

Supplemental materials

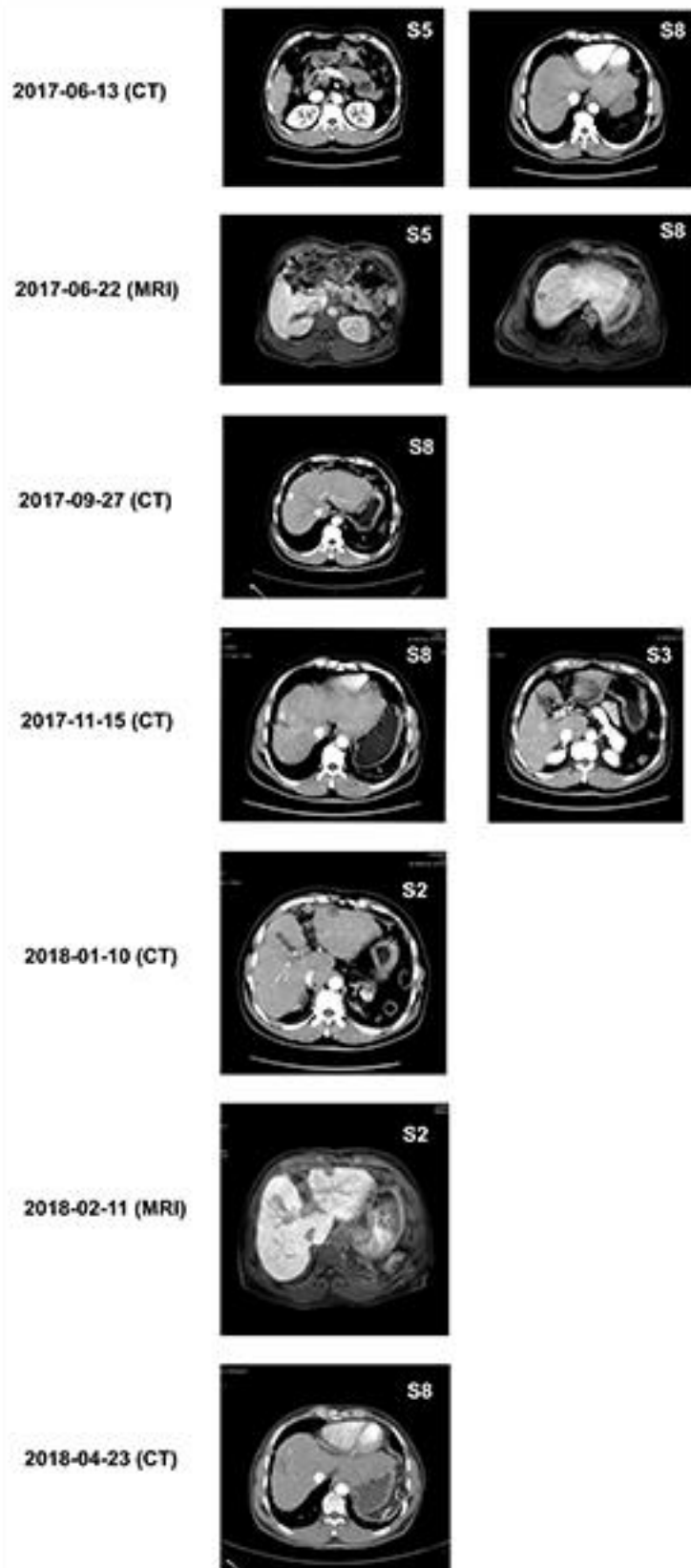


Figure 1. CT and MRI results of patient between June 2017 and April 2018.

- Detection of HEV infection

Anti-HEV serum immunoglobulin (Ig) G and Ig M detection were performed with FAME enzyme-linked immunosorbent assay (ELISA) (Hamilton, Switzerland), according to the manufacturer's instructions. RNA was extracted from the 100 ul of serum sample using a HEV nucleic acid purification kit (Acon, China) following the guideline of the company. Afterward, 20 ul HEV RNA was used for reversed transcription polymerase chain reaction (RT-PCR) assays by ABI 7500 (Hunan, China). A 20ul reaction solution including 18ul RT-PCR reaction mixture (Tris-HCl, KCl, MgCl, Dntp), 0.6 ul HEV fluorescent probe (HEV primer and fluorescent probe) and 1.4 ul mixed enzyme (taq enzyme and antibody, reverse transcriptase and inhibitor of RNA enzyme) was prepared for extracting HEV RNA. At the same time, RNA transcribed in vitro and TE buffer solution were applied as the positive control and negative control respectively. Subsequently, PCR amplification was carried on under the conditions of 50°C for 20 minutes at the first round, 70°C for 15 minutes at the second round, 94°C for 15 minutes or 60°C for 30 minutes lasting for 40 rounds. Primers (forward, 5'-CCGGTCAGCCGTCTGGC-3'. reverse, 5'GCGAAGGGGTTGGTTGGA-3') were used at every round of PCR amplification. Lastly, if HEV RNA was positive, a qRT-PCR would be conducted.

- Biological test

Serum samples were collected from the patients at each follow-up for different biological tests according to the manufacturer's instruction (Table 1), including tests for liver fibrosis (Lumo chemiluminescence immunoassay, China), liver cancer (ELISA, Hamilton, Switzerland), liver and renal function (AU5800, Beckman Coulter, America).Hepatitis B surface antigen (HBsAg), anti-HBs, hepatitis B e antigen (HBeAg), anti-HBe, hepatitis B core antibody (anti-HBc) were tested using chemiluminescence (Roche, China).

The normal references used in this study were: Alanine aminotransferase (ALT), 9~50(U/L); aspartate aminotransferase (AST), 15~40(U/L); gamma-glutamyltransferase (GGT),10~60(U/L); alkaline phosphatase (ALP),45~125(U/L); total bile acid (TBA),1~14(umol/l). CIV, 0~95(ng/ml); LN, 0-130(ng/ml); PIIINP, 0-15(ng/ml); HA, 0~120(ng/ml); HBsAg, 0~1(COI); anti-HBs, 0~10(IU/L); HBeAg, 0~1(COI); anti-HBe, 0(COI); anti-HBc, 0(COI); AFP, 0~9(ng/ml); CEA, 0~5(ng/ml).