitis group

The ADA and TB-DNA levels in the non-tuberculous meningitis group were lower than those in the tuberculous meningitis group, and the differences were statistically significant ($P<0.05$), as shown in Table 2 and Figure 1.
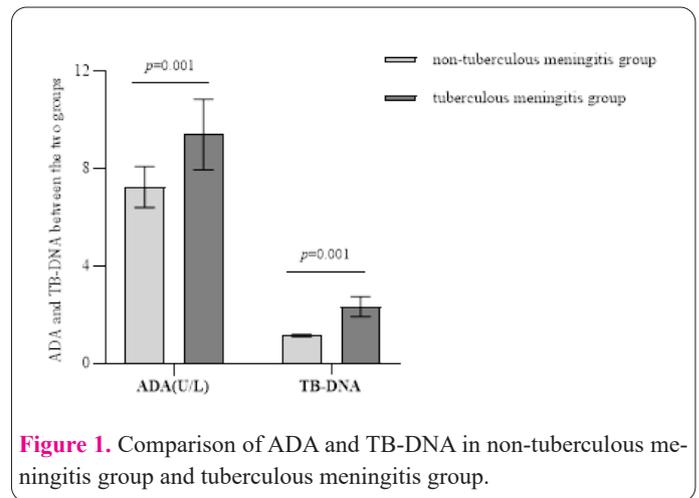

Figure 1. Comparison of ADA and TB-DNA in non-tuberculous meningitis group and tuberculous meningitis group.

Table 1. Comparison of Gene Xpert MTB/RIF positive rate between two groups.

Group	Number of cases (n)	Gene Xpert MTB/RIF		Positive expression rate
		Positive	Negative	
non-tuberculous meningitis group	100	8	92	8.00
tuberculous meningitis group	102	24	78	23.54
t-value				26.188
P-value				0.001

Table 2. Comparison of ADA and TB-DNA between the two groups ($\bar{x}\pm s$).

Group	Number of cases (n)	ADA (U/L)	TB-DNA
non-tuberculous meningitis group	100	7.25±0.84	1.16±0.04
tuberculous meningitis group	102	9.41±1.45	2.34±0.41
t-value		24.456	125.140
P-value		0.001	0.001

Comparison of ADA and TB-DNA in different disease classifications

Compared with grades I-II tuberculous meningitis, grade III patients had higher levels of ADA and TB-DNA, with statistical differences (P<0.05). Compared with grade III tuberculous meningitis, patients with grade VI tuberculous meningitis had higher levels of ADA and TB-DNA, possessing statistical differences (P<0.05), as shown in Table 3 and Figure 2.

Logistic regression analysis of the correlation between Gene Xpert MTB/RIF, ADA, and TB-DNA and the progression of tuberculous meningitis

The occurrence of TB meningitis in progress was used as the dependent variable (assignment: not occurring=0, occurring=1). Gene Xpert MTB/RIF (assigned values: ≤180ng/ml=0, >180ng/ml=1), ADA (assigned values: ≤0.88mg/L=0, >0.88=1), and TB-DNA (assigned values: ≤198U/L, >198U/L) were used as independent variables. The three were included in a dichotomous multifactorial logistic regression model for analysis. The results showed that Gene Xpert MTB/RIF, ADA, and TB-DNA were factors occurring in the progression of tuberculous meningitis, and all three were associated with the progression of tuberculous meningitis, possessing statistical differences (P<0.05), as shown in Table 4.

Correlation analysis of Gene X-pert MTB/RIF, ADA, TB-DNA in the progression of tuberculous meningitis

Pearson correlation analysis showed the presence of positive correlations for Gene Xpert MTB/RIF, ADA; Gene X-pert MTB/RIF, TB-DNA; ADA, TB-DNA, respectively, with correlation coefficients of (r=0.296, P=0.002); (r=0.422, P=0.001); (r =0.366, P=0.001).

Discussion

With the rapid development of society in recent years, people's diets have undergone significant changes, leading to a rising trend in the incidence of tuberculous meningitis disease. As a common disease in the nervous system, tuberculous meningitis can directly destroy brain tissue and cause secondary brain damage, affecting the formation of the microenvironment in the body and seriously threatening the physical and mental health and life safety of patients (9,10). Therefore, early prediction and timely treatment are clinically important for the recovery and
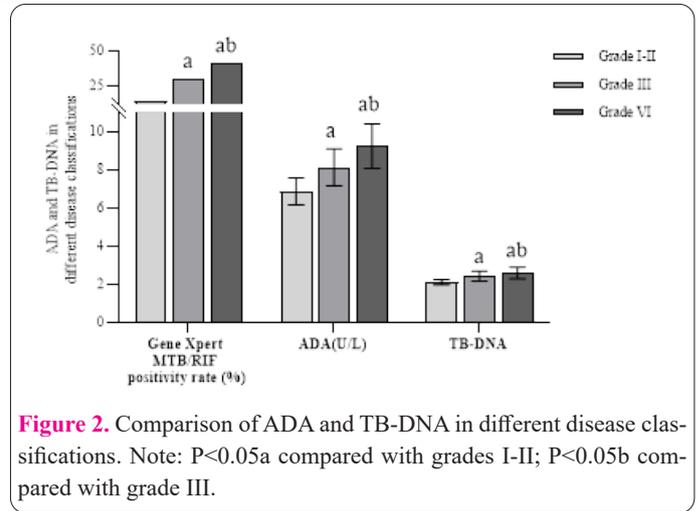

Figure 2. Comparison of ADA and TB-DNA in different classifications. Note: P<0.05a compared with grades I-II; P<0.05b compared with grade III.

prognosis of patients (11).

Gene Xpert MTB/RIF, one of the molecular markers of thrombin production, has the ability to directly reflect the production of thrombin within the body and the anticoagulant response of the body (12). Moreover, the coagulation-fibrinolytic system has an important role in regulating the mechanism of coagulation disorders in cardiovascular and cerebrovascular diseases. Clinical studies have shown that when patients developed tuberculous meningitis, localized hematoma fluid appeared, which led to an abnormal rise in inflammatory-related factors. Inflammatory factors and hematoma secretions can lead to a rapid increase in Gene Xpert MTB/RIF (13,14). In this study, Gene Xpert MTB/RIF was found to be highly expressed in tuberculous meningitis, and the level of Gene Xpert MTB/RIF increased with the amount of hemorrhage, indicating that Gene X-pert MTB/RIF is closely related to tuberculous meningitis. Furthermore, in this study, it was found that the serum Gene Xpert MTB/RIF showed an increasing trend with the severity of nerve damage. Thus, Gene Xpert MTB/RIF is not only closely related to the amount of bleeding, but also strongly correlated with neurological impairment.

Effective and timely treatment can stop the progression of tuberculous meningitis. Related studies have shown that during early tuberculous meningitis infection, more factors mediate the recognition of Mycobacterium tuberculosis and turn on the innate immune response to infection (15,16). Factors such as leukotriene A4 hydrolase gene polymorphisms are associated with the progression of the disease, among which the expression of ADA, has

Table 3. Comparison of ADA and TB-DNA in different disease classifications ($\bar{x}\pm s$).

Disease classification	Number of cases (n)	Gene Xpert MTB/RIF positivity rate (%)	ADA (U/L)	TB-DNA
Grade I-II	57	8 (14.04)	6.89±0.71	2.13±0.14
Grade III	37	11 (29.73)[a]	8.14±0.97[a]	2.44±0.26[a]
Grade VI	12	5 (41.67)[ab]	9.25±1.16[ab]	2.61±0.31[ab]

Note: P<0.05a compared with grades I-II; P<0.05b compared with grade III.

Table 4. Correlation of Gene Xpert MTB/RIF, ADA, and TB-DNA in the progression of tuberculous meningitis.

Variables	Regression coefficient	Standard error	Wald x^2 value	*P value*	*OR value*	*95% CI*
Gene X-pert MTB/RIF	1.267	0.529	9.568	0.001	0.659	0.306-1.263
ADA	1.629	0.694	7.521	0.001	0.751	0.385-1.741
TB-DNA	1.529	0.854	7.995	0.001	0.768	0.431-1.623

a close link with the regulatory capacity of the immune system of the patient's organism (17,18). In this study, we found that ADA showed high expression in tuberculous meningitis, and the level of ADA increased with the progression of the disease. This indicates that ADA is closely related to tuberculous meningitis. Moreover, in this study, it was found that the serum ADA of patients showed an increasing trend with the severity of nerve damage.

When patients develop tuberculous meningitis, it affects the microenvironment of the patient's organism, releasing large amounts of inflammatory factors, which in turn aggravate brain damage (19,20). TB-DNA is a clinically important inflammatory factor. When tuberculous meningitis occurs, it is able to activate the activity of TB-DNA, which consequently enhances the degree of the inflammatory environment of the patient's neuronal cells (21,22). In this study, we found that TB-DNA showed high expression in tuberculous meningitis, and the level of TB-DNA increased with the amount of bleeding, indicating that TB-DNA is tightly related to tuberculous meningitis. Besides, in this study, it was found that the serum TB-DNA of patients showed an increasing trend with the severity of nerve damage. As a result, TB-DNA is not only closely related to the amount of bleeding but also highly correlated with neurological impairment. Also, Gene Xpert MTB/RIF, ADA, and TB-DNA were found to be positively correlated in tuberculous meningitis in this study.

In conclusion, Gene Xpert MTB/RIF, ADA, and TB-DNA showed abnormally high expression in tuberculous meningitis, and the positive expression rate of Gene Xpert MTB/RIF was high, indicating that all three were positively correlated with the presence of tuberculous meningitis. Moreover, the correlation study of the three found that they showed positive correlation in tuberculous meningitis.

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