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# OUTBREAK OF VIRAL HEPATITIS A IN VINNITSYA REGION IN WARTIME PERIOD AMONG PEDIATRIC POPULATION: CLINICAL AND LABORATORY FEATURES

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#### Summary

**Introduction.** HAV is a ubiquitous viral disease with a faecal-oral transmission mechanism, characterized by liver lesions. According to global data reposts, the frequency of HAV outbreaks all over the world had increased. From the beginning of the outbreak of HAV (Vinnytsia, Ukraine) in 2023 incidence rate of HAV was detected as 27,25 per 100,000 (in 2022-0,35 per 100,000). Having analyzed these data, an outbreak of HAV was also registered among the pediatric population (88 out of 454 cases during outbreak; 19,3 %). Incidence and evidence rates of HAV have increased, especially in regions with increased rates of military activities, furthermore, these findings have highlighted the increasing impact of HAV on the burden faced by the public health system.

**Aim.** To estimate the peculiarities of clinical, laboratory features, genotype distribution of HAV among pediatric patients of Vinnitsya region during outbreak in 2023-2024 years.

**Materials and methods.** Among 88 pediatric patients with HAV during the outbreak, 31 children were examined in the course of the study and formed the study group (average age-12,84±0,16 years). Clinical examination of all patients, laboratory and instrumental assays, ELISA, PCR-testing were performed in accordance with the standard of management. All cases of HAV (n=31; 100 %) were confirmed by ELISA and additionally PCR-testing was performed for 16,1 % (n=5) of examined. Statistical data analysis was performed using «SPSS v.22.0». The data were considered significant at p<0.05.

**Results.** During the outbreak, out of all 454 cases of HAV, 46 serum samples were tested by PCR with genome sequencing and in 78,2 % (n=36) was detected IB genotype of HAV, while in 21,8 % (n=10) – IA genotype. Among pediatric patients (n=5), IB genotype was detected in serum of 80 % (n=4) of tested, while IA – in 20 % (n=1). Clinical course of HAV had a severe course in 100 % (n=31) of study group according to laboratory indexes. Frequency of clinical symptoms was detected in the study group. The level of total bilirubin, ALT, AST among study group in the serum was estimated with clinical significance. Severe laboratory course of HAV among the study group could be explained by spreading of IB genotype of HAV during outbreak.

**Conclusions.** The outbreak of HAV with a severe clinical course in the Vinnitsya region in 2023-2024 in the population was associated with IB genotype of HAV. Among the examined paediatric patients IB genotype of HAV was detected in 80 %. The clinical course of HAV in 100 % (n=31) of the main group was severe according to laboratory parameters.

Keywords: viral hepatitis A, children, liver, clinical and laboratory features, genotypes

#### INTRODUCTION

Today the world suffers from a big amount of wars, conflicts and other military activities. In case of global conflicts outbreaks of infectious diseases were registered as result of displacement, overcrowding, healthcare collapse, destruction of critical water and sanitation infrastructure, and malnutrition [1]. These outbreaks remind us of factors such as social, cultural, political, antimicrobial, economic, and environmental elements that affect the clinical course of infectious diseases [2].

Nowadays, outbreaks of various nosological forms are reported globally during conflicts and wars, including African trypanosomiasis, Marburg fever, plague, yellow fever, poliomyelitis, malaria, cutaneous leishmaniasis, leishmaniasis, tularemia, leptospirosis, coccidioidomycosis, cholera, West Nile virus, mucormycosis, ZIKV, melioidosis, typhus [3].

Outbreaks of waterborn diseases caused by Campylobacter, Cryptosporidium, Escherichia coli, Giardia, Norovirus, Shigella, and Salmonella as well as outbreaks caused by hepatitis A virus, were registered globally [3].

According to Lee G. Y. et al., HAV was estimated as most reasonable causative agent of hepatitis worldwide. Having analyzed obtained data, it was reported that genetic analysis of HAV strains was conducted for seven genotypes including human (I-III and VII) and simian (IV-VI) groups. Additionally, HAV strains are classified into six sub-genotypes (IA, IB, IIA, IIB, IIIA, and IIIB), which are typically observed in humans [4].

This pathogen mainly could be transmitted by direct close contact and also by ingestion of contaminated food or water. Nowadays, outbreaks of HAV mostly had been registered in low-income countries with poor sanitary and sociodemograpic conditions, but have also been identified as a potential causative agent of hepatitis in outbreaks occurring during wartime [5].

According to the USA reports, over 37,000 new outbreak-related cases with more than 22,600 hospital admissions and approximately 350 HAV-related deaths were reported in the period 2016-2020. In 24 European countries, 19,947 cases of HAV were reported between January and December 2017, which is four time higher, compared with data from period between 2012 and 2015 [6]. Also, it was reported that in the USA and in European countries most cases of HAV were caused by IA genotype of the virus.

So, globally we had registered a positive tendency in increasing of total amount of HAV outbreaks, that's why it's important to analyze clinical, laboratory features and genotype distributions to perform proper clinical and epidemiological characteristics of outbreaks nowadays. Ukraine is not an exception. Before the beginning of the war in Ukraine, the incidence rate was low, but with the beginning of military activities, the incidence rates increased sharply, and therefore outbreak of HAV in the Vinnytsia region appeared and was registered.

So, incidence and evidence rates of HAV had increased, especially in regions with increased rates of military activities, furthermore, these findings have highlighted the increasing impact of HAV on the burden faced by the public health system.

#### AIM

To estimate the peculiarities of clinical, laboratory features, genotype distribution of HAV among pediatric patients of Vinnitsya region during outbreak in 2023-2024 years.

#### MATERIALS AND METHODS

According to «State institution Vinnytsia region center for diseases control and prevention of the Ministry of Health of Ukraine» reports, during the outbreak of HAV in 2023-2024 in Vinnitsya region 454 cases of HAV were confirmed and reported, 19,3 % (n=88) were registered among pediatric population. Among 88 pediatric patients, who were hospitalized in the infectious-box department of communal non-profit enterprise «Vinnytsia regional clinical children's infectious diseases hospital, Vinnytsia regional Council» with HAV during the outbreak, in the process of scientific research 31 children were included and examined (males-51,6 % (n=16), females-48,4 % (n=15); average age-12,84 $\pm$ 0,16 years), forming the study group. Clinical examinations of all patients, along with laboratory and instrumental assays, ELISA and PCR testing with genome sequencing, were performed according to standards of management. Clinical symptoms, biochemical markers of cytolytic (ALT, AST) and cholestatic (fractional bilirubin) syndromes of HAV were estimated and performed. Clinical symptoms were evaluated at the time of admission to the hospital. Laboratory assays were performed on the 1<sup>st</sup> day of admission to the hospital, on the 5<sup>th</sup> day and before discharge from the hospital (10th day after admission to the hospital). All cases of HAV (n=31; 100 %) were confirmed by ELISA by detection of anti-HAV IgM and additionally PCR-testing was performed for 16,1 % (n=5) of examined patients of the study group. All medical interventions were performed in accordance with standard operating procedures.

Statistical analysis was performed by «SPSS v.22.0» software, using the methods of descriptive statistics. Parametric data were presented in the format of mean (M) and its error (m). The clinical reliability between parametric data was assessed using the paired Student's t-test. Reliability was considered significant at p<0.05. The study was conducted in compliance with ethical standards.

#### RESULTS

According to the data of the Public health center of the MoH of Ukraine, it was determined that in Ukraine in the period 1970-1996 years, high incidence rates of HAV was registered (maximum rate in 1983 - 398,9 per 100,000), however, since 1997, the incidence rates had a downward trend (Fig. 1).

In the Vinnytsia region, the incidence rates of HAV for the period from 2013 to 2023 were higher compared to the incidence rates in Ukraine. Since the beginning of military activities in the Vinnytsia region, the incidence rate of HAV increased by 77.85 times: in 2023, it was 27.25 per 100,000 population, while compared to 2022, it was 0.35 per 100,000 (Fig. 2).



Figure 1. Incidence of HAV in Ukraine during the period of 1970-2012.



Figure 2. Incidence rates of HAV in Ukraine and Vinnytsia region for the period 2013-2023.

During the outbreak of HAV, among 454 patients which were hospitalized with HAV, among 46 of them serum samples were additionally tested by PCR with genome sequencing. In the course of the scientific study, IB genotype of HAV was detected in 78.2 % (n=36) of patients, which is atypical for the European region, while IA genotype was found in 21.8 % (n=10) of those examined. Among pediatric patients (n=5), IB genotype was detected in serum of 80 % (n=4) of tested, while IA – in 20 % (n=1).

Among 88 pediatric patients which were hospitalized with HAV in CNE «Vinnytsia regional clinical children's infectious diseases hospital, Vinnytsia regional Council», in the course of the study, 31 patients with HAV were examined. Taking into account laboratory reports, HAV in 100 % (n=31) had a severe course. Icteric clinical form of HAV was reported in 96,8 % (n=30) of examined patients, while the unicteric clinical form was observed in 3.2 % (n=1).

According to the analysis of the duration of the preicteric clinical stage of HAV among the examined patients, it was estimated that 16.1 % (n=5) had a duration of approximately 1-3 days, 61.4 % (n=19) had a duration of 4-7 days, and 22.5 % (n=7) had a duration of 8-14 days. Clinical course of HAV in preicteric stage was mainly mixed in 77,5% (n=24) of patients, dyspeptic -16,1% (n=5), flu-like in 3,2% (n=1), astenic -3,2% (n=1) of the examined patients.

Table 1

According to the clinical examination of hospitalized patients with HAV who were enrolled in scientific studies,

the frequency of the main clinical syndromes was reported (Tab. 1).

Main clinical symptoms which were registered in patients with HAV	Patients of the study group (n=31)			
	Abs.	%		
Intoxication syndrome				
Fever	30	96,7		
Malaise	26	83,7		
Anxiety	14	45,1		
Dyspeptic syndrome				
Vomiting	26	83,7		
Loss of appetite	25	80,6		
Diarrhea	5	16,1		
Abdominal syndrome				
Abdominal pain	30	96,7		
Enlargement of the liver	28	90,3		
Abdominal pain in right upper quadrant	26	83,7		
Enlargement of spleen	26	83,7		
Cholestatic syndrome				
Icretic colour of the skin	30	96,7		
Icteric colour of the sclerae	29	93,5		
Changing of the urine colour	25	80,6		
Paleness of the stool	22	70,9		
Pruritus of the skin	7	22,6		

Frequency of clinical symptoms of HAV among pediatric patients (%)

Among the examined patients of the study group, the frequency of the main clinical symptoms during the outbreak was assessed within the main clinical syndromes, such as intoxication, dyspeptic, abdominal and cholestatic.

Having analysed the data obtained, symptoms of intoxication syndrome, such as fever, were recorded in 96,7 % (n=30) of children, malaise - in 83,7 % (n=26) of patients. Vomiting was registered among 83,7 % (n=26) and loss of appetite within 80,6 % (n=25) of children of the study group.

It was noted, that enlargement of liver was reported in 90,3 % (n=28) and spleen – in 83,7 % (n=26) of the examined patients, which was not typical for classical course of HAV. Such symptoms as jaundice (96 %; n=30), icteric colour of the sclerae (93,5 %; n=29), changing of the urine colour (80,6 %; n=25) also were reported during the study.

After evaluation of clinical symptoms and syndromes among patients of the study group, laboratory reports of these children were analysed (Table 2).

Table 2

# Dynamics of laboratory indexes in patients of the study group with HAV during the outbreak in the Vinnytsia region $(M\pm m)$

Laboratory indexes	Day 1 (day of admission to hospital) (n=31)	Day 5 (from the day of admission to hospital) (n=31)	Before discharge from the hospital (n=31)
Total bilirubin, µmol/l	132,48±12,65	137,72±21,95	67,11±8,73 <sup>##,!!!</sup>
Direct bilirubin, µmol/l	46,46±4,54	45,87±7,96	25,88±4,26 <sup>#,!!</sup>
Indirect bilirubin, µmol/l	85,44±8,22	86,28±14,01	41,17±5,21 <sup>##,!!!</sup>
ALT, IU/I	2193,93±218,42	1165,33±157,65***	433,58±42,3###,!!!
AST, IU/l	1351,51±160,72	490,71±90,09***	151,31±15,65 <sup>###,!!!</sup>

Note:

\*\*\* (p1) – significant difference between indexes on the 1st and 5th day after admission to the hospital with p<0,001; # (p2) – significant difference between indexes on the 5th day after admission and before discharge from the hospital with p<0,05;

# (p3) – significant difference between indexes on the 5th day after admission and before discharge from the hospital with p<0,01;

### (p4) – significant difference between indexes on the 5th day after admission and before discharge from the hospital with p<0,001;

!! (p5) – significant difference between indexes at the time of admission and before discharge from the hospital with p<0,01; !!! (p6) – significant difference between indexes at the time of admission and before discharge from the hospital with p<0,001. During laboratory assays, the level of total bilirubin in the serum of the study group was measured as follows: (day 1: 132,48±12,65 µmol/l; day 5: 137,72±21,95 µmol/l; before discharge: 67,11±8,73 µmol/l; p6<0,001; p3<0,01). It should be noted, that during the outbreak of HAV indirect bilirubin level was significantly higher at the time of admission (p<0,001), on the 5<sup>th</sup> day (p<0,001) and before discharge from the hospital (p<0,05), compared with direct bilirubin level. The level of ALT in the serum was estimated (day 1: 2193,93±218,42 IU/l; day 5: 1165,33±157,65 IU/l; before discharge: 433,58±42,3 IU/l; p1<0,001; p4<0,001; p6<0,001). The level of AST in the serum was estimated (day 1: 1351,51±160,72 IU/l; day 5: 490,71±90,09IU/l; before discharge: 151,31±15,65 IU/l; p1<0,001; p4<0,001; p6<0,001).

According to the analysis of the obtained data, deviations in these laboratory indexes indicated a severe course of HAV and changes in the morpho-functional activity of the liver parenchyma in the examined patients. It should be noted that abnormally high levels of cytolytic syndrome markers (such as ALT and AST) and increased levels of total bilirubin (with a predominance of the indirect fraction) may be associated with the spreading of the IB genotype of the HAV virus, which was isolated from hospitalized patients during the outbreak in the Vinnytsia region in 2023-2024.

#### DISCUSSION

The HAV virus has modified its clinical course over the past four decades, both in Ukraine and in European countries, taking into account epidemiological, clinical, and laboratory features. Having analized the peculiarities of the modern course of HAV, it is necessary to modify the system of epidemiological control and prevention strategies, especially during outbreaks, on a global scale [7].

Given the peculiarities of the etiological factor of HAV during outbreaks, it is very important to assess the genomic epidemiology of HAV, which plays a crucial role in identifying and tracking the source of pathogens during outbreaks and their geographical distribution [4].

Taking into account the global genotypes distribution of HAV, it was estimated that in European countries and in the USA, the IA genotype of HAV was detected during epidemiological studies, and the clinical course of the disease has been mild. Compared to the obtained data, it was reported that the IB genotype of HAV, which was detected during the outbreak in the Vinnytsia region in 2023-2024, predominantly circulates in Spain, Jordan, Egypt, Turkey, and other countries of the Mediterranean region [8, 9].

According to the analysis of clinical and laboratory data of examined patients during the HAV outbreak, it was noted that the clinical course of HAV was severe and mostly was associated with the IB genotype of HAV, both among adults and the pediatric population. It was reported that the risk of severe and fulminant courses of HAV was associated with genotypes other than IA [10].

Having analyzed reports of the Polish authors, it should be noted that high incidence rates of HAV were also registered among the pediatric population, and the incidence rates of HAV was increased during the phase of migration of war refugees from Ukraine in recent years. Thus, population migration typically raises incidence rates of HAV, especially during wars and other emergencies globally. A similar trend of a sharp rise in HAV cases has been detected in Lebanon, mainly attributed to the influx of Syrian refugees [11, 12].

Having summarized all the data obtained during the HAV outbreak in the Vinnytsia region in 2023-2024 and compared these findings with scientific reports from other authors, we found that our results were similar to those collected in previous studies.

#### CONCLUSIONS

The outbreak of HAV with a severe clinical course in the Vinnytsia region during 2023-2024 among the population was associated with the IB genotype of HAV, in both the adult and paediatric populations, mainly adolescents.

The main clinical manifestations in patients with HAV were reported such as signs of intoxication (96.7 % of examined patients had an increase in body temperature up to 38-39 °C), abdominal (abdominal pain was registered in 96.7 % of children), and cholestatic (jaundice was reported in 96.7 % of patients) syndromes.

In 90,3 % of children, with HAV hepatomegaly was registered, in 83,7 %—splenomegaly was reported, which is not specific for the typical course of HAV.

Clinical course of HAV in all patients of the study group was severe according to evaluation of laboratory indexes.

During admission to the hospital, cholestatic syndrome was laboratory manifested in children of the study group as an increase in the level of total bilirubin (in 61,3 % of examined it was in the range of  $81-160 \mu mol/l$ ), predominantly was formed by indirect bilirubin (in 58 % of patients it was in the range of 17,2-100  $\mu mol/l$ ) and by abnormally high levels of cytolytic syndrome indexes in the form of increased levels of ALT (in 51,6 % of children it was more than 2000 U/l) and AST (in 67,7 % of patients it was more than 700 U/l) both at the time of admission to the hospital and before discharge.

**Prospects for further researches:** investigation of the relations between genotypes of HAV and clinical, laboratory, immunological course of HAV among pediatric

population with estimation of possible predictors of severe course of the disease.

## COMPLIANCE WITH ETHICAL REQUIREMENTS

The study was conducted in compliance with the main statements of the Declaration of Helsinki of 1975, revised in 2000. The manipulations and evaluation of clinical results were performed and approved by the bioethics commission of the National Pirogov Memorial Medical University, Vinnytsia, and the Communal Nonprofit Enterprise «Vinnytsia Regional Clinical Children's Infectious Diseases Hospital, Vinnytsia Regional Council.» Clinical and laboratory examinations were conducted after the patients' representatives signed the appropriate informed consent form (ICF) for medical examination and management.

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### Резюме

# СПАЛАХ ВІРУСНОГО ГЕПАТИТУ А У ВІННИЦЬКІЙ ОБЛАСТІ ПІД ЧАС ВІЙНИ СЕРЕД ДИТЯЧОГО НАСЕЛЕННЯ: КЛІНІКО-ЛАБОРАТОРНІ ОСОБЛИВОСТІ

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Вступ. ВГА – убіквітарне вірусне захворювання з фекально-оральним механізмом передачі, що характеризується ураженням печінки. Відповідно до даних глобальних звітів, частота спалахів ВГА у всьому світі зросла. З початку спалаху ВГА (м. Вінниця, Україна) у 2023 році захворюваність на ВГА становила 27,25 на 100 тис. (у 2022 році – 0,35 на 100 тис.). Проаналізувавши ці дані, спалах ВГА зареєстрований також й серед дитячого населення (88 із 454 випадків за час спалаху; 19,3 %). Рівень захворюваності та поширеності ВГА зросли, особливо в регіонах із підвищеним рівнем військових дій, також ці факти показали зростаючу роль ВГА, як обтяжливого чинника на систему охорони здоров'я.

**Мета.** Визначити клінічні, лабораторні особливості, особливості розподілу генотипів ВГА серед пацієнтів дитячого віку Вінницької області під час спалаху ВГА у 2023-2024 рр.

**Матеріали та методи.** Серед 88 пацієнтів дитячого віку з ВГА під час спалаху в процесі наукового дослідження було обстежено 31 дитину, які сформували досліджувану групу (хлопчики – 51,6 %; середній вік – 12,84±0,16 років). Всім хворим проведено клінічне обстеження, лабораторно-інструментальні дослідження, ІФА, ПЛР-тестування відповідно до стандарту лікування. Усі випадки ВГА (n=31; 100 %) підтверджено методом ІФА та додатково проведено ПЛР-тестування у 16,1 % (n=5) обстежених. Статистичну обробку даних проводили за допомогою «SPSS v.22.0». Дані вважали достовірними при p<0,05.

Результати. Під час спалаху серед усіх 454 пацієнтів із ВГА, 46 зразків сироватки крові досліджено методом ПЛР із секвенуванням геному та у 78,2 % (n=36) обстежених виявлено ІВ генотип ВГА, а у 21,8 % (n=10) – генотип ІА. Серед пацієнтів дитячого віку (n=5) генотип ІВ виявлений у сироватці крові у 80 % (n=4) обстежених, а ІА – у 20 % (n=1). У 100 % (n=31) обстежених пацієнтів за даними лабораторних показників клінічний перебіг ВГА був важким. Серед дітей досліджуваної групи виявлено частоту клінічних симптомів. Клінічно значущими визначені рівні загального білірубіну, АЛТ, АСТ у сироватці крові серед дітей досліджуваної групи. Важкий лабораторний перебіг ВГА серед обстежених дітей можна пояснити поширенням ІВ генотипу НАV під час спалаху.

**Висновки.** Спалах ВГА з тяжким клінічним перебігом у Вінницькій області у 2023-2024 роках серед населення був асоційованим з поширенням ІВ генотипу ВГА. Серед обстежених дітей ІВ генотип НАV виявлений у 80 %. Клінічний перебіг ВГА у 100 % (n=31) осіб основної групи за оцінкою лабораторних показників був тяжким.

Ключові слова: вірусний гепатит А, діти, печінка, клініко-лабораторні особливості, генотипи