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RESEARCH ARTICLE

Seroprevalence and genotype distribution of hepatitis A virus in the pre-vaccine era in South Transdanubia, Hungary (2010–2020)

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ABSTRACT

In this study, the age-related seroprevalence of hepatitis A virus (HAV) infection was investigated in the population in South-Transdanubia, Southwest Hungary (Central Europe) between years 2010 and 2020. Up to the age of 40, the HAV seropositivity was less than 18% in all age groups indicating a low level of HAV endemicity in this part of the country in the covered study period. The HAV seropositivity started to increase at the age group 41–45 years, reaching the ~50% at age group 56–60, and 75–80% at age group 66–70, respectively. A total of 43 (0.2%) of the 21,106 tested sera were HAV IgM-positive (the annual percentage range of HAV IgM-positivity was 0.046–0.6%). Total of 24 (55.8%) of the 43 HAV IgM-positive samples tested RT-PCR-positive confirmed as HAV sub-genotypes IA ($N = 17$; 70.8%) and IB ($N = 7$; 29.2%), respectively. Imported HAV infections (three cases from Romania, and one-one case from Austria and Italy), two small outbreaks and 11 cases of a genetically identical sub-genotype IA strain (GenBank number of the prototype strain: KM657825) from 2012 to 2014 were identified later connected directly to the enormous HAV outbreak initiated among men who have sex with men (MSM) at the end of 2011 in the capital Budapest.

In summary, low endemicity but high and increased susceptibility for HAV infection was found in the population in Southwest Hungary, where repeated introduction of sub-genotypes IA and IB HAV strains were identified between 2010 and 2020.

INTRODUCTION

Hepatitis A virus (HAV) is a well-known etiological agent of viral hepatitis caused by one serotype of HAV of the genus *Hepatovirus*, family *Picornaviridae* [1]. Based on the genome analysis, HAV has been classified on seven genotypes (I–VII) including four human (I, II, III and VII) groups. Most human strains belong to the genotype I, which has been divided into sub-genotypes IA and IB [2]. HAV is transmitted by faecal-oral route from human-to-human through direct contact with an infected person, contaminated food and water [3]. The geographic distribution of the infection is closely related to hygienic and sanitation standards, as well as socio-economic levels and vaccine coverage. This may mean that the seroepidemiology of HAV in a given geographic area may change over time. The HAV endemicity level for a population is defined by the results of age-seroprevalence surveys that measure the proportion of each age group that has acquired immunity to HAV, either through infection or immunization, as demonstrated by the presence of IgG anti-HAV antibodies in serum. Countries have been classified as high, intermediate, and low endemicity, defined as ≥90% of the population immune by age of 10 years, ≥50% by the age of 15 years, and ≥50% by the age of 30 years, respectively [4].

In Hungary, the HAV endemicity changed in the previous 50 years. The epidemiological changes of HAV herd-immunity have been registered first in 1969 in the country [5, 6]. Countrywide HAV seroepidemiological surveys have been performed based on serum samples taken in 1982, 1987, 1994 and 1999 from healthy people in Hungary [7]. Results

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obtained during this survey showed that the proportion of HAV seropositivity decreased from 69.25% to 18% within 17 years [7]. However, no further data are available since 1999 about the HAV seroprevalence in Hungary.

Policies for public health control are dependent on an understanding of the changing epidemiology of HAV infection. In this study, the age-specific rates of immunity to HAV in Southwest Hungary were reviewed between 2010 and 2020 supplemented with the determination of the circulating HAV genotypes.

MATERIALS AND METHODS

Collection of specimens

The seroepidemiological analysis is based on a retrospective analysis of the serological laboratory results of the HAV IgM and HAV total antibody tests between January 1, 2010, and December 31, 2020. Blood samples were originally sent by physicians (university/county hospitals and general practitioners) from patients with a history of hepatitis or without hepatitis for routine clinical virological HAV testing to the Laboratory of Virology, Department of Medical Microbiology and Immunology, University of Pécs (Pécs, Hungary) covering a population of ∑894,000 persons (9.1% of the total population of Hungary in 2017) in three counties (Baranya, Somogy and Tolna) in South Transdanubia, Southwest Hungary.

Serological methods

Serum samples were tested by ELISA method using the HAV IgM (Dia.Pro Diagnostic Bioprobes, Sesto San Giovanni, Italy) and HAV total Ab (Dia.Pro, Diagnostic Bioprobes, Sesto San Giovanni, Italy) test kits according to the manufacturer's instructions.

Molecular methods

HAV IgM-positive serum samples were tested by RT-PCR for HAV. Viral RNA was isolated by TRIzol™ LS Reagent (ThermoFisher Scientific, Waltham, MA, USA) method according to the manufacturer's instructions. Screening primers newRT/BR5 [8] were used for the detection and characterization of the viral VP1/2A genomic regions recommended common consensus sequencing region of HAVNET, a global network of HAV laboratories [9]. PCR-products were directly sequenced and run on an automated sequencer (3,500 Genetic Analyzer, Applied Biosystems/Hitachi, Hitachinaka, Japan).

RESULTS

A total of 21,106 serum samples were tested for HAV IgM and HAV total antibodies collected from three counties (Baranya, Somogy and Tolna) in South Transdanubia, Southwest Hungary between 2010 and 2020. The yearly distribution of samples were between 1,371 (in the year 2011) and 2,660 (in the year 2019) specimens (Fig. 1A); the average was 1,918 specimens. Most of the specimens originate from age groups 36–40 (8.14%) and 61–65 (11.26%) years (Fig. 1B). The fewest samples are from age groups over 86 years. Figure 1B shows the detailed distribution of the specimens in percentage by age groups (Fig. 1B). Between 2010 and 2020, there is a decreasing tendency in the percentage of average HAV antibody (Ab) positivity by year (Fig. 1C). During the study period, the highest (46.02%) average HAV antibody seropositivity was observed in the year 2011 and the lowest (26.81%) in the year 2020 (Fig. 1C). The yearly percentage of age-specific HAV antibody positivity (HAV %Ab+) shows that until the age of 40 the HAV seropositivity was less than 18% in all age groups, in all years (Fig. 1D). The seropositivity is starting to increase at the age group 41–45 years, reaching the ~50% at age group 56–60, and 75–80% at age group 66–70, respectively (Fig. 1D).

Between the years 2010 and 2020, a total of 43 (0.2%) of the 21,106 specimens tested HAV IgM-positive; the percentage range per years were: 0.046–0.6% (Fig. 2). All the 43 patients had symptomatic hepatitis. There was an HAV IgM-positive specimen in every year during the study period. The highest number of HAV IgM-positive specimens originated from the years 2013 ($N = 10$) and 2014 ($N = 8$). In the years 2011, 2012 and 2015, there was only one HAV IgM-positive sample per year (Fig. 2). The yearly distribution of the number of HAV IgM-positive serum samples and the yearly percentage of the HAV IgM-positive samples per tested total specimens correlates well to each other (Fig. 2). The average age of the HAV-infected patients was 35.9 years (between 10 and 81 years). Among the HAV-infected patients there were 77% men and 23% women.

With the confirmatory molecular test, 24 (55.8%) of the 43 HAV IgM-positive samples tested RT-PCR-positive for HAV at the time of the specimen sampling. Based on the analysis of VP1/2A nucleotide and amino acid sequences these strains represent HAV sub-genotypes IA ($N = 17$; 70.8%) and IB ($N = 7$; 29.2%), respectively. HAV strains were deposited in HAVNET [10] and GenBank databases under accession numbers OL830105-OL830127 and KM657825. The yearly distribution of the HAV RT-PCR-positive specimens with HAV genotypes were 4 (3 IA/1 IB), 1 (1 IA/0 IB), 1 (1 IA/0 IB), 7 (7 IA/0 IB), 3 (2 IA/1 IB), 1 (1 IA/0 IB), 0, 3 (1 IA/2 IB), 2 (1 IA/1 IB), 2 (0 IA/2 IB) and 0 between 2010 and 2020, respectively.

Based on the combined epidemiological and molecular data 5 imported HAV infections (three cases from Romania, and one from Austria and Italy) were found. A total of two HAV outbreaks were detected: two small clusters with 2-2 symptomatic HAV cases with sub-genotype IB strains occurred in a family in 2017 and in a bake house in 2019, respectively. In addition, a total of 11 symptomatic HAV cases, caused by a genetically identical sub-genotype IA strain (the prototype strain is KM657825) from 2012 to 2014, connected directly to the enormous HAV outbreak imported and started among men who have sex with men (MSM) at the end of 2011 in the capital (Budapest).

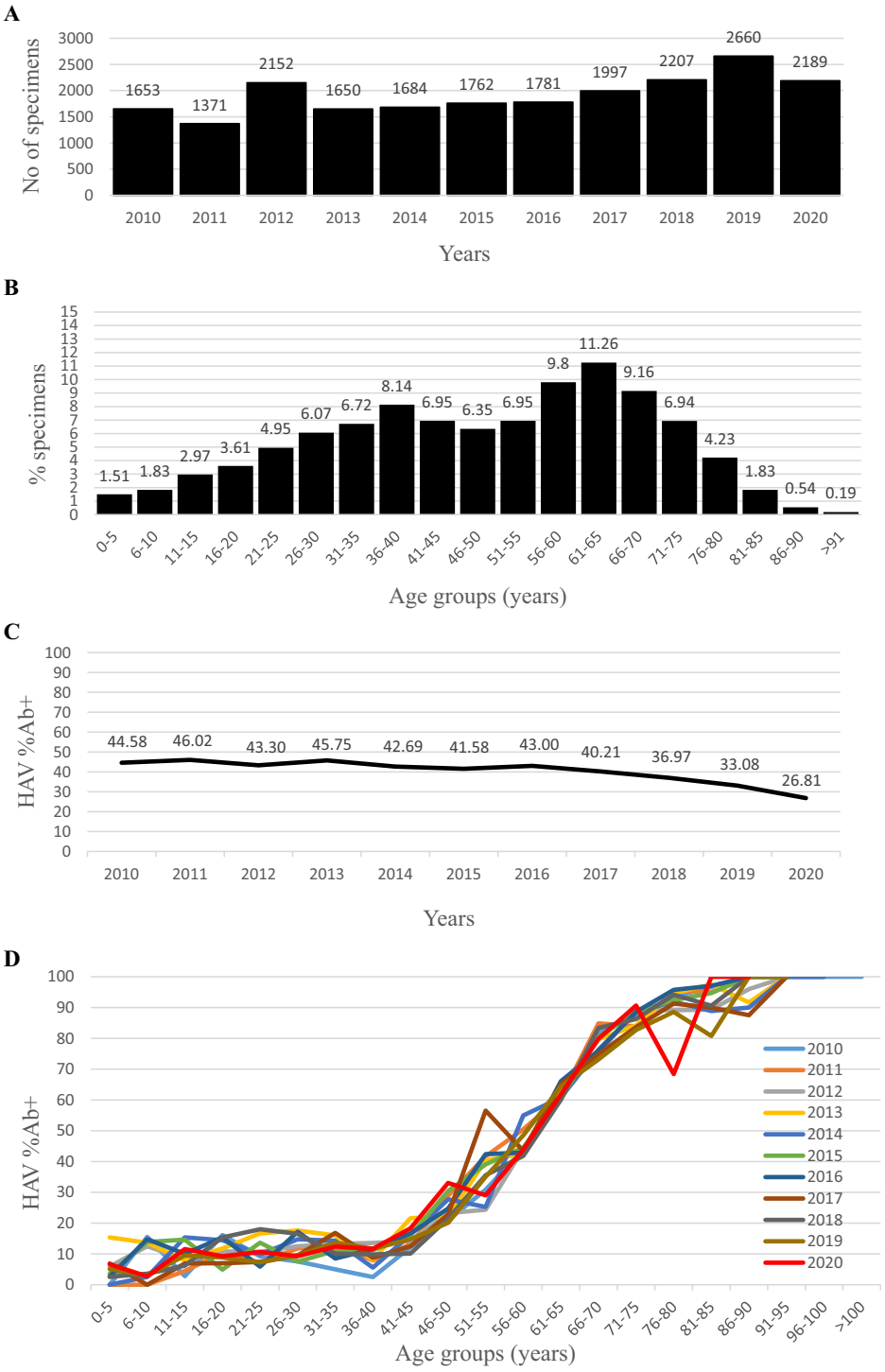

Fig. 1. **A**) Yearly distribution of serum samples tested for HAV in South-Transdanubia, Southwest Hungary between 2010 and 2020. **B**) Distribution of the specimens in percentage by age groups. **C**) Percentage (%) of average HAV antibody positivity (HAV %Ab+) of serum samples by year between 2010 and 2020. **D**) Yearly percentage (%) of age-specific HAV antibody positivity (HAV %Ab+) between 2010 and 2020 in Southwest Hungary. Each age group covers 5 years between the ages of 0 and 100 years

DISCUSSION

In this study, the seroprevalence of HAV infection was investigated in the population in South-Transdanubia, Southwest Hungary (Central Europe) between the years 2010 and 2020. Up to the age of 40, the HAV seropositivity was less than 18% in all age groups among the population that has been in contact with health care. This means that the endemicity level of HAV was constantly low in this part of the country in the covered study period.

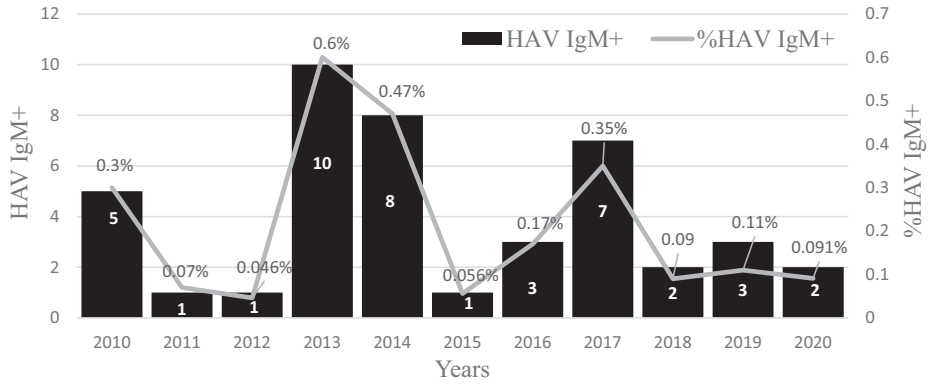

Fig. 2. Yearly distribution of the number of HAV IgM-positive (HAV IgM+) serum samples (black column) and the yearly percentage (%) of the HAV IgM-positive (%HAV IgM+) samples per tested total specimens (grey line) in South-Transdanubia, Southwest Hungary between 2010 and 2020

Only very few and old research studies are available related to the incidence, epidemiology, seroepidemiology (published more than 20 years ago), and genetic diversity of HAV and there is no study on the vaccination coverage of HAV from Hungary. The seroepidemiological survey showed that the average proportion of seropositivity significantly decreased in healthy blood donors between 1982 (69.25%) and 1999 (18%) in the country [7]. For example, the seroprevalence of HAV was found to be 29.16% at the age groups 20–29 years in 1982, but only 5.9% in 1999 [7]. In our study, the seroprevalence of this age group is higher (5.88–18.03%) in all years between 2010 and 2020. Within 10 years (between 2011 and 2020) the average HAV seropositivity decreased by nearly 20% (from 46.02% to 26.81%). This decline has accelerated since 2017. The higher age shift of HAV infections is also indicated by the fact that the mean age of acute HAV infections (patients with HAV IgM-positive serum samples) falls in the middle-aged (35.9 years).

Three previous studies reported the molecular epidemiological investigations of a total of four HAV outbreaks from years 2003/2004 [8, 11] and 2006 [12] in Hungary; two of these studies published only in Hungarian. These research studies showed the circulation of sub-genotype IA in North East Hungary (endemic HAV area) and the presence of sub-genotype IB HAV in South Transdanubia (low endemic study area) in the beginning of the 2000 [8, 11, 12]. In this study, the reported number of HAV infections were less than 10 cases per year in Southwest Hungary. Based on the present data, continuous introduction of imported HAV strains caused cases and outbreaks occurred in the investigated geographic region where both sub-genotypes IA and IB HAV were identified. The peak of the confirmed HAV cases associated with a genetically identical sub-genotype IA HAV strain was observed in the years 2013 and 2014. This HAV strain was originally imported from The Netherlands by a pre-symptomatic person (data not shown; Vennema H personal communication) and started to spread in the capital (Budapest) among the MSM population at the end of 2011 confirmed as the first documented sexually transmitted HAV infections in the country [13, 14]. From August 2012, this HAV strain affected a wider range of populations first in the capital (e.g. homeless people, social workers, students, penitentiary institute, sex workers, food-borne and transfusion-associated cases) [14, 15] and then, as the related cases show in the Southwest Hungary, caused a country-wide HAV cases and outbreaks in the subsequent years. The impact of this event on the number of reported HAV cases in the country is well illustrated by the fact that while only 82 cases of HAV were reported in 2011 (the lowest reported number since the year 1950), until then there were 331, 1,132 and 1,556 cases between 2012 and 2014, respectively.

There are some factors which lead to research bias in this study. This seroepidemiological study was conducted among people who were placed in health care and not among the general population or blood donors. Some age- and health-affected age groups are potentially overrepresented in the HAV seroepidemiology. This HAV seroprevalence study reflects the sub-national situation (Southwest Hungary) but not for the potential regional differences within the country. In addition, the HAV vaccination coverage is not known in the population, assumed to be low. HAV vaccine was first introduced in Hungary in the early 1990's but was not included in the national immunization programme for children or any other age or professional risk groups. HAV vaccine is mostly used among contacts of confirmed HAV-infected people during the epidemiological investigation (since the year 2007) [12], health-conscious travellers and in narrow group of health care workers.

In summary, the susceptible population for HAV is high and increasing in Southwest Hungary, where repeated introduction of sub-genotypes IA and IB HAV strains were identified between 2010 and 2020. HAV can be present in this population in low endemicity but cluster of HAV infections occur from time to time and the risk of epidemic circulation is increased. Knowledge of current age-specific HAV seroprevalence rates in each country and region is important in order to establish public health priorities and to adopt appropriate active vaccination policies [4].

Conflict of interest: No conflict of interest was present.

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