Acta virologica **62**: 208 – 213, 2018 doi:10.4149/av_2018_215

Evidence of hepatitis A virus infection in the patients with acute encephalitis syndrome in Gorakhpur region, North India

M. S. JOSHI*, B. V. TANDALE, M. M. GORE, S. BHALLA, Y. K. GURAV, G. N. SAPKAL, K. P. KUSHWAHA, A. C. MISHRA, S. D. CHITAMBAR*

Enteric Viruses Group, National Institute of Virology, 20-A, Dr. Ambedkar Road, P. O. Box No. 11, Pune 411 001, India

Received August 21, 2017; accepted November 29, 2017

Summary. – The etiological agent remained unidentified in a large number of patients hospitalized for acute encephalitis syndrome (AES) in 2008–2009 in Uttar Pradesh and Bihar, north India. All patients were found to present with fever and altered sensorium, while 28%, 19% and 13% showed hepatomegaly, splenomegaly and meningeal signs, respectively. Involvement mostly of children with abnormal hepatic features prompted us to undertake an exploratory study on viral hepatitis A to determine its association, if any, with hepatic derangements. AES patients (n = 2515) and healthy children (n = 167) were investigated for the presence of serum anti-hepatitis A virus (anti-HAV) IgM and anti-Japanese encephalitis (anti-JE) virus IgM by ELISA. Cerebrospinal fluids (CSFs, n = 595) and rectal swabs (n = 182) were examined for anti-HAV IgM and/or HAV RNA. Anti-HAV IgM was detected in the sera of 14.6% patients as against 6.6% of healthy children (p = 0.0042). Anti-JE virus IgM positivity was <10% and 0%, respectively, among these groups. Anti-HAV IgM seropositivity was significantly high in patients with hepatomegaly and raised liver enzyme levels. Among anti-HAV IgM seropositive patients, CSF anti-HAV IgM positivity was detected in 56.9%, of which 18% had evidence of fecal excretion/viremia of HAV. CSF anti-HAV IgM positivity in AES patients was associated with pleocytosis and low glucose levels in CSF. The data of this study suggest association of HAV infection with increase in abnormal hepatic functions of AES patients and warrants enhanced hospital and community-based surveillance on hepatitis A for implementation of preventive measures to reduce the HAV infections and their impact on AES in north India.

Keywords: acute encephalitis syndrome; cerebrospinal fluid; hepatitis A virus; anti-HAV IgM; non-Japanese encephalitis

Introduction

Hepatitis A is a major public health problem worldwide. Globally, the disease has been reported to occur in approximately 1.5 million cases per year. Hepatitis A virus (HAV), the causative agent of hepatitis A, is a member of the genus *Hepatovirus*, the family *Picornaviridae* ,known to be transmitted mainly through fecal-oral route. The majority of hepatitis A patients recover completely and the rate of mortality due to HAV infection has also been described to be low (0.1%–2.1%) (Hollinger *et al.,* 1996). However, about 15% of patients have been reported to experience prolonged jaundice and/or relapses over several months (Sjogren *et al.,* 1987) while some have been described to develop cholestatic hepatitis, fulminant liver failure and acute kidney injury (Jung *et al.,* 2010). Neurological syndromes such as myelitis, peripheral neuropathy and Guillian-Barre' syndrome have been described occasionally in association with hepatitis A (Adams and Asbury, 1980; Chitambar *et al.,* 2006).

Acute viral encephalitis can occur either in sporadic or outbreak forms and is known to be caused by a variety of

*Corresponding authors. E-mail: ms_joshi2004@yahoo.co.in, dr-shobha.niv@gmail.com; phone: +91-20-26006237.

Abbreviations: AES = acute encephalitis syndrome; BRD Medical College Hospital = Baba Raghav Das Medical College Hospital; CSFs = cerebrospinal fluids; HAV = hepatitis A virus; JE = Japanese encephalitis; S/Co = sample optical density (OD)/cut off value; SGPT = serum glutamic pyruvic transaminase; SGOT = serum glutamic oxaloacetic transaminase

viruses such as herpes virus, alpha virus, flavi virus, Chandipura virus, rabies virus, influenza A virus, measles and enterovirus (Kennedy 2004; Sapkal *et al.,* 2009). In addition to these viruses, association of hepatitis A virus with sporadic cases of encephalitis and menigoencephalitis has also been reported (Hammond *et al.,* 1982; Mathew *et al.,* 2012).

In 2005–2006, large outbreaks of acute encephalitis syndrome (AES) were reported in eastern Uttar Pradesh and Bihar, north India (Parida *et al.,* 2006; Sapkal *et al.,* 2009). Subsequent to these eventualities, a Japanese Encephalitis (JE) vaccination campaign was launched among children in UP by the Government of India. In 2008–2009, a decline in JE seropositivity from 38.8% noted in 2005 to 9.8% was reported. However, AES cases continued to occur (Kumari and Joshi, 2012).

As per the records at the Baba Raghav Das (BRD) Medical College Hospital, Gorakhpur in north India, 4382 AES cases were reported during 2008–2009 predominantly from Gorakhpur and 5 adjoining districts of eastern Uttar Pradesh and 2 adjoining districts of Bihar, the locations where JE is known to be endemic in north India. Laboratory tests performed to detect the etiological agents known to cause acute viral encephalitis were positive for JE (<10%), enterovirus (2%) and negative for West Nile virus, alphavirus, Chandipura virus, herpes simplex virus and measles virus. The etiological agent remained unidentified in a large number of AES patients. Clinical features of the patients included fever, altered sensorium (100%), hepatomegaly (28%), splenomegaly (19%) and meningeal signs (13%). The data available from 42% of the AES cases showed hepatomegaly and abnormal serum glutamic pyruvic transaminase (SGPT)/ serum glutamic oxaloacetic transaminase (SGOT) levels in 30%. We conducted an exploratory study in the subsets of cases from 2008–2009 for viral hepatitis A to determine its relation, if any, with AES mainly recorded in children.

Materials and Methods

A retrospective study was carried out among children and adolescents (n = 2067) aged 1 to 18 years (median age 5 years), and adults (n = 448), aged 19 to 90 years (median age 25 years) admitted at BRD Medical College Hospital for AES in 2008–2009. An individual of any age, who was admitted to the hospital at any time in the year for the acute onset of fever and a change in the mental status such as confusion, disorientation, coma or inability to talk, was considered as a case of AES. Individuals suffering from simple seizures were not included in the study. A total of 2515 serum samples, and 595 cerebrospinal fluids (CSFs) and 182 rectal swabs paired with serum samples were collected for laboratory analysis. Serum samples from 167 healthy subjects, aged 2 to 15 years (median age 6 years), available from JE vaccination program in Gorakhpur, were also tested. As the study involved use of pre-existing, left over, and stored samples, the requirement of informed consent was waived and the study was approved by Institutional Ethics Committee as per the national guidelines.

HAV-specific IgM was detected using IgM class capture ELISA as described earlier (Chitambar *et al.,* 1994). This test has been reported to be highly specific for HAV with non-reactivity of sera from healthy individuals, patients with other types of hepatitis and rheumatoid factor and has been also used to investigate the acute HAV infection in a patient with Guillain-Barre syndrome (Chitambar *et al.,* 2006). Anti-JE virus IgM was detected by the ELISA as reported earlier (Thakare *et al.,* 2002).

The viral RNA was extracted by using viral RNA mini kits (Qiagen, Hilden, Germany). HAV RNA was detected by RT-PCR targeting partial RNA polymerase region as described previously (Joshi *et al.,* 2014). PCR products were analyzed by gel electrophoresis and sequenced in ABI PRISM 3130XL Genetic Analyzer using ABI PRISM Big Dye Terminator cycle sequencing ready reaction kit (Applied Biosytems, CA, USA).

Statistical analysis was carried out in PASW 18. Chi-square test was used for comparison of features of AES patients with and without serological evidence of anti-HAV IgM. Cochran-Mantel-Haenszel chi-square test was employed for comparison of hepatitic features of patients tested for both anti-HAV IgM and anti-JE IgM. The association between the S/Co (sample optical density (OD)/ cut off value) ratios determined for serum and corresponding CSF sample was assessed by calculating the correlation coefficient.

Results

Of the 2515 sera collected from AES patients, 367(14.6%) samples from 354 children/ adolescents and 13 adults showed presence of anti-HAV IgM while 240 (9.6%) samples from 175 children/adolescents and 65 adults with AES were positive for anti-JE virus IgM, a marker known for acute JE virus infection. In comparison, serum samples from healthy children (n = 167) showed significantly low positivity (6.6%, p = 0.0042) to anti-HAV IgM and no positivity to anti-JE virus IgM.

Among 2515 patients, data on hepatomegaly, raised SGPT and SGOT levels was available from 1154, 988 and 882, respectively, and hence, only these patients were examined for the distribution of hepatitic features with respect to anti-HAV IgM status (Table 1). Among specific features, age group of 1–5 years patients was included due to predominance of HAV infections observed in pediatric population below 5 years of age in India. Comparison of hepatitis-specific features between different subsets of AES patients showed a significantly higher frequency of hepatomegaly and raised SGPT and SGOT levels in anti-HAV IgM-positive than in anti-HAV IgM-negative patients (Table 1). A significantly higher number of sole anti-HAV IgM-positive patients with palpable liver and raised (>120 IU/l) SGPT level than that in sole anti-JE virus IgM-positive patients (Table 2) and a mark-

Table 1. Comparative analysis of salient features of AES patients with and without serological evidence of anti-HAV IgM

Feature of AES patients*	Specific feature	Anti-HAV IgM-positive patients**	Anti-HAV IgM-negative patients**	p value
Age group (n = 2515)	1–5 years	260/367 (70.8%)	817/2148 (38%)	0.0001
Hepatomegaly (n = 1154)	Liver palpable	210/234 (89.7%)	733/920 (79.6%)	0.0006
	Liver >2.5cm below costal margin	118/234 (50.4%)	408/920 (44.3%)	0.0956
SGPT (n = 988)	>40IU/l	132/197 (67.0%)	422/791 (53.3%)	0.0008
	>120IU/l	42/197 (21.3%)	92/791 (11.6%)	0.0006
SGOT (n = 882)	>40IU/l	144/194 (74.2%)	430/688 (62.5%)	0.0027
	>120IU/l	53/194 (27.3%)	110/688 (15.9%)	0.0005

*: Figures in the column indicate total number N of patients with data available; **: Figures in the column indicate number of patients with defined specific feature/number of patients with data available (percentage).

Table 2. Comparison of hepatitic features of AES patients tested for both anti-HAV IgM and anti-JE IgM

Feature of AES patients	Specific feature	Anti HAV IgM positive alone (percentage)[a*]	Anti-JE virus IgM alone (percentage)[b*]	Positive for both anti-HAV IgM and anti-JE IgM (percentage)[c*]	p value a vs. b	p value b vs. c
Hepato-megaly	Liver palpable	134/143 (93.7%)	48/62 (77.4%)	27/28 (96.4%)	0.0009	0.0261
	Liver >2.5cm below costal margin	67/143 (46.8%)	20/62 (32.2%)	19/28 (67.8%)	0.0529	0.0019
SGPT	>40IU/l	93/135 (68.9%)	39/63 (61.9%)	23/29 (79.3%)	0.3329	0.098
	>120IU/l	31/135 (22.9%)	7/63 (11.1%)	9/29 (31.0%)	0.0493	0.02
SGOT	>40IU/l	100/132 (75.7%)	42/57 (73.7%)	23/29 (79.3%)	0.7628	0.5682
	>120IU/l	39/132 (29.7%)	9/57 (15.8%)	11/29 (37.9%)	0.0463	0.0225

*: Figures in the column indicate number of patients with defined specific feature/ number of patients with data available (percentage).

edly higher number of patients with hepatomegaly and raised (>120 IU/l) SGPT and SGOT levels associated with positivity to both anti-HAV IgM and anti-JE virus IgM than that with sole anti-JE virus IgM positivity (Table 2).

Of the 595 CSF samples tested for anti-HAV IgM, 202 and 393 were collected from anti-HAV IgM seropositive and seronegative AES patients, respectively. CSF samples from most of the patients were clear and without color after centrifugation. Among 202 seropositive patients, 115 (56.9%) showed CSF anti-HAV IgM positivity. All seronegative patients (n = 393) were negative for anti-HAV IgM in CSF samples. The mean S/Co ratios obtained for anti-HAV IgM reactivity in serum and CSF specimens of AES patients were 3.3±2.0 and 2.5±1.53 respectively, and the correlation between the paired serum and CSF S/Co ratios was found to be significant ($r = 0.82$, $p < 0.01$)(Fig.).

In 115 AES patients with CSF anti-HAV IgM positivity, 67.8% and 76.1% showed hepatomegaly and raised SGPT-SGOT levels, respectively, and 18% showed presence of HAV RNA in rectal swabs and/or serum samples. Eleven percent of anti-HAV IgM seropositive but CSF-negative patients also showed presence of HAV RNA in the rectal swab and/or serum samples. Nucleotide sequence analysis of the amplified partial RNA polymerase region revealed presence of HAV genotype IIIA in all of the HAV strains detected in rectal swabs/sera. HAV RNA was not detected in any of the 120 CSF samples tested positive for anti-HAV IgM in the study.

Among seropositive AES patients, the data for CSF cell count, CSF glucose levels and CSF protein levels was available for 67 patients, of which 43 were positive for CSF anti-HAV IgM and 24 were negative. CSF anti-HAV IgM-positive group

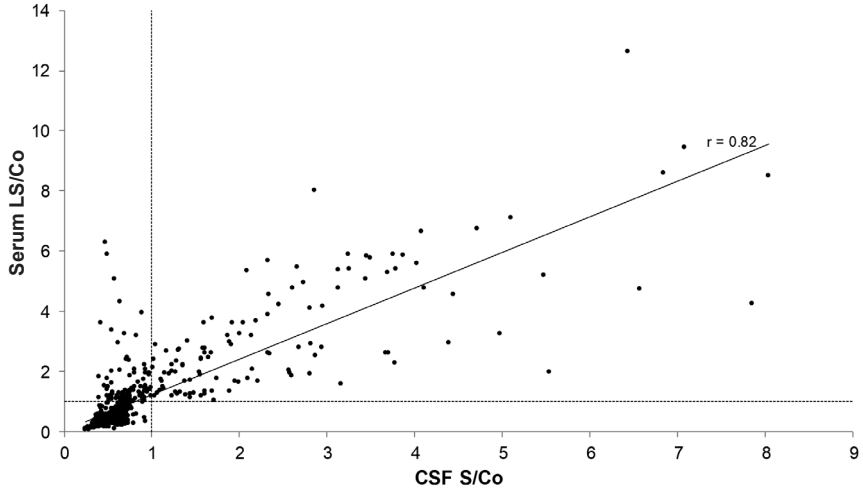

Fig. 1

Scatter diagram displaying anti-HAV IgM activity in serum and CSF specimens collected from acute encephalitis patients (n = 595)

(n = 43) showed significantly higher proportion of patients with high CSF cell count and low glucose level as compared to those in CSF anti-HAV IgM-negative patients group (n = 24) (cell count >5 cell/µl: 77.6% vs 39%; glucose < 50 mg/dl: 60.5% vs 20.8%; p <0.05 for each parameter). CSF protein levels above 50 mg/dl were recorded in 46.5% and 29.2% of the CSF anti-HAV IgM-positive and CSF anti-HAV IgM-negative patients respectively. However, the difference in the proportions was not statistically significant (p = 0.16).

Discussion

In the present study, a significant proportion of AES patients were found to present with clinical signs and/or biochemical parameters that were suggestive of hepatic involvement. Interestingly, among these patients 14.6% displayed presence of anti-HAV IgM, the serological marker of acute HAV infection. Remarkable (54%) positivity to this marker was also observed in corresponding CSF samples. The acute infection of HAV was confirmed by viremia and fecal shedding of the virus in the seropositive AES patients. Further, association of abnormal liver functions was more frequently found in the anti-HAV IgM-positive patients than in patients with anti-HAV IgM negativity. Abnormal liver functions were observed more frequently when patients were positive for both anti-HAV and anti-JE virus IgM as compared to anti-JE virus IgM alone.

Lower glucose (< 50 mg/dl) and relatively higher protein (> 50 mg/dl) levels noted in anti-HAV IgM-positive CSF samples were in agreement with those reported earlier in acute infections with herpes, varicella zoster and mumps viruses (Hammond *et al*., 1982; Ekmekci *et al.,* 2013). Further, CSF pleocytosis associated with anti-HAV IgM/IgG positivity in the CSF of AES patients was in concurrence with that of the previously reported HAV-associated encephalitis cases (Davis *et al.,* 1993; Lee *et al.,* 2011) and suggested active inflammatory response in this group of patients. A few case reports have been described earlier on the occurrence of encephalitis following the preceding/prodromal stage of hepatitis A (Hammond *et al.,* 1982; Davis *et al.,* 1993; Cam *et al.,* 2005; Lee *et al.,* 2011; Yis *et al.,* 2013). This is the first report on the role of hepatitis A demonstrating increase in the severity of the AES, in sizeable group of AES patients. However, this observation could not be supported by the evidence of intrathecal antibody synthesis due to insufficient quantities of serum / CSF samples.

The set of patients investigated in the study lacked evident jaundice, and, therefore, the possibility of occurrence of anicteric hepatitis prior to the onset of AES needs to be taken into consideration (Verma *et al.,* 2012). It may also be noted that the BRD medical college hospital wherein the study was carried out is the only tertiary care hospital in Gorakhpur district of North India, and hence, communities residing in this region prefer to seek medical treatment in this hospital (Murhekar *et al.,* 2016). As a result, possible hindrance due to referral bias in the study could have been minimal.

AES, a major public health problem causing significant mortality in Uttar Pradesh, may be a primary manifestation or a secondary complication of certain viral infections or vaccinations. The possible mechanisms responsible for this neurologic damage may include a post infectious autoimmune state, in which immune system may attack central nervous system antigens that resemble the proteins of the

infectious agent(s), or the virus may invade the blood vessels directly (Verma *et al.,* 2012). To date, a single patient with encephalitis has been described for the presence of anti-HAV IgM and HAV RNA in the CSF during the course of HAV infection (Cam *et al.,* 2005). In the present study, positivity to CSF anti-HAV IgM was highly evident; however, no HAV RNA was detected in any of the CSFs, rendering the definite causal relationship of this agent with AES uncertain. In India, HAV infections have been reported to be in the intermediate status of endemicity with mixed pockets of immune and susceptible populations and increased symptomatic infections (Verma and Khanna, 2012). In view of this, patients with neurological disturbances associated with abnormal liver function tests may be investigated for HAV infection.

The present exploratory study was restricted to the analysis of data on hepatitic features available from the limited number of AES patients and healthy children. Also, the study was performed on the samples transported and stored frozen at the National Institute of Virology, Pune, India. In such a status, possibility of loss of IgM or viral RNA during transportation and storage of samples needs to be considered. Nevertheless, the high frequency of CSF anti-HAV IgM positivity in AES patients suggests the need for enhanced surveillance and seroepidemiological studies on hepatitis A in hospital and community settings in Gorakhpur and adjoining districts from North India. This would be useful to adopt the strategies of prophylaxis and help reduce the HAV infections and their influence on AES.

Acknowledgment. We thank Dr. D.T. Mourya, Director, NIV, Pune for all the support; the Directorate of Health Services, Government of Uttar Pradesh, India for help and cooperation; Mr. A. Walimbe for statistical analysis; M. Shinde, P. More, R. More and staff members of the National Institute of Virology Unit, Gorakhpur, for technical support; Funds were provided by the host institute, Indian Council of Medical Research - National Institute of Virology, Ministry of Health and Family Welfare, Government of India.

References

Adams RD, Asbury AK (1980): Diseases of peripheral nervous system. In Isselbacher KJ, Braunwald E, Wilson JD, Martin JB, Fauci AS (Eds): Harrisons Principles of Internal Medicine. New York: MacGraw Hill, pp. 2097–3030.

Cam S, Ertem D, Koroglu OA, Pehlivanoglu E (2005): Hepatitis A virus infection presenting with seizures. Pediatr. Infect. Dis. J. 24, 652–653. https://doi.org/10.1097/01.inf.0000168754.24478.6d

Chitambar SD, Fadnis RS, Joshi MS, Habbu A, Bhatia SG (2006): Case Report- Hepatitis A Preceding Guillain-Barre Syndrome. J. Med. Virol. 78, 1011–1014. https://doi.org/10.1002/jmv.20656

Chitambar SD, Murthy-Grewal S, Bokil MP, Arankalle VA, Gore MM, Banerjee K (1994): Indigenous anti-hepatitis A virus IgM capture ELISA for the diagnosis of hepatitis A. Indian J. Med. Res. 99, 243–251.

Davis LE, Brown JE, Robertson BH, Khanna B, Polish LB (1993): Hepatitis A post-viral encephalitis. Acta. Neurol. Scand. 87, 67–69. https://doi.org/10.1111/j.1600-0404.1993.tb04078.x

Ekmekci H, Ege F, Ozturk S (2013): Cerebrospinal fluid abnormalities in viral encephalitis. In Tkachev S (Eds): Encephalitis. InTech, pp. 292. https://doi.org/10.5772/54590

Hammond GW, MacDougall BK, Plummer F, Sekla LH (1982): Encephalitis during the prodromal stage of acute hepatitis A. Can. Med. Assoc. J. 126, 269–270.

Hollinger FB, Ticehurst JR (1996): Hepatitis A virus. In Fields BN, Knipe DM, Howley PM, (Eds): Fields Virology. 3rd ed Philadelphia : Lippincott-Raven, pp. 735–782.

Joshi MS, Bhalla S, Kalrao VR, Dhongade RK, Chitambar SD (2014): Exploring the concurrent presence of hepatitis A virus genome in serum, stool, saliva, and urine samples of hepatitis A patients. Diag. Microbiol. Infect. Dis. 78, 379–382. https://doi.org/10.1016/j.diagmicrobio.2013.12.013

Jung YM, Park SJ, Kim JS, Jang JH, Lee SH, Kim JW, Park YM, Hwang SG, Rim KS, Kang SK, Lee HS, Yun HS, Jee YM, Jeong SH (2010): Atypical manifestations of hepatitis A infection: a prospective, multicenter study in Korea. J. Med. Virol. 82, 1318–1326. https://doi.org/10.1002/jmv.21822

Kennedy PG. (2004): Viral encephalitis: causes, differential diagnosis and management. J. Neurol Neurosurg. Psychiatry 75, 10–15. https://doi.org/10.1136/jnnp.2003.034280

Kumari R, Joshi PL (2012): A review of Japanese encephalitis in Uttar Pradesh, India. WHO South East Asian J. Public Health 1, 374–395. https://doi.org/10.4103/2224-3151.207040

Lee JJ, Kang K, Park JM, Kwon O, Kim BK (2011): Encephalitis associated with acute hepatitis A. J. Epilepsy Res. 30, 27–28. https://doi.org/10.14581/jer.11005

Mathew T, Aroor S, Nadig R, Sarma G (2012): Focal meningoencephalitis of hepatitis A: A clinic-Radiologic picture. Pediatr. Neurol. 47, 222–223. https://doi.org/10.1016/j.pediatrneurol.2012.05.023

Murhekar MV, Mittal M, Prakash JA, Pillai VM, Mittal M, Girish Kumar CP (2016): Acute encephalitis syndrome in Gorakhpur, Uttar Pradesh, India - Role of scrub typhus. J. Infect. 73, 623–626. https://doi.org/10.1016/j.jinf.2016.08.014

Parida M, Dash PK, Tripathi NK, Ambuj, Sannarangaiah S, Saxena P, Agarwal S, Sahni AK, Singh SP, Rathi AK, Bhargava R, Abhyankar A, Verma SK, Rao PV, Sekhar K (2006): Japanese encephalitis outbreak, India, 2005. Emerg. Infect. Dis. 12, 1427–1430. https://doi.org/10.3201/eid1209.060200

Sapkal GN, Bondre VP, Fulmali PV, Patil P, Gopalkrishna V, Dadhania V, Ayachit VM, Gangale D, Kushwaha KP, Rathi AK, Chitambar SD, Mishra AC, Gore MM (2009): Enteroviruses in patients with acute encephalitis, Uttar Pradesh, India. Emerg. Infect. Dis. 15, 295–298. https://doi.org/10.3201/eid1502.080865

Sjogren MH, Tanno H, Fay O, Sileoni S, Cohen BD, Burke DS, Feighny RJ (1987): Hepatitis A virus in stool during clinical relapse. Ann. Intern. Med. 106, 221–226. https://doi.org/10.7326/0003-4819-106-2-221

Thakare JP, Rao TLG, Padbidri VS (2002): Prevalence of West Nile virus infection in India. Southeast Asian J. Trop. Med. Public Health 33, 801–805.

Verma R, Khanna P (2012): Hepatitis A vaccine should receive priority in National Immunization Schedule in India. Human Vaccin. Immunother. 8, 1132–1134. https://doi.org/10.4161/hv.20475

Yis U, Carman KB, Yılmaz-Ciftdogan D, Yıldırım C, Yis R, Akar E (2013): Hepatitis A infection presenting with recurrent seizures and widespread cerebral white matter lesions. Turkish J. Pediatr. 55, 118–120.