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BIOCHEMICAL INVESTIGATIONS IN THE ASSESSMENT OF HEALTH RISKS FOR OVER 35-YEAR-OLD PATIENTS AFFECTED BY ENVIRONMENTS WITH *HEPATITIS A* VIRUS

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Abstract

Hepatitis A virus (HAV) is well known as a major public health problem, with a high index of occurrence especially in regions and environments with poor sanitation and water quality. From a biochemical viewpoint, acute hepatitis A virus infection is revealed by the considerably elevated serum transaminase levels and by bilirubin metabolism disorders, since it may often be clinically asymptomatic. Our paper is aimed at analyzing the evolution and significance of biochemistry tests for this type of infection in over 35-year-old patients in the Infectious Diseases Hospital of Iași, Romania (group 1 – 28 cases) as compared to younger patients (group 2 – 34 cases). The first group represents 7% of the total number of virus A hepatitis cases diagnosed in our hospital in 2015. The over 35-year-old patients came mostly from urban areas (78 vs. 25% in group 2, $p=0.0001$). The peak aminotransferases values were comparable in the two groups. The anicteric case percentage was significantly higher in the second group (44.3% vs. 3.6%, $p<0.0001$), whereas among the icteric cases, the cholestasis syndrome was more pronounced in the 1st group (peak total bilirubin values 9.1 vs. 4.1 mg/L, $p<0.0001$). The first group had more often severity markers and longer hospitalization periods (16.9 vs. 11 days, $p=0.001$). During the first month after hospital discharge, the first group included more patients with clinical symptoms or hepatocytolysis relapse (21.4 vs. 2.8%, $p=0.04$). Therefore, over 35-year-old adults make up a mere minority of the patients diagnosed with HAV in our hospital, as their clinical picture and laboratory variables are often different from the ones of pediatric patients; they distinguish themselves by a more important cholestatic syndrome, and by an extended, sometimes severe or undulating evolution. No significant differences were found between the patients in the two groups in what concerns the extent of the hepatocytolysis syndrome. Thus, these adults require a closer and prolonged medical monitoring and more intense therapy measures.

Key words: cholestase, hepatocytolysis, jaundice, transaminases

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1. Introduction

Hepatitis A is present worldwide, with incidence rates varying widely from region to region and country to country. The hepatitis A virus (HAV) is mainly enterically transmitted to previously non-immunized persons, after exposure to environmental sources such as contaminated drinking water and pit toilets or outhouses (Gibellini et al., 2017; Uzunovic-Kamberovica et al., 2005). It's incidence rate is

closely related to the social-economic conditions and to the level of development of the area where it occurs: the better these conditions (i.e. clean water supply to the population, modern sanitation systems, education, large scale education etc.), the lower this incidence rate (Gossner et al., 2015).

An excessive population density, a lack of elementary sanitary and hygiene precautions, malnutrition or improper supply of drinking water are known risk factors associated with HAV infection and

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contribute to the of epidemic spread of numerous other diseases (Giardina et al., 2016; Leach et al., 1999; Manciu et al., 2018; Uzunovic-Kamberovica et al., 2005). A low level of secondary education is one indicator of a lower socioeconomic status, which is also associated with HAV infection.

Several types of vaccine are available (since 1979) with good efficacy and safety profile (Hilleman, 1993; WHO, 2012) and have been widely used in some regions to reduce the burden of the disease (Bialek et al., 2004; Dagan et al., 2005). Beside immunization, other preventive measures, such as the improvement of hygiene or sanitary education in a defined general population, may contribute to a reduced incidence of HAV infection (Morales et al., 1992).

HAV infections are usually mild and self-limiting (Curry and Chopra, 2010), but sometimes (0.8-5% of cases) can generate severe, life-threatening forms of disease (Taylor et al., 2006). Initial clinical manifestations are frequently insufficient for the prediction of the severity of the disease to follow. Thus, the need to have more quantitative and reliable information at the beginning of the disease which can predict the outcome. Liver impairment assessment is usually done by determining particular biochemical markers, which include: the serum transaminase level (ALT, AST, GGT), total bilirubin and its (conjugated, unconjugated) fractions levels, the prothrombin index, serum protein fractions, cholesterol or glycemia. Other experimental early markers of severity have been proposed (Shin et al., 2014).

The most affected groups during epidemic events in our country are children - young patients (with a maximum incidence for the age group 5 to 9 years – 149 cases/100000 inhabitants), but also 35-year-old people (incidence rate below 20 cases/100000 inhabitants). Epidemiological investigations and research are mainly the responsibility of public health authorities. Also, the hospitals and universities from the area, can develop disease reports and statement, based on specific analyses of patient data, as a scientific support for decision making in the view of minimizing the incidence of this disease.

Considering all the above, we aimed at studying the evolution and significance of biochemistry tests for this type of infection in over 35-year-old patients hospitalized in the Infectious Diseases Hospital of Iași City, as means to provide scientific support for assessment and management of human health risks generated for exposed people to HAV, as well as for policy and decision makers in the area of infectious diseases mitigation and control.

2. Material and methods

We conducted a retrospective research based on the data in the medical records of patients diagnosed with acute virus A hepatitis (AAH) and hospitalized in the Infectious Diseases Hospital of Iași City, Romania, during 2015 (01 January - 31

December). We gathered data on the annual number of cases, demographic (age, sex, background), epidemiological and clinical (onset symptoms, jaundice) data, as well as laboratory variables (biochemical, hematological and immunological tests). We also collected data on the annual number of cases and certain demographic patient data extracted from the classical and electronic archives of the Hospital for the 1978-2015 period.

We divided the patients into two groups, in order to better keep track and compare of the patients' specific characteristics:

- Group 1: included all the over 35-year-old patients diagnosed with AAH in 2015 (28 cases)
- Group 2: included the first 34 patients aged 35 and below, and diagnosed with AAH in 2015

AAH diagnosis confirmation required the simultaneous occurrence of a significant hepatocytolysis syndrome expressed by an ALT value exceeding 1000 UI/L, the detection of anti-HAV antibodies (PR-2100 BIO-RAD-ELISA); the detection of jaundice and/or other clinical manifestations was optional. Where at least one of the following criteria was met, the disease was considered severe: total serum bilirubin > 15 mg%, Quick index < 50%, clinical symptoms such as hemorrhagic syndrome, fever, altered consciousness, leukocytosis (without other causes). The patients were followed-up 3 months after their discharge from the hospital; a more than 3-fold increase in their ALT levels or the re-appearance of the jaundice were used as criteria for disease recurrence. The database thus achieved was statistically analyzed using descriptive statistical tests: means, median, 95% confidence interval and analytical tests: chi-squared test, t-Student, by means of the Microsoft Excel and Analyze-it software. Any $p < 0.05$ was considered significant.

3. Results

Whereas historically speaking, Romania and our region saw a progressive and almost constant drop in the number of virus B hepatitis cases since 1994 (Văță et al, 2013), the same does not hold for hepatitis A, where the number of cases varied significantly, with short inter-epidemic periods of 3 years at the most. In the Infectious Diseases Hospital of Iași, Romania, the 1980-1990 decade saw epidemic peaks of almost 2000 cases/year. The 1997-2006 period witnessed a significant diminution of the prevalence of the disease, with a maximum of 1200 cases/year. Starting with 2007, we noted a marked decrease of the number of cases, from over 500 each year, to only 7 in 2007. Nevertheless, in 2014, the number of cases started to increase again rather abruptly, and went as high as 453 in 2015 (Fig. 1).

In the high or intermediate endemicity areas, AAH is a child's disease, as most adults are immunized, symptomatically or otherwise. Our findings in Iași City were similar, since only 7% of the AAH patients diagnosed in 2015 were over 35. These 28 patients were included in the 1st group. The

percentage of over 35-year-old patients varied considerably over the last 10 years – 2.3% in 2007 to 21.7% in 2013 (with a 9.5% mean), and it was higher in the years with fewer cases (Table 1).

Table 1. Main characteristics of the patients in the 2 groups

	group 1	group 2
Median age (years)	33	9
Sex ratio (M/F)	1.3	1.6
Living conditions (Urban/rural)	3.7	0.4
Average ALT (UI/l)	3401	2692
Average AST (UI/l)	2851	2144
Average total bilirubin (mg/dl)	8.8	2.2
Jaundice (%)	96	76
Severe disease (%)	21	3
Disease relapse (%)	21	6
Hospitalisation (days)	16.9	11.1

Most adults were aged 35 to 40 (53%), the oldest being 58. The 2nd group included mainly 8 to 15 year-old children (61%), with a 9 year median. The male sex prevailed in both groups (57% in the 1st group vs. 61% in the 2nd group). Most of the patients in the 2nd group had rural extraction – 71%, whereas most of the patients in the 1st group came from urban

areas – 79%. The disease occurred throughout the year in both groups, with morbidity peaks in September and November for the 2nd group and October and December for the 1st one (Fig. 2).

All patients were Romanian nationals, with no abroad travel history in the last 2 months (except 1 adult who had a short stay in Italy 2 weeks prior to disease onset). The epidemiological investigation carried out at the time of the diagnosis setting revealed contacts with other known patients in more than half (59%) of the pediatric cases (2nd group), mainly with relatives or school mates (13 brothers and sisters, 1 aunt, 4 school mates, 1 neighbor). Most of the cases in the 1st group had unknown origin (89%), the possible sources of infection being 2 grandchildren and a neighbor. Water can be an important vehicle for the transmission of HAV. 56.5% of the patients had access to the public water distribution system (71.4% in group 1 and 44.1% in group 2). Only 16.1% of the patients declared that they were drinking only bottled or boiled water (28.6% of group 1 and 5.8% of group 2). 67.7% of the patients were using indoor toilet facilities (85.7% in group 1 vs. 53.9% in group 2, p=0.006).

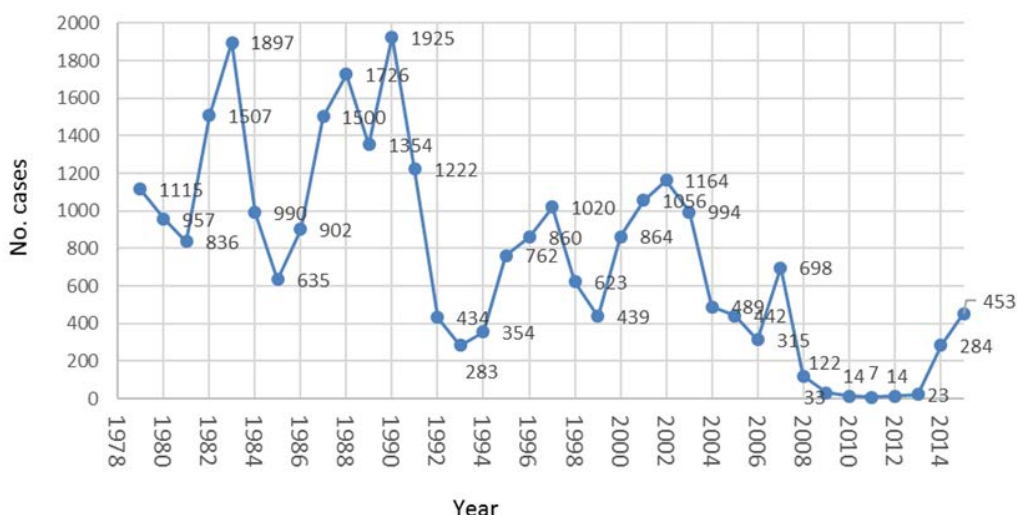


Fig. 1. Annual number of AAH cases diagnosed in the Infectious Diseases Hospital of Iași City (1978-2015)

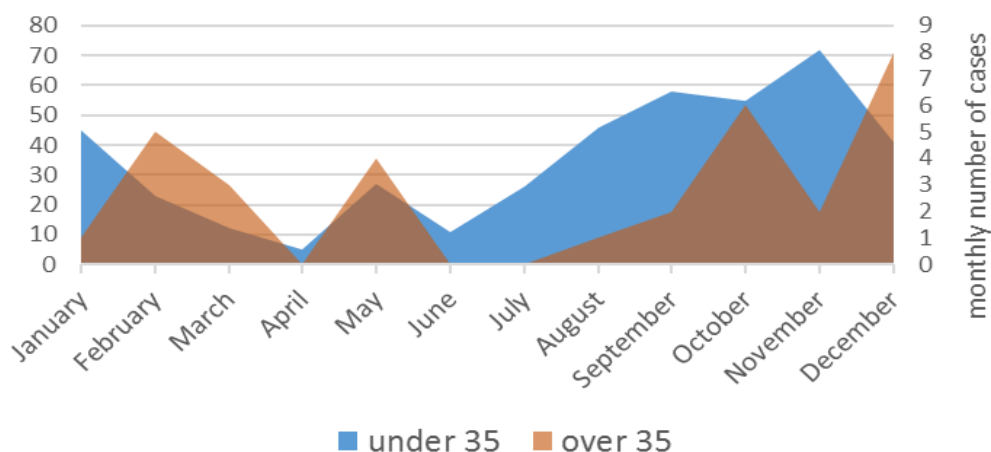


Fig. 2. Seasonal AAH diseases in the research groups

The preicteric period manifestations were similar in the two groups (Fig. 3). Children complained mainly of abdominal pain, whereas asthenia was felt more acutely by adults. This period lasted slightly longer in the 1st group: 6.4 days vs. 4.9 days in the 2nd group ($t = 1.174, p = 0.24$). Jaundice, an important clinical sign of AAH, occurred in 96% of the patients in the 1st group and in only 76% of the patients in the 2nd group. In the icteric forms of the disease, it lasted significantly longer in the adult patients in the 1st group (17.8 vs. 10 days, $p=0.0008$).

The peak ALT values reached during the hospitalization period were similar in the two groups, 3401 UI/L in the 1st group and 2692 UI/L in the 2nd group. The 2nd group showed a high percentage of patients with maximum ALT values ranging from 2000 to 3000 UI/L – 41.2% vs. 25% in the 2nd group, whereas the 1st group had a higher number of patients with values exceeding 5000 UI/L (Fig. 4). Yet, the differences were not statistically significant, probably due to the small number of cases.

The 1st group of patients included 4% of mild disease forms, 75% of moderate disease forms and 21% of severe disease forms, whereas the 2nd group had 47% of mild disease forms, 50% of moderate

disease forms and 3% of severe disease forms. In Romania patients with hepatitis A require mandatory hospitalization during the acute phase of the disease. The average hospitalization time was 16.9 days in the 1st group and 11.1 days in the 2nd group. Disease recrudescence, manifested by hepatocytolysis syndrome or cholestasis syndrome reoccurrence after a period of apparent recovery, was detected in 21% of the cases in the 1st group and in only 6% of the cases in the 2nd group.

4. Discussions

Most of the EU member states have a very low or low incidence rate of hepatitis A (below or around 2 cases/100000 inhabitants). Romania (among other 3 states) has one of the highest incidence rates for this disease - over 20 cases/100000 inhabitants (ECDC, 2016).

As of the mid 1990’s, in Romania, HAV hepatitis incidence is measured with only 2 digits, and since 2000 it has been relatively constantly decreasing. The disease incidence rate hit the bottom in 2011, with less than 20 cases/100000 inhabitants (CNSCBT, 2015).

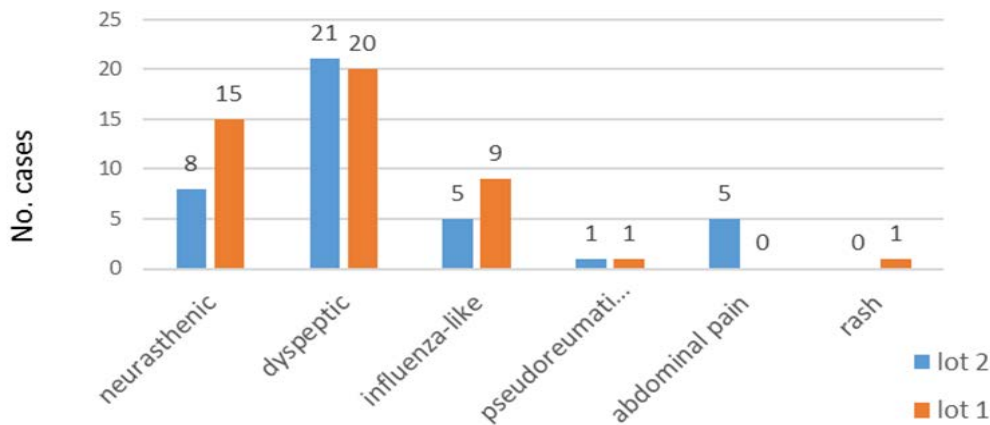


Fig. 3. Clinical syndromes in the preicteric period

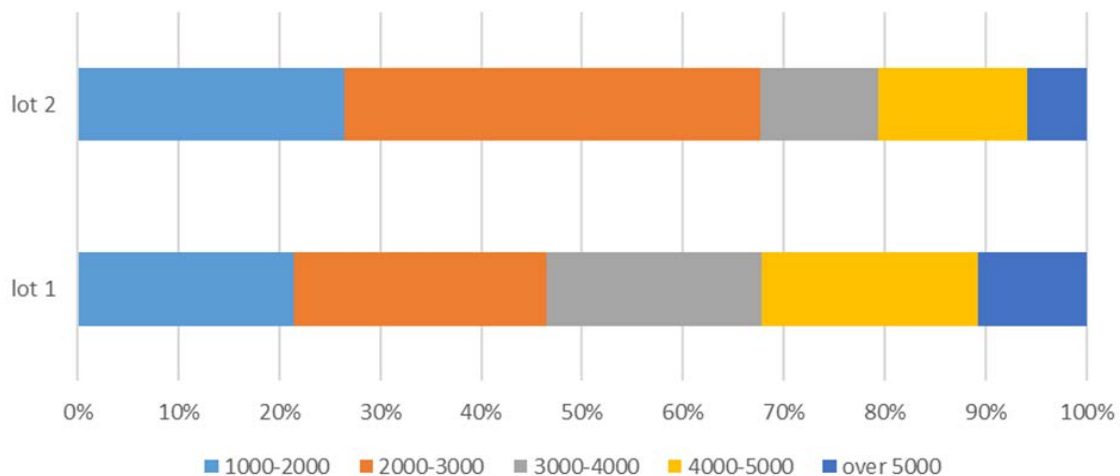


Fig. 4. Peak ALT values reached by the patients in the two groups (UI/L)

No environmental, immigration law or traveling policy changed significantly during this period in order to influence the appearance of the disease. The decrease was thought to be the consequence of better sanitation or improved education of the population, but might have been just an inter-epidemic, low-incidence interval.

Our hospital is the largest in the region and considered the referral center for the six counties in Nord-Eastern Romania. A similar rise in the incidence of HAV infections has also been reported country-wide since 2013 (CNSCBT, 2015).

It is a well-known fact that AAH is generally more frequent in the male sex (Antony and Celine, 2014; CDC, 2011; Wu et al., 2001). We noted that in both research groups (M/F ratio – 1st group: 1.33 vs. 2nd group: 1.61), without any statistically significant differences between them ($\chi^2=0.13$, $p=0.71$). AAH is usually associated with poor hygiene conditions, with difficult access to drinking water and rural environment (WHO, 2012; Wu et al., 2001) which is fairly noticeable among the 2nd group patients. The situation of the adults is completely different, with only 79% of them being of urban extraction, the difference being statistically significant ($\chi^2=14.85$, $p=0.0001$).

AAH is usually seasonal in countries with intermediate endemicity (Rakadjieva et al., 2002), with a peak in the number of cases in the autumn and winter time (Al-Naaimi et al., 2012; Fares, 2015), being associated with the beginning of the non-vaccinated children's attending schools and kindergartens. In our study, we noted such seasonality in the 2nd group (maximum number of cases in November and September), where the 1st group (adults above 35) reached the highest numbers one month later (peaks in October and December - the incubation period?) which may suggest an interrelation between the children's and adults' disease. The peak in the number of cases occurring in May in both children and adults is harder to explain.

The precise origin of the disease is hard to prove scientifically (where advanced virologic tests are necessary), yet it may be often suspected, since this is an enterically transmitted disease, which requires close contact with other patients (symptomatic or asymptomatic) or with their contaminated objects. We noted a significant difference between the two groups of patients, the origin of the infection being detected in 11% of the patients in the 1st group vs. 59% in the 2nd group ($\chi^2=15.2$, $p=0.00009$).

Jaundice in AAH is usually a sign that worries the patient and sends him/her to the doctor. The frequency of the anicteric forms was significantly higher in the 2nd group - 24 vs. 4% ($p=0.03$). The hepatocytolysis syndrome is detected during the disease regardless of the patient's age, yet it is not usually associated with disease severity or prognosis. No significant differences were found as concerns the maximum ALT value in the two groups ($p=0.09$).

Disease severity depends on jaundice intensity, prothrombin index or several clinical symptoms in the disease manifestation period. We thus noted that 97% of the cases in the 2nd group were mild or moderate, as compared to 21% in the 1st over 35-year-old patient group, the difference being statistically significant ($p=0.03$). AAH, the isolation of which in the hospital is still compulsory by law, is associated with rather long hospitalization times, with an average of 13 days over the last few years. This means, in addition to the individual suffering and discomfort, school performance decreases and material and productivity losses for active adults. Moreover, we noted that disease evolution is often slower in adults, the hospitalization mean in the 1st group being significantly higher than in the 2nd children's group 2 ($p=0.001$). Probably more than other viruses, AAH sometimes has an undulating evolution, with apparent recoveries followed by subsequent inflammatory process reoccurrence. A two-phase evolution with clinical or biochemical relapses was found in 6% of the children in the 2nd group and in 21% of the adults in the 1st group, the difference not being statistically significant ($p=0.069$), possibly also due to the small number of patients included in the groups.

The limited number of cases, the regional origin of the cases and the lack of the inclusion of healthy matched controls for the risk factor analysis are some of the limitation of the current paper. More in-depth, nation-wide studies are necessary for completely clearing these issues.

Considering the current situation, maybe our country should follow the example of other nations in which the introduction of mass vaccination campaigns against HAV has led to a dramatic decrease of the disease incidence (Bialek et al., 2004, Dagan et al., 2005). WHO recommends large-scale anti-HAV vaccination in countries with intermediate endemicity (WHO, 2012), which is also currently the case of Romania (CNSCBT, 2015).

5. Conclusions

The number of AAH cases in Iași County, Romania has increased considerably since 2013 and the phenomenon does not seem to decline. The disease affects mostly children and only a minority of adults, which often have specific demographic characteristics, a clinical picture and laboratory variables different from those of pediatric patients, and it distinguishes itself by a more marked cholestasis syndrome, and prolonged, sometimes severe or undulating, progress. These adults require a closer and prolonged medical monitoring and more intense therapeutical measures.

Keeping in mind the characteristics of AAH in adults over 35 and the recommendations of WHO for intermediate endemic regions, public health authorities should take into consideration the need for a large-scale vaccination program in our area.

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