Biochemical Features of an Acute Viral Hepatitis A Outbreak

SIMONA CLAUDIA CAMBREA[1], OANA CRISTINA ARGHIR[1]*, AGRIPINA RASCU[2], CRISTIAN LUCIAN PETCU[3]

[1] Ovidius University of Constanta, Faculty of Medicine, 124 Mamaia Blvd, 900527, Constanta, Romania

[2] Carol Davila University of Medicine and Pharmacy, Medicine Faculty, 1 Dr Grozovici Str., 02115, Bucharest, Romania;

[3] Ovidius University of Constanta, Faculty of Dentistry, 124 Mamaia Blvd, 900527, Constanta, Romania

In developing countries, outbreaks of acute hepatitis A virus (HAV) infection have a cyclic recurrence and almost 90% of children go through disease by the age of 10. Although the evolution of HAV is rarely severe, it can cause significant economic and social losses. In order to analyze clinical and biochemical characteristics of acute HAV reported diseases in Constanta county, South Eastern Romania, during the last pediatric outbreak, all cases of hospitalized children, less than 13 years old (n=578), mostly boys with residence in urban cities, were included. Cases were divided into 295 isolated cases, mean aged 6.939 years, and 283, mean aged 6.587 years, diagnosed in different foci of the outbreak. Clinical and biochemical features of an acute HAV outbreak in the foci childrenŒ§s collectivities and families consisted in mild form of disease with frequent hepatomegaly, lack of jaundice and lower levels of aminotransferases and bilirubin.

Keywords: acute hepatitis A virus, outbreak, biochemistry, aminotransferases, bilirubin

The liver play an important role in metabolism and detoxification by eliminating toxic substances. Liver present an important functional reserve so damage of the organ may not affect its activity [1]. It is very difficult to make a difference between different types of acute viral hepatitis just based on clinical aspects. Biochemistry reveals that liver damages produce changes of specific serum enzymes like: alanine aminotransferases (ALT) or aspartate aminotransferases (AST) [2]. In acute viral hepatitis, levels of both these amino transaminases are high, increasing more than 10-20 times normal as a consequence of the hepatocellular damage. Increased values of aminotransferases are common in acute viral hepatitis. In absence of epidemiological tracers for acute viral hepatitis, clinicians have to take into consideration non-alcoholic or alcoholic steatohepatitis, parasitic diseases, or a possible hepatotoxicity induced by different medication or therapy, recreational drugs, occupational exposure or herbal remedies [1, 3-5]. The results of standard biochemical liver functions tests cannot provide a precision diagnosis, as these changes are common in so different situations, including liver damages caused by other hepatitis viruses [6, 7]. Biochemical tests for revealing liver damages are non-invasive, easily accessible, cheap, and very effective in establishing liver disease, guiding other supplementary investigations. Acute hepatitis A virus (HAV) is mostly evolving as mild or moderate form, exceptionally as a fulminant form. Transmission of HAV is possible through direct contact with an infected person or by ingestion of contaminated food or water [8]. In developing countries, there is a cycling recurrence of epidemics and nearly 90% of children go through HAV before 10 years. Although the evolution of the disease is rarely severe, it can cause significant economic and social losses. Recovery time post HAV till returning to school or work and daily activities can take up to one month. The most effective way to prevent this disease consists in improving sanitation, hand washing, safe water supply and vaccination for HAV [8].

Experimental part

In order to evaluate the biochemistry characteristics of the last HAV outbreak, we performed a 3- year survey among children hospitalized in Clinical Infectious Diseases Hospital of Constanta, Romania, in the last HAV epidemics that evolved from August 2013 to May 2015. There were included all cases of children aged less than 13 years old (limits from 3 months to 12 years and 10 months) diagnosed with acute VHA, by ELISA method determining IgM anti-HAV. All 578 cases were analyzed by general demographic data (age, sex, residence and environmental area), clinical data (fever, jaundice, hepatomegaly, splenomegaly) and biochemical investigations [total bilirubin serum level, concentration of prothrombin - Quick time, serum level of alanine (ALT) and aspartate aminotransferases (AST)]. Study population was split in two groups: patients from foci (group 1) and isolated cases (group 2), considering familial or kindergarten as foci if there were at least 3 cases diagnosed with acute HAV. This research followed the ethics and deontology standards and it was performed with the approval of Local Ethics Committee of Clinical Infectious Diseases Hospital of Constanta. The statistical analysis of data was assessed by using the IBM SPSS Statistics 23 version. For statistical evaluation, we take into considerations normal values for Quick time (70-100%) and total bilirubin (less than 1.2 mg/dL) and, for aminotransferases, the cut-off value of 1,000 IU/L was consider the elevated value (20 times more than normal) suggestive for the diagnosis of acute HVA) [8, 9]. The procedure used was non-parametric statistical tests (the χ^2 association test, the relationship between two categorical variables, with the determination of the risk chance- odds ratio (OR) and 95% Confidence Interval. It was considered statistically significant if $p < \alpha = 0.05$.

Results and discussions

In a period of consecutive 3 years (2014-2017), an outbreak of acute viral hepatitis, type A, evolved in Constanta County of Romania and 578 children, less than 13 years old, were diagnosed with acute HAV. The highest

number of cases were recorded in 2014 (n=415; 71.8%), followed by 88 cases in 2015 (15.22%) and 75 in 2013 (12.98%). From all these cases, 283 (48.96%) were identified from different foci (group 1) and 295 (51.04%) were isolated cases (group 2). The mean age of children from foci was lower (6.587 years) versus isolated cases (6.939 years) (table 1). For both groups of children, the median age was 7 years old. In this HAV epidemic, cases repartition by gender evidenced more boys (n=361; 62.5%) comparative with girls (n=217; 37.5%), with a sex ratio M/F of 1.3. Almost a half of boys (n=178; 49.3%) and girls (n=105; 48.4%) had foci distribution versus isolated cases (183 boys; 50.7% and 112 girls; 51.6%) ($\chi^2 = 0.046$, df = 1, p = 0.83) (table 1). Repartition by residence (table 1) revealed more patients with acute HAV from urban cities (n=341; 59 %) comparative with children from rural area 237 (41 %). Foci was the predominant urban transmission pattern of Constanta HAV infection outbreak (n=185; 54.3%) comparative with urban isolated cases (n= 156; 45.7%).

The most clinical manifestation of HAV was hepatomegaly (n=447; 77.3%) in both groups (n1=231/283; 81.62% and n2=216/295; 73.22%), (χ^2 = 5.822, df = 1, p = 0.016), with a 1.625 higher risk of hepatomegaly in group 1 [OR = 1.625; 95% IC for OR = (1.093, 2.414)]. Splenomegaly was reported in 147 children (25.1%), mostly in group 1 (n=86/283; 30.38%) versus group 2 (n=61/295; 20.67%) (χ^2 = 7.182, df = 1, p = 0.007), with a 1.675 higher risk of splenomegaly among children from foci than isolated cases [OR = 1.675; 95% IC for OR = (1.146, 2.446)]. Digestive onset (anorexia, nausea, vomiting, and abdominal pain) was present in almost all cases of both groups (n1=252/283; 89%; n2=272/295; 92.2%). Fever was reported in a quarter of children in both groups (n1=74/283; 26.1% and n2=73/295; 24.7%) and jaundice was more frequent in group 2 (n=122/295; 55.2%) than group 1 (n=99/283; 44.8%). Digestive symptoms often noticed in patients with acute viral hepatitis type A are fatigue, nausea and vomiting or anorexia [1, 8]. In our study, these symptoms were noticed in almost all cases (89% of foci cases and in 92.2% of isolated cases). Severe cases can rapidly progress to acute liver failure by decreasing synthetic function of the liver. Fulminant liver failure and hepatic encephalopathy with brain edema, as a result of impaired osmoregulation in the brain can be possibly fatal complication [1, 8]. One of the biomarkers of HAV severe evolution is considered decreased QT below than 50%. In our study, QT between 50-70% was suggestive for a moderate form of hepatitis and it was reported in only 22.83% children, while a QT over 70% was noticed in mild forms of HAV (75.08%), among cases of both groups (n1=189/283; 66.78% and n2= 221/295; 74.91%) (table 2).

A decreased Quick time (QT) below 50% was noticed in 2% of all cases (n=12/578), suggesting severe form of HAV, mainly in patients with other previous viral liver disease induced by Coxsakie virus (n=2), Cytomegalovirus (n=3), Epstein Barr virus (n= 5). There were 2 identified carriers of hepatitis B virus. There is a risk of 1.48 higher to identify a child with a mild form of evolution in group 1 than in group 2 [OR = 1.485; 95% IC for OR = (1.035, 2.132)]. In addition, we noticed a significant relation of dependence between QT and source of HAV infection (foci or isolated) (χ^2_{calc} = 4.631, df = 1, p = 0.031). The elevated serum values of total bilirubin (TB) higher than 1.2 mg/dL were noticed more frequent in group 2 (n= 207; 70.16%), with a mean value of 2.778 mg/dL +/- 2.142 std.dev (limits:

Patients (n)	Gender (n)		Age (yrs)	Residence (n)	
	Boys	Girls		Urban	Rural
Group 1 (n1 = 283)	178	105	6.58±3.05*	185	98
Group 2 (n2 = 295)	183	112	6.93±2.79*	156	139

Table 1
DEMOGRAPHIC CHARACTERISTICS OF TWO GROUPS

Legend: n = number, n1= cases from group 1; n2= cases from group 2; yrs = years; *standard deviation

Table 2
PATIENTS DISTRIBUTION ACCORDING WITH QUICK TIME VALUE

Patients (n)	Quick Time		χ^2_{calc}	p	OR	95% Confidence Interval for OR	
	< 70%	≥70%				Lower	Upper
Group 1 (n1 = 283)	94	189	4.631	0.031	1.485	1.035	2.132
Group 2 (n2 = 295)	74	221					

Legend: n = number, n1= cases from group 1; n2= cases from group 2; χ^2_{calc} = Chi-Square Test of association; p – value; OR = Odds Ratio;

Table 3
PATIENTS DISTRIBUTION ACCORDING WITH TOTAL BILIRUBIN LEVEL

Patients (n)	Total bilirubin		χ^2_{calc}	p	OR	95% Confidence Interval for OR	
	<1.2mg/dL	≥1.2 mg/dL				Lower	Upper
Group 1 (n1 = 283)	108	175	4.474	0.034	1.452	1.027	2.052
Group 2 (n2 = 295)	88	207					

Legend: n = number, n1= cases from group 1; n2= cases from group 2; χ^2_{calc} = Chi-Square Test of association; p – value; OR = Odds Ratio;
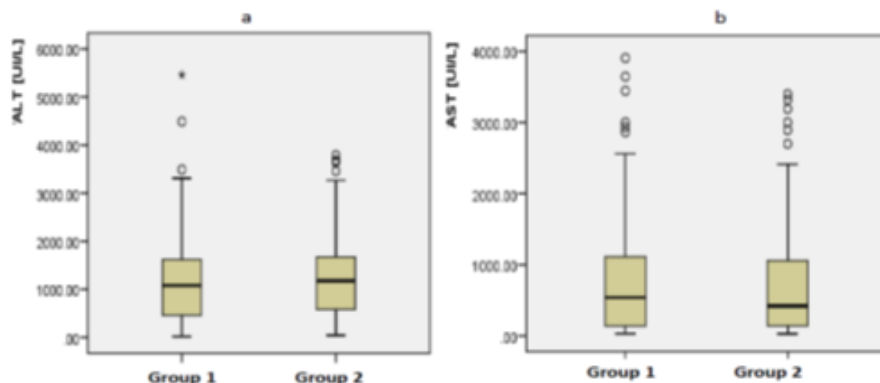
Fig. 1. Distribution of alanice (ALT) (a) and aspartate aminotransferases (AST) (b) values among groups of HAV cases

0.32- 11.00) versus group 1 (n= 175; 61.83%), with a mean value of 2.238 mg/dL +/- 1.821 std.dev. (limits 0.6- 9.65). Analyzing the blood distribution of TB by groups, a significant association was noticed (χ^2_{calc} = 4.474, df = 1, p = 0.034 <α = 0.05), with a chance of 1.452 higher to find a person with TB level less than 1.2 mg/dl in group 1 than in group 2 [OR = 1.452; 95% IC for OR = (1.027, 2.052)] (table 3). These results sustained the predominant cases without jaundice in foci than as isolated cases during the HAV epidemic.

Regarding serum ALT levels, the mean value was above 1,000 UI/L, greater in isolated cases (1,221.53 UI/L +/- 781.464 std dev; limits: 46- 3,479) than in children from foci collectivities (1,159.98 UI/L+/-841.179 std.dev.; limits: 21- 5,460) (fig. 1a). The median value was 1,178 UI/L in group 2 and 1,080 UI/L in group 1. The serum levels of ALT above 1,000 IU/L were reported in 353 cases (n1=157/283; 55.47% and n2=168/295; 56.94%) and below 1,000 IU/L in 225 children (n1= 126/283; 56% and n2= 99/295; 44%), with a dependence relation between elevated serum levels of ALT and source of infection (foci or isolated) (χ^2_{calc} = 7.3, df = 1, p = 0.007) (table 4). The chance to find one child with ALT below 1000 IU/L is 1.58 higher in group 1 than in group 2 [OR = 1.58; 95% IC for OR = (1.13, 2.22)]. Regarding biochemical changes of AST, the mean value was greater in children from foci collectivities (741.98 UI/L+/- 708.674 std.dev.; limits: 32- 3,907) than isolated cases (679.64 UI/L +/- 672.834 std dev; limits: 30- 3,3397) (fig. 1b). The median value was 539.3 UI/L in group 1 and 422 UI/L in group 2. Elevated levels above 1,000 UI/L were observed in 329 cases and lower in 249 children, especially in foci cases (n=135/249; 54.21%) (table 5), with a dependence relation with the type of source – cases from

foci or isolated cases (χ^2_{calc} = 4.834, df = 1, p = 0.028). The chance to find one child with ALT less than 1000 IU/L was 1.448 higher in foci than isolated case. [OR = 1.448; 95% IC for OR = (1.041, 2.016)].

According to Samji et al study, icteric acute viral hepatitis type A can be present in 10% of children less than 6 years, in 40-50% of older children and in 70-80% of adults [10]. The overall reported mortality rate in acute HAV is 0.002% in small children (younger than 5 years) [9, 10]. During the outbreak of pediatric acute HAV in Constanta, no one fulminant hepatic failure or death was reported. Elevated serum values of bilirubin and the presence of jaundice were more frequently noticed among isolated cases and older children (37.33%) versus small children (29.25%). The occurrence and spreading of HAV outbreaks can be related with ethnicity, urban area and difficulty in current water supply were the main determinant factors [11]. In urban cities, in the last 20 years, are living more people and children than in rural area [12, 13]. As a consequence, more and more children collectivities (kindergarten, primary schools) were developed and poor hygiene in the favor of HAV infection spreading and viral hepatitis epidemics were facilitated. In our 3 year survey study, isolated HAV cases were more frequent in isolated children with rural residence (n=139; 58.6%) versus foci (n= 98; 41.4%), with a significant association between isolated cases and rural residence pattern of HAV (χ^2 = 9.314, df = 1, p = 0.002). In Kim et al study, severe clinical cases of HAV, reported in 87 patients (12.2%), with 1.4% fulminant form of evolution, were correlated with older age, alcohol intake and chronic viral hepatitis type B [14]. In our study, 12 cases (2%) with severe HAV forms had a positive personal history of a previous liver damage caused by other viruses. International

Patients (n)	ALT		χ^2_{calc}	P	OR	95% Confidence Interval for OR	
	< 1000IU/L	≥1000IU/L				Lower	Upper
Group 1 (n1 = 283)	126	157	7.303	0.007	1.589	1.135	2.225
Group 2 (n2 = 295)	99	196					

Table 4
PATIENTS DISTRIBUTION ACCORDING WITH ALT LEVEL

Legend: n = number, n1= cases from group 1; n2= cases from group 2; ALT = alanine aminotransferases; χ^2_{calc} = Chi-Square Test of association; p – value; OR = Odds Ratio;

Patients (n)	AST		χ^2_{calc}	p	OR	95% Confidence Interval for OR	
	< 1000IU/L	≥1000IU/L				Lower	Upper
Group 1 (n1 = 283)	135	148	4.834	0.028	1.448	1.041	2.016
Group 2 (n2 = 295)	114	181					

Table 5
PATIENTS DISTRIBUTION ACCORDING WITH AST LEVEL

Legend: n = number, n1= cases from group 1; n2= cases from group 2; AST = aspartate aminotransferases; χ^2_{calc} = Chi-Square Test of association; p – value; OR= Odds Ratio;

studies revealed seven genotypes identified for HAV [14]. Out of these genotypes, 4 (I, II, III and VII) were recovered from humans and 3 (IV, V, VI) from simians [15, 16]. Furthermore, genotype I, II and III, identified in humans, are subdivided each into two groups A and B, which differ in sequence in less than 15% of nucleotide positions [15-18]. Genotype I is responsible for almost 80% of all acute hepatitis type A, isolated in most countries, genotype IA being more frequently than IB [18-20]. Genotype IIIA is more prevalent in central Asia [19]. In countries with low prevalence (Western Europe and United States), genotype IA is more often reported. In some situations, can be present coinfections with two subtypes and severe cholestasis and prolonged severe evolution can happened, like Coppola N et al described [17]. In Bulgaria, during a period of 3 years (2012-2014), almost in the same period with our Romanian South Eastern outbreak, subtype IA was found as dominant (74%) versus subtype IB (26%) [20]. In a study performed by Yilmaz in children from Turkey, the predominant subtype was IB and, in addition, two children were diagnosed with subtype IA and IIIA and the child with HAV subtype IIIA travelled from Turkey to Afghanistan and had a severe evolution [21]. Although many studies suggest that subtype IA of HAV is predominant in Europe, further studies in order to evidence the exactly subtype of HAV circulating in Romania are required [19, 20]. No Romanian national study regarding HAV genotyping was performed, only for HBV [22]. During Constanta HAV outbreak study, no case with important cholestasis and prolonged evolution was diagnosed. Coinfection with different subtypes was less likely in our area during the last epidemics.

Conclusions

During an outbreak of acute HAV, in the foci, there were more HAV cases characterized by mild form of disease, higher risk of developing hepatomegaly and lower risk of jaundice. Biochemical characteristics of low serum levels of bilirubin and aminotransferases (ALT and AST) sustained the mild forms with lower risk of jaundice especially among children from urban cities, identified in foci, versus isolated cases from rural area.

References

1.MARSHALL, W.J, BANGERT, S.K., The Liver in Clinical Chemistry, 6th Ed, Mosby Elsevier, 2008, p. 95;
2.De RITIS, F., GIUSTI, G., PICCININO, F., CACCIATORE, L., Bull World Health Organ, 32, nr. 1, 1965, p. 59.
3.STANCIU, L.E., PASCU E.I., IONESCU, E.V., CIRCO, E., OPREA, C., ILIESCU, M.G., J Environ Prot Ecol 18, nr 2, 2017, p. 728.
4.STOICESCU, R.M., MIHAI, C.M., ARGHIR, O, CAMBREA, C, HALICHIDIS, S., LILIOS, G., J Environ Prot Ecol 15, nr 1, 2014, p. 321.
5.OTELEA, M, HANDRA, C, RASCU A. Rom J Leg Med 23, 2015, p. 279.
6.COMAN, M., CHISCOP, I., MATEI, M.N., GHIBU, L., MIFTODE, E., DOROBAT, C., NECHITA, A., EARAR K., ILIE, M., MOISEI M, LECA, D. Rev. Chim. (Bucharest) 66, no.12, 2015, p. 2144.
7.GHEORGHE, D.N., RUSU, D., HERASCU, E., POPESCU, D.M., SURLIN, P., ROGOVEANU, I. Rev. Chim. (Bucharest), 68, no. 6, 2017, p. 1252
8.*** http://www.who.int/mediacentre/factsheets/fs328/en/;
9.*** Centers for Disease Control and Prevention. Viral Hepatitis Surveillance United States, 2014 (revised: 9/26/16). Available at https://www.cdc.gov/hepatitis/statistics/2014surveillance/pdfs/2014hepsurveillancerpt.pdf.
10.SAMJI, N.S., Viral hepatitis. Medscape, 2017, June 12; Available at https://emedicine.medscape.com/article/775507-overview;
11.CAMBREA, S.C., ILIE, M,M, CARP, D.S., DUMEA, E, HALICHIDIS, S., PETCU, L.C., J Environ Prot Ecol 15, nr 1, 2014, p. 340.
12.BAETICA, R., Romania between modernism and traditionalism. Eurostat Statistics Explained. Available at: http://ec.europa.eu/eurostat/statistics-explained/index.php/Marriages_and_births_in_ Romania;
13.NATIONAL INSTITUT OF STATISTICS. Romania 2015. Romania in figures. Available at: http://www.insse.ro/cms/files/publicatii/Romania_in_figures_2015.pdf;
14.KIM, J.I., KIM, Y.S., JUNG, Y.K., KWON, O,S, KIM, Y.S., KU, Y.S., CHOI, D.J., KIM, J.H., Korean J Hepatol, 16, nr 3, 2010, p. 295.
15.ROBERTSON, B.H., JANSEN, R.W., KHANNA, B., TOTSUKA, A., NAINAN, O.M., SIEGL, G., WIDELL, A., MARGOLIS, H.S., et al, J Gen Virol 73, 1992, p. 1365.
16.TSAREV, S.A., EMERSON, S.U., BALAYAN, M.S., TICEHURST, J., PURCELL, R.H., J Gen Virol 72, 1991, p. 1677.
17.COPPOLA, N., GENOVESE, D., PISATURO, M., TAFFON, S., ARGENTINI, G., PASQUALE, G., SAGNELLI, C., PICCININO, F., RAPICETTA, M., SAGNELLI, E., Clin Infect Dis 44, nr 9, 2007, p. e73–e77; https://doi.org/10.1086/513430 .
18.de PAULA, V.S., BAPTISTA, M.L., LAMPE, E., NIEL, C., GASPAR, A.M.C., J Med Virol 66, 2002, p. 22.
19.DESBOIS, D., COUTURIER, E., MACKIEWICZ, V., GRAUBE, A., LETORT, M.J,, DUSSAIX, E., et al., J Clin Microbiol 48, nr 9, 2010, p. 3306.
20.BRUNI, R., TAFFON, S., EQUESTRE, M., CELLA, E., Lo PRESTI, A., COSTANTINO, A., CHIONNE, P., MADONNA, E., GOLKOCHEVA-MARKOVA, E., BANKOVA, D, CICCOZZI, M., TEOHAROV, P., CICCAGLIONE, A.R., BMC Infect Dis 17, 2017; p. 497.
21.YILMAZ, H., KARAKULLUKCU, A, TURAN, N., CIZMECIGIL, U.Y., YILMAZ, A., OZKUL, A.A., AYDIN, O., GUNDUZ, A., METE, M., ZEYREK, F.Y., et al. BMC Infect Dis 17, Nr 1, 2017, p. 561.
22. URSU, R.G., LUCA C.M., LUCA, A.S., TOADER E., SIMION, L., IANCU, L.S. Rev.Chim.(Bucharest), 67, no. 12, 2016, p. 2614