



Herpes Simplex Virus and Tuberculosis-Associated Encephalitis in Northeast of Iran

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Abstract

Background: Encephalitis is associated with high rates of mortality and morbidity; however, its pathophysiology is yet to be determined.

Objectives: A prospective cohort study of patients with clinically diagnosed encephalitis was performed to investigate herpes simplex virus (HSV) or tuberculosis infection in encephalitis patients.

Methods: Overall, 114 patients were enrolled according to clinical profile and were tested for the presence of HSV-1 and 2 or tuberculosis using TaqMan real-time PCR method on cerebrospinal fluid (CSF).

Results: Thirty patients were clinically diagnosed with HSV infection, whose CSF samples were sent for HSV qRT-PCR test. The results showed that 10 (33.3%) subjects were HSV-1 or 2-positive. Out of 84 patients who were tested for tuberculosis encephalitis, and tuberculosis qRT-PCR was carried out on their CSF, 3 (3.6%) cases were positive. No significant differences were found in gender and age between HSV or tuberculosis-negative and positive groups. The main symptoms in HSV positive subjects were fever and depressed level of consciousness. No cases of tuberculosis infection were diagnosed in direct CSF smear test.

Conclusions: Since infectious encephalitis is a life-threatening problem and tuberculosis and HSV are progressing in the developing countries, health authorities, physicians and researchers should address this problem more seriously

Keywords: Cerebrospinal Fluid, Herpes Simplex Virus 1, Herpes Simplex Virus 2, Infectious Encephalitis, *Mycobacterium tuberculosis*, Polymerase Chain Reaction, Iran

1. Background

Encephalitis is a complex, severe neurological dysfunction, defined by the presence of an acute regularly diffuse inflammatory process affecting the central nervous system (CNS). This disease is associated with significant morbidity and mortality (1). Infection-related encephalitis is caused by direct effect of pathogenic infectious agents and autoimmune-mediated encephalitis is possibly caused by vaccination or a recent viral infection (2, 3). In spite of extensive testing on patients, the main etiology remains unknown in about 60% of the cases (4, 5).

Herpes simplex encephalitis (HSE) is the most frequent cause of acute encephalitis followed by varicella zoster virus (VZV) and *Mycobacterium tuberculosis* (6). Herpes sim-

plex encephalitis is a life-threatening consequence (7). The annual incidence rate of HSE is 1 to 2.5 cases per one million population, but 70% of these patients die due to lack of treatment, which can be reduced to 11 - 19% with immediate antiviral (7, 8).

The frequency and distribution of viruses noticeably varies according to geographical region, with large differences seen between Europe, Asia, and the USA (9). A study of over 3,000 CNS infection patients from Finland showed that the most frequently detected virus was VZV (29%) followed by HSV (11%) and influenza A virus (7%) (5, 10). In a study conducted among Middle-eastern patients with encephalitis, HSV-1 DNA was detected in 30% and cytomegalovirus (CMV) and human herpes virus 6 (HHV-6) DNAs were each detected in 3% of patients. Thus, HSV-2 and

HHV-7 PCRs were negative (11, 12).

Meningeal tuberculosis is a highly devastating form of tuberculosis and accounts for approximately 1% of all cases of tuberculosis (13, 14). Due to its relative rarity and the diverse nature of the manifestations, CNS tuberculosis remains a vigorous diagnostic challenge (14, 15). To assess the impact of tuberculosis encephalitis and to determine its etiology, large epidemiological studies have been conducted in several countries. A study from Spain showed that CNS tuberculosis accounted for 3.2% of tuberculosis deaths in 1993, a rate that has declined over the past 20 years (16). In a large study in Taiwan between 1997 and 2001, it was reported that 1.5% of tuberculosis deaths were related to CNS involvement (17). A recent study has shown that tuberculosis meningitis should be detected by more accurate techniques with high sensitivity and specificity such as PCR method (18).

Since neurotropic pathogens and associated diseases are usually common, the CNS infectious diseases in the developing countries have been relatively neglected and have not been addressed by researchers and health authorities. Therefore, epidemiological studies and rapid and more reliable tests are needed for early diagnosis and medical intervention. In fact, more studies should be conducted to understand the infection rate and behavior of HSV and tuberculosis pathogens for finding precise and rapid tests that can lead to proper and successful treatment of these life-threatening diseases.

2. Objectives

To the best of our knowledge, there is a scarcity of data on the causes of encephalitis in Iran and the ways of its differentiation from other causative agents. Furthermore, the conventional tests used for diagnosis are not suitable for such life-threatening diseases. Hence, this study was conducted to evaluate the prevalence of HSV and tuberculosis-associated encephalitis using the most reliable PCR method, TaqMan real-time PCR. The study was carried out in two main referral neurologic and infectious diseases centers affiliated to Mashhad University of Medical Sciences, which are the referral centers in northeast of Iran.

3. Methods

3.1. Study Population and Sample Size

This prospective cohort study was performed among 114 patients with encephalitis who were referred to the infectious diseases ward in Imam Reza Hospital or Neurology Ward in Ghaem Hospital affiliated to Mashhad University of Medical Sciences during March-2014 to September-2016. Each subject was evaluated by two specialists and using the routine laboratory tests. Being clinically diagnosed with

HSV or tuberculosis, the patients' cerebrospinal fluid (CSF) specimens were immediately sent for more evaluations to a referral molecular medical lab. The clinical and demographic characteristics of the patients were recorded. The inclusion criteria were standard clinical criteria for encephalitis (19) and lack of any autoimmune diseases, HBV, HIV and HCV infections.

3.2. Sampling Procedures

Cerebrospinal fluid (lumbar puncture) specimens were taken by a neurologist for performing the routine laboratory tests. Half of the sample was also sent for qRT-PCR test. DNA was extracted from CSF using DNA mini kits (QIAGEN, Germany), according to manufacturer's instructions. All the DNA samples were kept at -20°C until PCR amplifications.

3.3. qRT-PCR TaqMan Method

In the present study, molecular diagnosis of encephalitis was confirmed according to HSV-1 and 2 or tuberculosis real-time PCR on CSF. Primers and labeled probes for tuberculosis qRT-PCR, TaqMan method, were used as previously described (20). The standard TaqMan master mix (Takara, Japan) was used for qRT-PCR amplifications. A commercial HSV-1&2 kit (Novin Gene, Iran) was used for the detection and quantification of HSV. Rotor-Gene Q 6000 Machine (QIAGEN, Germany) and Rotor Gene software were used for data collection and analyzing the results.

3.4. Statistical Analysis

Statistical analysis was performed using SPSS version 16.0 (SPSS, Chicago, IL). Normality of the data for each variable was checked prior to data analysis using Kolmogorov-Smirnov test. If the data had normal distribution, Student's *t*-test was used, but when variables were not normally distributed, the nonparametric Mann-Whitney U test was employed for data analysis. Chi-square test was run when one of the dependent or independent variables was subjective. Correlation analysis was performed using Spearman's test to detect the association of the variables. P value less than 0.05 was considered statistically significant.

4. Results

4.1. Clinical Findings

Analyses of the demographic data and clinical manifestations as well as laboratory findings were performed in all the patients with the signs and symptoms of encephalitis. Clinical diagnosis of encephalitis was confirmed in 114 patients according to the standard clinical criteria (19). More than half of the patients under the study (53.3%) had fever and 43.3% of the patients had headache. Furthermore, 60%

of the HSV-diagnosed and 85% of tuberculosis-diagnosed patients had depressed level of consciousness on admission. This difference did not meet 95% confidence interval (CI), $P = 0.1$ and $CI = 90\%$, it seems that unconsciousness is more frequent in tuberculosis encephalitis than in HSV. Overall, depressed level of consciousness, convulsion, delusion, nausea, altered mental status, aphasia and fatigue were reported in 76.6%, 50.0%, 20.0%, 33.3%, 20%, 16.6% and 16.6% of the patients, respectively (Table 1).

4.2. HSV and Tuberculosis Associated Encephalitis

The results of HSV qRT-PCR on CSF samples were negative for 20 (66.7%) patients who were diagnosed and treated for HSV encephalitis. On the other hand, of the 84 cases who were introduced for more molecular evaluations for tuberculosis, 3 (3.6%) had positive tuberculosis results using real-time PCR TaqMan method on CSF. However, the routine laboratory tests failed to detect these patients with tuberculosis encephalitis. No significant difference existed in gender and mean age between HSV-negative (27.2 ± 22.8) and HSV-positive (35.3 ± 27.15) patients. No significant difference was observed between the mean age of the tuberculosis-negative group (45.3 ± 21.1 years) and the tuberculosis-positive group (38.6 ± 22.7 years). According to Chi-square test, no significant difference was observed in gender distribution, as 42 (50%) were male and 42 (50%) were female.

5. Discussion

Encephalitis can become a severe public health problem. Neurotropic pathogens are usually common in the developing countries, it can be expected that the prevalence of CNS infectious diseases should be more in the developing countries. Therefore, it seems that these diseases have been relatively neglected and not seriously addressed by researchers and health authorities. The diagnosis of encephalitis is always confirmed by standard microbiological tests. In some studies, conventional PCR is also used to detect the infectious agents because of their importance as risk factors for encephalitis. However, in this study qRT-PCR TaqMan method was employed for HSV-1 and 2 and tuberculosis in patients newly diagnosed with encephalitis.

Conventional PCR for HSV detection in CSF in the 1990s has made a massive contribution towards improving the diagnosis of many microbial diseases. Because of its effectiveness, promptness, low invasiveness and sensitivity, most cases are diagnosed with the use of PCR that has surpassed the "Gold standard" of brain biopsy (21, 22). Indeed, CSF PCR was assessed and found to be reliable for diagnosing several CNS infectious diseases and has become the reference technique for prompt diagnosis of HSE (10, 23). However, negative PCR results are not reliable to interpret and do not eliminate clinical suspicion, and the

timing of the CSF sampling should be taken into account (12, 24). Therefore, more sensitive and specific molecular methods must be applied for such life-threatening CNS diseases. In the present study, we applied the real-time PCR TaqMan method for detecting HSV and tuberculosis infections.

The demographic characteristics of the patients in the present study were similar to those reported in other studies; age was a non-significant factor in our patients with HSV encephalitis and non-HSV encephalitis, which is in accordance with other studies and confirms that age is an independent prognostic factor of the disease and there is no gender tendency (25, 26). It is noteworthy that some sources have mentioned the bimodal age distribution, with a smaller peak among the youth and a larger peak among the elderly (27).

Out of 30 patients included in the current study, 10 (33.33%) cases were diagnosed with HSV. This is in agreement with a report from France showing that the most frequent etiological agent of encephalitis was HSV (28). However, a report from England indicated that the incidence rate of HSV encephalitis was 19% (29). Also, in Taiwan, HSV is still considered as the main viral source of encephalitis (30). However, a study from Spain showed that HSV was positive in 92% of hospitalized patients with encephalitis (26). The high rate of HSV infection in that research may be due to the high quality of sampling and laboratory diagnostic methods. A report from Shiraz, a city in south of Iran, showed that the detection rate of encephalitis was 9.3% (31). Nevertheless, a report from Babol, a northern city in Iran, revealed a high rate (34%) of patients with encephalitis were HSV-positive (32).

Although tuberculosis meningitis is well illustrated, prominent encephalitic features are less commonly described. Mortality and morbidity associated with neurotuberculosis are extremely dependent on the stage of disease; prompt diagnosis and treatment correlate with better outcomes (33). In a recent study, a higher proportion of encephalitis due to tuberculosis (3.6%) was seen than in other studies (no *M. tuberculosis* in the study by Koskiniemi et al. (5) and 1% for *M. tuberculosis* in the study by Glaser et al. (4)). We are confident that our tuberculosis patients had encephalitis rather than meningitis because the case definitions included clinical patterns specifically encountered in encephalitis, such as focal neurologic signs.

In the current study, fever was the most common symptom at the time of hospitalization, such that more than half of patients under the study (53.3%) presented with fever and 43.3% of the patients had headache. Nonetheless, HSV detection in viral encephalitis is still critical because there is effective treatment for it. Absence of positive PCR in CSF samples does not preclude HSV diagnosis, particularly early in the disease (27); further prospective studies using direct DNA sequencing are needed.

Table 1. Demographic Data of the Patients with Herpes Encephalitis and Non-Herpes Encephalitis^a

Variables	HSV-Encephalitis (n = 10)	Non-HSV Encephalitis (n = 20)	P Value
Age, y (mean ± SD)	35.3 ± 27.15	27.2 ± 22.8	0.396
Gender (F/M)	5/5	10/10	1.000
Clinical Symptoms			
Fever	9 (90)	7 (35)	0.007
Headache	5 (50)	8 (40)	0.705
Decreased level of consciousness	6 (60)	17 (85)	0.181
Convulsion	4 (40)	11 (55)	0.700
Delusion	2 (20)	4 (20)	1.000
Nausea	2 (20)	8 (40)	0.419
Altered mental status	3 (30)	3 (15)	0.372
Aphasia	3 (30)	2 (10)	0.300
Fatigue	1 (10)	4 (20)	0.640

Abbreviations: F, female; M, male.

^aValues are expressed as No. (%) unless otherwise indicated.

5.1. Conclusions

In sum, as HSV and tuberculosis encephalitis are treatable and qRT-PCR TaqMan method provides a rapid and reliable diagnosis in CNS infections (34), it has to be considered for the management of encephalitis patients. In this study, one third of the patients had received unnecessary HSV treatment and three patients with tuberculosis infection were not diagnosed. Our research provides insight into the nature of the clinical and etiologic patterns of encephalitis in this geographic region. It may reduce the hospitalization rate and the use of unnecessary therapies and improve the health system.

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Footnotes

Authors' Contribution: Performing the experiments and manuscript drafting: Raheleh Miri, Sanaz Ahmadi Ghezdasht, Vajihah Mohamadinezhad, Arman Mosavat and Faezeh Sabet; research advisor: Seyed Aliakbar Shamshian; research directors, study conception and design, and data analysis: Seyed Abdolrahim Rezaee and Payam Sasannejad. All the authors read and approved the final manuscript.

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Ethical Considerations: This study was conducted in accordance with the latest Declaration of Helsinki guidelines

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