Hepatitis E virus infection in acute non-traumatic neuropathy: A large prospective case-control study in China

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A B S T R A C T
Neurological manifestations are potentially associated with hepatitis E virus (HEV) infection in Europe, mainly attributed to genotype (GT) 3 HEV infection. In this study, we determined the frequency and causal relationship of HEV in patients with non-traumatic neurological disorders in China, where GT4 HEV is prevalent. 1117 consecutive patients diagnosed with neurological illnesses in a hospital in eastern China and 1475 healthy controls who took routine examination in the same hospital were tested for HEV by serology and molecular methods. Anti-HEV IgM antibodies were detectable in 6 (0.54%) of the patients and 10 (0.68%) of the healthy controls (P = 0.651). Serum HEV RNA was detected in all of the 16 individuals with positive anti-HEV IgM. The six patients with HEV infection included two viral encephalitis, two posterior circulation ischemia, one peripheral neuropathy and one Guillain–Barré syndrome. They had no symptoms of acute viral hepatitis except two patients of viral encephalitis that showed mildly transaminitis. Additional, 39.51% patients and 35.63% controls without acute HEV infection were positive for anti-HEV IgG (P = 0.144). Anti-HEV IgG positivity was more frequent in male and elderly in both the patients and control groups, but unrelated to the incidence of any non-traumatic neurological illness, hospital stay or treatment outcome, except linking to better outcome of hemorrhagic stroke disease. These data demonstrated that HEV appears not to contribute to acute neurological disorders in China. Nevertheless, we cannot exclude a possible causative role, suggesting that testing HEV in this population, especially in situations of unexplained deregulated liver function would be warranted.

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1. Introduction

Hepatitis E virus (HEV) is the most common cause of acute viral hepatitis worldwide [1]. At least four main genotypes of HEV have been identified. Genotype (GT) 1 and GT2 HEV are restricted to human beings and transmitted via contaminated water sources, which are prevalent in developing countries. GT3 and GT4 HEV are zoonotic and commonly spread through consumption of undercooked pork or game products, which are endemic in both developing and developed countries [2]. Although HEV infection is asymptomatic in approximately 95% cases, it may result in wide-range clinical events, including hepatitis, acute pancreatitis, renal injury, neurological disorders and other immune-mediated manifestations [3–6].

As the most common extra-hepatic complication, >150 cases of HEV-associated neurological syndromes have been reported in developing and developed countries, mostly as case reports or small cohort series. The most frequently reported HEV-related neurologic syndromes include Guillain–Barré syndrome (GBS), neuralgic amyotrophy (NA),
Thus, the roles of GT4 HEV in neurologic diseases remain largely unknown due to lack of large case-control cohort study in endemic area. China is considered an HEV-endemic region where GT4 is prevalent. However, the roles of GT4 HEV in neurologic diseases remain largely unknown due to lack of large case-control cohort study in endemic area.

**Research in context**

**Evidence before this study**

Although hepatitis E virus (HEV) infection is asymptomatic in approximately 95% cases, it may result in wide-range clinical events, and neurological injury is the most common extrahepatic manifestation in patients with HEV infection. >150 cases of HEV-associated neurological syndromes have been reported in developing and developed countries, mostly as case reports or small cohort series and mainly attributed to genotype (GT) 3 HEV infection. China is considered an HEV-endemic region where GT4 is prevalent. However, the roles of GT4 HEV in neurological diseases remain largely unknown due to lack of large case-control cohort study in endemic area.

**Added value of this study**

The current study is the first large case-control cohort study to investigate the association of HEV infection with acute non-traumatic neurological diseases in China, where GT4 HEV is predominant. In this study, six of 1117 (0.54%) non-traumatic neuropathy patients and ten of 1475 (0.68%) controls had evidence of HEV infection \( (P = 0.651) \). This result is in contrast to all the cohort studies performed in Europe that the prevalence of HEV viraemia in neurological patients are at least 10 times higher than that documented in local blood donors. The six patients with HEV infection included two viral encephalitis, two posterior circulation ischemia, one peripheral neuropathy and one Guillain-Barré syndrome. They had no symptoms of acute viral hepatitis except two patients of viral encephalitis that showed mildly transaminitis. Anti-HEV IgG positivity was more frequent in male and elderly in both the patients and control groups, but unrelated to the incidence of any non-traumatic neurological illness, hospital stay or treatment outcome, except linking to better outcome of hemorrhagic stroke disease.

**Implications of all the available evidence**

These data demonstrated that HEV appears not to contribute to acute neurological disorders in China. Nevertheless, we cannot exclude a possible causative role, suggesting that testing HEV in this population, especially in situations of unexplained deregulated liver function would be warranted.

**2. Patients and methods**

**2.1. Patients and controls**

From September through November 2017, consecutive patients diagnosed with acute non-traumatic neurological illnesses in the First People's Hospital of Yancheng City, Jiangsu Province, China, were enrolled in the study. All patients were evaluated and managed by experienced clinical neurologists. Serum samples were obtained onset of diagnosis in the acute phase of disease before treatment. Serums from consecutive individuals visiting the physical examination center of this hospital within the same period for routine examination were collected as healthy controls. All samples were stored at \(-80\) °C before testing.

**2.2. Ethics**

The standard protocol, ethical approvals and registrations of this study were approved by ethical standards committee of The First People's Hospital of Yancheng City and all patients provided informed consent that allow future testing of archived bio-samples. The protocol number of the study is [2018]-[K020].

**2.3. HEV serological test**

All serum samples were tested for the presence of anti-HEV IgM and IgG antibodies using commercially available HEV ELISA Kit (Wantai, Beijing, China) according to manufacturer’s instructions. Samples with signal-to-noise ratio (S/N ratio) > 1.0 were considered positive.

**2.4. HEV molecular test**

Serums with positive anti-HEV IgM were subjected to HEV RNA detection and identification. Total RNA was extracted using QIAamp Viral RNA Mini Kit (QIAGEN, Germany) according to the manufacturer’s instruction. Next, total RNA was tested for HEV RNA by means of a Diagnostic Kit for Hepatitis E Virus RNA (Jinhao, Beijing, China) according to the manufacturer’s instruction. Specifically, according to the principle of real-time fluorescence quantitative polymerase chain reaction (qPCR) detection, the conserved region of open reading frame (ORF) 3 was identified by HEV-specific Taqman probe. The viral load of each sample was estimated by qPCR according to series diluted artificial pseudovirus as standards. For genotype identification, nest-PCR was performed to produce a 348-nucleotide amplicon from HEV ORF2 for sequencing as previously described [15].

**2.5. Statistical analysis**

Differences in proportions were tested using Chi-squared or Fisher exact test; differences in continuous variables of normal distribution were tested using the Student’s t-test, and the data with skewed distribution were compared using the Mann-Whitney U test. Logistic regression was performed to determine odds ratios (ORs). A two-sided \( P \) value <0.05 was considered to be statistically significant. Statistical analysis was performed using SPSS version 23.0.
3. Result

3.1. Study population

Between September and November 2017, 1117 patients including 626 men and 491 women were diagnosed with acute non-traumatic neurological injury and enrolled in this study. The mean age was 67 years (range 14–94 years). The wide range of neurological illnesses were divided into different clinical categories based on onset position and pathogenesis. Detailed categories were summarized in Table 1.

3.2. HEV infection in patients with neurological injury

Anti-HEV IgM antibodies were positive in 6 (0.54%) of the non-traumatic neuropathy injury patients, compared with 10 (0.68%) of the healthy controls (Fig. 1, Table 2). The OR after adjustment for age and sex showed a similar result (Table 2). The incidence of HEV infection and S/N ratio of positive anti-HEV IgM showed no significant difference between cases and controls (Fig. 2). HEV RNA was detected in all of the 16 subjects with positive anti-HEV IgM, and the viral loads ranged from $3.68 \times 10^3$ to $2.58 \times 10^5$ copies/mL. One sample with the highest viral load was successfully sequenced and confirmed as GT4 strain. All the 6 patients and six out of 10 health controls with HEV infection were also showed positive anti-HEV IgG.

3.3. Neurological presentations

The mean age of the 6 neurological patients with HEV infection was 53 years (range 27–86 years) compared to 65 years (range 14–94 years) in the remaining 1111 patients with negative anti-HEV IgM ($P = 0.038$). Of the six patients that had evidence of HEV infection, two had viral encephalitis, two had posterior circulation ischemia, one had peripheral neuropathy and one had GBS. None of the patients presented with jaundice. Only two patients with viral encephalitis presented with a modestly raised alanine aminotransferase (ALT) level. Five patients were eventually recovered from neurological syndrome. Only one patient was well-followed up and found symptoms relief along with HEV IgM seronegative conversion. An overview of the clinical characteristics and therapeutic regimens of the 6 patients with HEV infection was summarized in Table 3, and specific descriptions are as below.

3.3.1. Case 1

A 46-year-old woman had undergone surgery to remove a mammary gland fibroma 6 month previously. The patient presented with a 5-day history of fever associated with headache and nausea. Initial treatment with mannitol, cefuroxime, ribavirin, and dexamethasone was unsuccessful. Her limbs had a Medical Research Council (MRC) score of 5, and her tendon reflexes were normal. Liver function tests (LFTs) showed slightly elevated serum ALT and aspartate aminotransferase (AST). Routine blood examination showed abnormal total bilirubin (TBil). The patient showed seronegative conversion of anti-HEV IgM after 1 week.

3.3.2. Case 2

A 42-year-old woman presented with a 2-month history of numbness in her left upper extremities without apparent cause. Physical examination showed that the patient was anicteric and that she had normal superficial lymph nodes and tendon reflexes. Serological testing showed slightly increased levels of γ-glutamyltranspeptidase (GGT) and uric acid. Cerebral MRI revealed point ischemia nidus in the pallium. However, medullar MRI found no obvious abnormality in the cervical brachial plexus nerve. Electromyography showed normal peripheral nerves throughout the body. The cerebral vertebrae C4/5 and C5/6 showed intervertebral disc herniation, while C3/4 showed intervertebral disc bulging. Neither Brudzinski’s sign nor Murphy’s sign were present. The patient’s limbs had an MRC score of 5. Final diagnosis was peripheral neuropathy that caused by spinal degeneration. The symptom of numbness gradually improved without treatment, and the patient showed seronegative conversion of anti-HEV IgM after 1 week.

3.3.3. Case 3

An 87-year-old man presented with 1 month history of intermittent dizziness repeatedly. Physical examination revealed that he was anicteric, that he had no hemorrhage dots in the systemic skin, and that his superficial lymph nodes were normal. LFTs showed normal serum ALT and AST, with a slightly elevated total bilirubin (TBil). The level of uric acid was increased and the tendon reflexes were maintained. B-ultrasonography showed sclerosis with plaque formation at several sites in the bilateral carotid arteries and in the intervertebral disc section of the vertebral artery, as well as blood supply shortage to the right side of the vertebral artery and left carotid artery. Brain computed tomography showed lacunar infarction in the basal ganglia, and the final diagnosis was posterior circulation ischemia. The patient was treated with alprostadil and vinpocetine to improve blood circulation, and his symptoms of dizziness resolved after treatment.

3.3.4. Case 4

A 42-year-old man presented with sense of rotation, nausea and vomiting, as well as aggravating dizziness. However, tinnitus and deafness were not present. The patient had previously experienced the same symptoms repeatedly for 2 months. Cerebral MRI showed point ischemia nidus in the bilateral centrum semiovale. The patient’s limbs had an MRC score of 5 and the tendon reflexes were normal. Both LFTs and routine blood examination showed normal parameters. There were no pathological signs in either limb, and deep/shallow feelings were symmetrical. The patient was diagnosed with posterior circulation ischemia and treated with gastrodin and vinpocetine to improve blood circulation. He recovered after treatment.

3.3.5. Case 5

A 27-year-old man presented with headache that had lasted >10 days, after which it was accompanied by blurred vision. His symptoms improved when mannitol and dexamethasone were administered, but relapsed when treatment were withdrawn. The patient had a history of hypertension, but had not used his medications as directed. His serum ALT and GGT were mildly increased, while those of AST and TBil were normal, as well as his level of CRP. Cerebral MRI showed bilateral ventricular point ischemia nidus. Cervical vertebra MRI revealed physiological curvature and herniated disk at 4/5, 5/6 and 6/7. The abducens nerve was mildly limited on both sides, and an electrocardiogram (ECG) indicated T wave change and sinus rhythm. The CSF was transparent, but contained mildly elevated numbers of monocytes that had normalized after 3 weeks. Tests for hepatitis B virus (HBV), HCV, human immunodeficiency virus (HIV), HSV and treponema pallidum (TP) were all negative. Thus, the overall clinical picture suggested viral encephalitis, and the patient recovered after antiviral treatment with ganciclovir. He was also administered oxiracetam to protect the nerves.
| Type of acute neurological event | Number Tested(n=) | Anti-IgM positive number (%) |
|---------------------------------|-------------------|-------------------------------|
| Ischemia stroke                 |                   |                               |
| Vertebrobasilar insufficiency   | 191               | 0 (0%)                        |
| Posterior circulation ischemia  | 145               | 2 (1.33%)                     |
| Transient ischaemic attack      | 22                | 0 (0%)                        |
| Cerebral venous sinus thrombosis| 2                 | 0 (0%)                        |
| Ischemia stroke                 | 15                | 0 (0%)                        |
| Hemorrhagic cerebral infarction | 6                 | 0 (0%)                        |
| Massive hemispheric infarction  | 9                 | 0 (0%)                        |
| Brainstem infarction            | 5                 | 0 (0%)                        |
| Multiple infarction             | 2                 | 0 (0%)                        |
| Cerebral infarction             | 225               | 0 (0%)                        |
| Cerebellar infarction           | 5                 | 0 (0%)                        |
| Lacunar infarction              | 7                 | 0 (0%)                        |
| Basal ganglia infarction        | 1                 | 0 (0%)                        |
| Hemorrhagic stroke              |                   |                               |
| Subdural hemorrhage             | 1                 | 0 (0%)                        |
| Hypertension ventricular hemorrhage | 1           | 0 (0%)                        |
| Basal ganglia hemorrhage        | 18                | 0 (0%)                        |
| Cerebral hemorrhage             | 14                | 0 (0%)                        |
| Brainstem hemorrhage            | 12                | 0 (0%)                        |
| Intracranial hemorrhage         | 33                | 0 (0%)                        |
| Cerebellar hemorrhage           | 7                 | 0 (0%)                        |
| Thalamic hemorrhage             | 6                 | 0 (0%)                        |
| Iolar hemorrhage                | 5                 | 0 (0%)                        |
| Subarachnoid hemorrhage         | 30                | 0 (0%)                        |
| Intraventricular hemorrhage     | 4                 | 0 (0%)                        |
| Posterior circulation hemorrhage| 4                 | 0 (0%)                        |
| Hemorrhagic stroke              | 5                 | 0 (0%)                        |
| Neurodegenerative disease       |                   |                               |
| Guillain-Barre syndrome         | 3                 | 1 (33.3%)                     |
| Alzheimer disease               | 2                 | 0 (0%)                        |
| Myasthenia                      | 6                 | 0 (0%)                        |
| Peripheral neuropathy           | 12                | 1 (8.33%)                     |
| Ischialgia                      | 3                 | 0 (0%)                        |
| Neuralgia                       | 1                 | 0 (0%)                        |
| Migraine/headaches              | 32                | 0 (0%)                        |
| Dementia                        | 2                 | 0 (0%)                        |
| Dizziness/vertigo               | 28                | 0 (0%)                        |
| Parkison's disease              | 22                | 0 (0%)                        |
| Neurosis                        | 13                | 0 (0%)                        |
| Cranial/facial nerve palsies    | 7                 | 0 (0%)                        |
| Dyssomia                        | 3                 | 0 (0%)                        |
| Epilepsy                        | 8                 | 0 (0%)                        |
| Convulsions                     | 3                 | 0 (0%)                        |
| Limbs numbness                  | 2                 | 0 (0%)                        |
| Myelopathy                      | 5                 | 0 (0%)                        |
| Multiple system atrophy         | 2                 | 0 (0%)                        |
| Trigeminal Nerve Diseases       | 1                 | 0 (0%)                        |
| Oculomotor nerve injury         | 2                 | 0 (0%)                        |
| Hashimoto encephalopathy        | 1                 | 0 (0%)                        |
| Paraneoplastic syndrome         | 1                 | 0 (0%)                        |
| Hepatolenticular degeneration   | 1                 | 0 (0%)                        |
| Sensorineural deafness          | 1                 | 0 (0%)                        |
| Excessive shock response        | 1                 | 0 (0%)                        |
| Hunter's syndrome               | 1                 | 0 (0%)                        |
| Leukoencephalopathy             | 1                 | 0 (0%)                        |
| Subacute associated lesions     | 1                 | 0 (0%)                        |
| Central nervous system infections|               |                               |
| Unknown encephalitis            | 2                 | 0 (0%)                        |
| Toxicencephalitis               | 1                 | 0 (0%)                        |
| Viral encephalitis              | 26                | 2 (7.69%)                     |
| Myelitis                        | 3                 | 0 (0%)                        |
| Bacterial encephalitis          | 8                 | 0 (0%)                        |
| Toxic encephalopathy            | 2                 | 0 (0%)                        |
| Subdural haematoma              | 3                 | 0 (0%)                        |
| Sequela of cerebral infarction  | 57                | 0 (0%)                        |
| Sequela of cerebral hemorrhage  | 8                 | 0 (0%)                        |
| Sequela of cerebral apoplexy    | 4                 | 0 (0%)                        |
| Brain post-traumatic syndrome   | 7                 | 0 (0%)                        |
| Acute cerebrovascular disease   | 5                 | 0 (0%)                        |
| Intracranial space occupying lesion | 3           | 0 (0%)                        |
| Vertebro-basilar artery syndrome| 2                 | 0 (0%)                        |
| Arterial aneurysm               | 6                 | 0 (0%)                        |
| Arteriosclerotic encephalopathy | 5                 | 0 (0%)                        |
| Focal cerebral ischemia         | 2                 | 0 (0%)                        |
| Other\*                         | 33                | 0 (0%)                        |

* Other: rare diseases and unknown cause.
3.3.6. Case 6

A 71-year-old man presented with weakness of the left limb associated with slurred speech 3 days prior. He was initially admitted to the intensive care unit with a diagnosis of severe hypokalaemia and was then transferred to the neurology department when his symptoms had improved. He had previously undergone a gastric perforation operation. Physical examination revealed that the patient was anicteric, that he had no hemorrhage dot in the systemic skin, and that his superficial lymph nodes were normal. Furthermore, he maintained deep tendon reflexes. The patient’s serum levels of ALT and AST were normal, and his TBil was slightly elevated. His left and right limbs had MRC scores of 4 and 5, respectively. His CSF was transparent, but contained significantly elevated levels of monocytes, glucose, and CSF protein. Chloride level of was normal. The result of the Pany test was positive, confirming the increased levels of CSF protein. The overall clinical picture suggested

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Table 2

| Neurological injury (n = 1117) | Healthy controls (n = 1475) | OR (95% CI) | P value | Adjusted OR (95% CI) | Adjusted P value |
|-------------------------------|----------------------------|-------------|---------|----------------------|-----------------|
| Anti-HEV IgM positive 6 (0.54) | 10 (0.68) | 0.791 (0.287–2.183) | 0.651 | 1.396 (0.429–4.544) | 0.579 |
| Anti-HEV IgG positive 439 (39.51) | 522 (35.63) | 1.180 (1.005–1.864) | 0.044 | 0.861 (0.705–1.053) | 0.144 |

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Fig. 1. Flowchart of patients and healthy controls at enrollment.

Fig. 2. Anti-HEV IgM and IgG S/N ratios for patients with neurological injury and healthy controls. S/N ratios of in subjects with positive anti-HEV IgM (A) and IgG (B). Red dotted lines represent the cutoff ratio (1.0). The P values are calculated by t-test comparing S/N ratio of positives. HEV = hepatitis E virus; Ig = immunoglobulin; S/N = signal-to-noise.
Table 3
Demographic, clinical, and diagnostic features of neurological patients potentially associated with HEV infection.

| Case 1 | Case 2 | Case 3 | Case 4 | Case 5 | Case 6 |
|--------|--------|--------|--------|--------|--------|
| **Demographic characteristics** |        |        |        |        |        |
| Age, y | 46     | 42     | 87     | 42     | 27     | 71     |
| Sex    | F      | F      | M      | M      | M      | M      |
| **Liver function test** |        |        |        |        |        |        |
| Alanine aminotransferase (ALT), 5–40 U/L | 76     | 30.1   | 11.3   | 18.1   | 142.6  | 28     |
| Aspartate aminotransferase (AST), 8–40 U/L | 54     | 29.3   | 17.9   | 23.3   | 47     | 20     |
| γ-glutamyltransferase (GGT), 3–50 U/L | 37     | 51.2   | 38.2   | 56.8   | 88.5   | 17     |
| Alkaline phosphatase (ALP), 40–110 U/L | NT     | 88.3   | 74.5   | 93.5   | 78.3   | NT     |
| Total bilirubin (TBIL), 1.71–17.1 μmol/L | 9.6    | 6.68   | 24.07  | 23.69  | 7.32   | 28.6   |
| **Serum tumor/inflammation makers** |        |        |        |        |        |        |
| Carbohydrate antigen (CA) 19–9, 0–37 U/ml | NT     | 4.45   | NT     | NT     | 0.732  |        |
| CA125, 0–35 U/ml | 16.7   | 11.55  | NT     | NT     | NT     | 14.91  |
| CA-121, 0–3.3 U/ml | NT     | 3.17   | NT     | NT     | NT     | 1.93   |
| CA15–3, 0–25 U/ml | 0.673  | 7.78   | NT     | NT     | NT     | 1.24   |
| Neuron-specific enolase (NSE), 0–17 ng/ml | NT     | 6.69   | NT     | NT     | NT     | NT     |
| C reactive protein (CRP), ≤10 mg/L | 10.03  | 9.61   | NT     | NT     | 7.36   |        |
| **Viral serological testing** |        |        |        |        |        |        |
| Hepatitis B virus surface antigen (HBsAg) | –      | NT     | NT     | –      | –      | NT     |
| Hepatitis B virus surface antibody (HBsAb) | +      | NT     | NT     | –      | –      | NT     |
| HBeAg | –      | NT     | NT     | –      | –      | NT     |
| HBeAb | –      | NT     | NT     | –      | –      | NT     |
| Hepatitis B core antibody (HBcAb) | –      | NT     | NT     | –      | –      | NT     |
| Hepatitis C virus (HCV) | –      | NT     | NT     | –      | –      | NT     |
| Anti-HEV IgM S/N ratio | 9.01   | 4.25   | 3.53   | 2.83   | 3.28   | 1.37   |
| Anti-HEV IgG S/N ratio | 6.1    | 1.39   | 1.57   | 3.07   | 1.41   | 1.42   |
| Treponema pallidum antibodies (TPAB) | –      | NT     | NT     | –      | –      | NT     |
| Toxoplasma (Tox)-IgM | –      | –      | –      | –      | –      | NT     |
| Tox-IgG | –      | –      | –      | –      | –      | NT     |
| Cytomegalovirus (CMV)-IgM | –      | –      | –      | –      | –      | NT     |
| CMV-IgG | +      | +      | +      | +      | +      | +      |
| Herpes simplex virus (HSV)-IgM | –      | –      | –      | –      | –      | NT     |
| HSV-IgG | +      | +      | +      | +      | +      | +      |
| Rubella virus (RV)-IgM | +      | +      | –      | +      | +      | +      |
| RV-IgG | +      | +      | +      | +      | +      | +      |
| **Cerebrospinal fluid (CSF) testing** |        |        |        |        |        |        |
| CSF protein (CSF-PR), 0.15–0.45 g/L | 0.2    | NT     | NT     | NT     | 0.36   | 1.11   |
| CSF-CL, 120–132 mmol/L | 125.9  | NT     | NT     | NT     | 125.7  | 123.1  |
| CSF-Glu, 2.5–4.5 mmol/L | 4.49   | NT     | NT     | NT     | 4.41   | 5.32   |
| CSF-karyocyte count, (0–8) × 10⁶/L | 12     | NT     | NT     | NT     | 12     | 23     |
| C-TMD | transparent | NT     | NT     | NT     | transparent | transparent |
| C-Pandy | –      | NT     | NT     | NT     | –      | +      |

(continued on next page)
GBS, and adequate relief of symptoms was achieved after antibiotic treatment with ceftazidime.

3.4. Seroprevalence of past HEV infection

In the rest of patients without acute HEV infection, anti-HEV IgG antibodies were found in 439 (39.51%) patients, compared with 522 (35.63%) healthy controls (P < 0.001) (Fig. 1 and Table 2). After adjustment for age and sex, there was no difference of anti-HEV IgG seroprevalence between patients and controls (Table 2). S/N ratio of positive anti-HEV IgG was similar in the two groups (Fig. 2). Anti-HEV IgG positivity was more frequent in male and elderly in both groups, but unrelated to the incidence of any non-traumatic neurological illness (Table 4, S1), hospital stay or treatment outcome, except linking to better outcome of hemorrhagic stroke disease (Table 5).

4. Discussion

The current study is the first large case-control cohort study to investigate the association of HEV infection with acute non-traumatic neurological illness. In the rest of patients without acute HEV infection, anti-HEV IgG antibodies were found in 439 (39.51%) patients, compared with 522 (35.63%) healthy controls (P < 0.001) (Fig. 1 and Table 2). After adjustment for age and sex, there was no difference of anti-HEV IgG seroprevalence between patients and controls (Table 2). S/N ratio of positive anti-HEV IgG was similar in the two groups (Fig. 2). Anti-HEV IgG positivity was more frequent in male and elderly in both groups, but unrelated to the incidence of any non-traumatic neurological illness (Table 4, S1), hospital stay or treatment outcome, except linking to better outcome of hemorrhagic stroke disease (Table 5).

### Table 4

| Seroprevalence of IgG in patients of different types of acute neurological event. |
|---------------------------------|---------------------------------|
| Anti-HEV IgG+ (n = 439) | Anti-HEV IgG- (n = 672) |
| Age, years (64 ± 14) | 63 ± 15 |
| Sex | | |
| Male | 60.82% | 52.83% |
| Female | 39.18% | 47.17% |
| Ischemia stroke | 59.68% | 55.21% |
| Hemorrhagic stroke | 12.30% | 12.80% |
| Neurodegenerative disease | 13.44% | 15.48% |
| Central nervous system infections | 2.96% | 4.02% |
| Others | 11.62% | 12.50% |

| Sex | Male | 60.82% | 52.83% | 6.88 | 0.009 |
| | Female | 39.18% | 47.17% |
| | | | 2.167 | 0.141 |
| | | | 0.060 | 0.807 |
| | | | 0.880 | 0.348 |
| | | | 0.854 | 0.355 |
| | | | 0.194 | 0.660 |

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### Table 5

| Hospital stay and therapeutic outcomes of neurological injury patients with detectable versus undetectable anti-HEV IgG. |
|---------------------------------|---------------------------------|
| Type of neurological event | Anti-HEV IgG-N (%) | Anti-HEV IgG-N (%) |
| Ischemia stroke | 262 | 240 (95.04%) |
| Recovery/improvement | 371 | 350 (94.34%) |
| | 371 | 350 (94.34%) |
| Treatment failure | 1 (0.38%) | 0 (0%) |
| | 1 (0.38%) | 0 (0%) |
| Others | 12 (4.58%) | 21 (5.66%) |
| | 12 (4.58%) | 21 (5.66%) |
| Hospital stay (average, d) | 10.69 | 10.75 |
| | 10.69 | 10.75 |
| Hemorrhagic stroke | 54 | 56 (56.60%) |
| Recovery/improvement | 86 | 72 (83.72%) |
| | 86 | 72 (83.72%) |
| Others | 2 (3.70%) | 14 (16.28%) |
| | 2 (3.70%) | 14 (16.28%) |
| Hospital stay (median, d) | 10.19 | 10 (7.75–25) |
| | 10.19 | 10 (7.75–25) |
| Neurodegenerative disease | 59 | 60 (60.15%) |
| Recovery/improvement | 104 | 100 (96.15%) |
| | 104 | 100 (96.15%) |
| Others | 6 (1.69%) | 4 (3.83%) |
| | 6 (1.69%) | 4 (3.83%) |
| Hospital stay (average, d) | 13.21 | 13.18 |
| | 13.21 | 13.18 |
| CNS infections | 13 | 27 |
| Recovery/improvement | 27 | 26 (96.30%) |
| | 27 | 26 (96.30%) |
| Others | 0 (0%) | 1 (3.70%) |
| | 0 (0%) | 1 (3.70%) |
| Hospital stay (median, d) | 15 (95–25) | 14 (8–19) |
| | 15 (95–25) | 14 (8–19) |
| Others | 51 | 84 |
| | 51 | 84 |
| Recovery/improvement | 84 | 76 (90.48%) |
| | 84 | 76 (90.48%) |
| Treatment failure | 0 (0%) | 1 (1.19%) |
| | 0 (0%) | 1 (1.19%) |
| Others | 5 (9.80%) | 7 (8.33%) |
| | 5 (9.80%) | 7 (8.33%) |
| Hospital stay (average, d) | 9.94 | 13.02 |
| | 9.94 | 13.02 |

a Student t-test for normally distributed continuous variables whereas other continuous variables were expressed in median (interquartile range [IQR]); the data with skewed distribution were compared using the Mann-Whitney U test; Chi-squared or the Fisher’s exact test for categorical variables with excepted cell sizes less than five were used.

b There is no treatment failure patient in both anti-HEV IgG positive and negative cohorts.

The current study is the first large case-control cohort study to investigate the association of HEV infection with acute non-traumatic neurological illness.
and cytomegalovirus [21], or only positive for anti-HEV IgM were either coinfected with other pathogens, such as hepatitis B virus. 

Several sporadic cases of neurological injury that possibly linked to HEV infection have been documented in China [12–21]. However, these cases were either coinfected with other pathogens, such as hepatitis B virus and cytomegalovirus [21], or only positive for anti-HEV IgM [12,22,23]. Although there was one case of GT4 HEV related bilateral peripheral facial palsy reported in Japan [24], the causation with HEV infection is uncertain. Moreover, a small cohort study performed in China composed of 64 GBS patients and 21 encephalitis patients found one patient with possible acute HEV infection (only anti-HEV IgM was positive) and none of the encephalitis patients tested positive for anti-HEV IgM, and no HEV RNA was detected in serum and CSF [12]. Interestingly, a very recent Chinese study indicated that HEV viraemia was detected in 2.1% (4/188) MG patients and the all isolates were GT4 HEV [13]. Further studies are required to clarify the causality of GT4 HEV infection in neurological diseases.

GBS and NA have been identified as the most frequent HEV associated neurological disorders. Two previous reports conducted in the Netherlands and Bangladesh, where GT3 and GT1 HEV predominate, respectively, revealed that 5% and 11% of GBS patients had positive anti-HEV IgM [7,8]. More recently, a study conducted in Belgium found that 8% of the GBS cases were possibly associated with a recent HEV infection [25]. However, no confirmed GBS or NA attributing to GT4 HEV has been documented among the approximately 70 HEV-related GBS or NA cases [6]. In our study, one of the three GBS cases appeared to be associated with GT4 HEV. Nevertheless, the causal relationship remains to be established with larger GBS population.

The rates of anti-HEV IgM positivity and HEV viraemia showed no significant difference between the patients and controls. However, whether HEV is an etiology of the six non-traumatic neuropathy injury patients reported in the present study requires further investigation. The striking observation is that two of the 26 patients with viral encephalitis tested positive for HEV, and only these two patients among all patients with HEV showed abnormal LFTs. This is consistent with other studies involving GT3 HEV-associated encephalitis, in which the only two documented cases showed elevated ALT [26,27]. This observation indicates that viral encephalitis, unlike other HEV-related neurological illnesses, appears to present symptomatic acute hepatitis. Encephalitis/myelitis is one of the most common HEV-associated neurological disorders after GBS and NA, but all had conformed GT3 HEV infections. Recently, an experimental study in gerbils confirmed that HEV can break through the blood-brain barrier and replicate in the central nervous system [28]. However, one cohort study performed in China enrolled 21 patients with encephalitis found no positive anti-HEV IgM or detectable HEV RNA in the serum or CSF [12], probably because of geographical heterogeneity or small size of cohort. The present large cohort study indicated a causal relationship between HEV and viral encephalitis.

Patient 2 presented with a 2-month history of numbness in the left upper extremities and was diagnosed with peripheral neuropathy. She was positive for both anti-HEV IgM and IgG. The numbness of the left upper limb resolved without any treatment upon anti-HEV IgM sero-negative correlation, suggesting a potential causal association. In a previous study, viral status was shown to be associated with peripheral neuropathy in two cases of chronic HEV infection. In one case, neurological symptoms completely resolved, with successful HEV clearance, after treatment with a combination of Peg-IFN alpha and ribavirin [29]. In contrast, the other case, who failed to achieve viral clearance by Peg-IFN alpha treatment and reduction of immunosuppressants, had no improvement in symptoms and ultimately dying [30].

The relationship between HEV and ischemic neuropathy remains less clear. In our study, involving 635 patients with posterior circulation ischemia (PCI)/transient ischemic attack (TIA)/vertebrobasilar insufficiency (VBI)/stroke, two patients showed evidence of HEV infection. Similar cases were reported in a recent study, whereby four of 238 patients who suffered stroke/TIA presented positive HEV IgM and two of them had HEV viraemia [9]. The rate of HEV infection in ischemic neuropathy patients was even lower than that in the healthy population in the current study. There is no evidence to support a causal relationship between HEV and this neurological pattern.

Of note, HEV viraemia was observed in all the patients and controls who had positive anti-HEV IgM antibodies, among which only one sample was successful sequenced. We found the viral loads in most of the 16 samples were close to the detection threshold and the sample of successful sequencing had the highest viral load. Thus, the failure in sequencing of the other 15 HEV viraemic individuals is likely due to low viral load. The kit for HEV RNA detection used in the study is highly sensitive and specific [31,32] with detection limit of 1.42 × 10^3 copies/mL, which explains the high rate of HEV RNA detection and the low rate of successful sequencing.

Since >70 neurological conditions were included in the current study, it could be argued that the heterogeneity of the studied population may lead to involvement of potential confounding factors, which probably contribute to no more detected HEV infections in the cases group as compared to the controlled one. However, as an exploratory study, we incipently desired to know the full range and frequency of GT4 HEV-associated neurological injury, in addition to foregone HEV associated neurological syndromes, just as an pilot Europe study reported in 2017 that explored the role of HEV in a wide range of neurological pathologies of various aetiologies [9]. The provisional findings in our study have provided a hint of like or unlikely causality between specific neurological conditions and HEV infection. Nevertheless, further studies focusing on the defined neurological populations that HEV is likely a contributor are deserved in China to precisely address the relationship between GT4 HEV infection and specific neurological disorders.

Besides the lacking of the definite confirmation of HEV genotype for all the patients and heterogeneity of the studied population, there are some other limitations in our current study. First, the sex and age were not well-matched between the patient and control groups. Secondly, CSF samples were not collected for HEV RNA tests, thus lacking of the definite HEV status in most cases were missed, leading to a question whether the alleviation of symptoms is the result from recovery of HEV infection. In conclusion, this is the first large cohort study of HEV infection in a wide spectrum of neurological diseases in China, where GT4 is prevalent. We have demonstrated that 0.54% patients with acute non-traumatic neurological injury had HEV infection. The overall incidence of HEV infection was not significantly different between patients and healthy controls. Nevertheless, we cannot exclude a possible causative role, suggesting that testing HEV in this population, especially in situations of unexplained deregulated liver function would be warranted. Multicenter studies are warranted to further clarify the role of HEV infection in neurological diseases in GT4 prevalent regions.

Conflicts of interest

The authors have declared that no competing interests exist.
u thors contributions

Y.W. contributed to study concept and design, obtained funding, supervision of the study, literature search and writing of the manuscript; S.W. contributed to acquisition and analysis of data, making tables and figures, and drafting of the manuscript; J.W. and N.G. contributed to patients’ sample and medical information collection; Y.J. contributed to acquisition and analysis of data; S.L. and H.L. contributed to statistical analysis. C.Y. and H.T. contributed to statistical analysis; M.P.P. contributed to study concept; Q.P. contributed to study concept and critical revision of the manuscript; J.Z. contributed to study concept and study supervision.

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