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Chapter

Epidemiological Aspects of Hepatitis A: Endemicity Patterns and Molecular Epidemiology

Saba Gargouri, Lamia Fki-Berrajah, Imen Ayadi, Amel Chtourou, Adnene Hammami and Héla Karray-Hakim

Abstract

Improvements in hygiene and socio-economic conditions in many parts of the world have led to an epidemiological shift in hepatitis A with a transition from high to low endemicity. Consequently, in these areas, higher proportion of symptomatic disease among adolescents resulting in large-scale community outbreaks has been described. In Tunisia, an increase in the average age at the time of infection has been reported, hence resulting in regular outbreaks, especially household and primary school epidemics. Molecular investigation of such outbreaks, based on the determination of viral genotype and genetic relatedness between hepatitis A virus (HAV) strains, is a useful tool to identify the potential source of HAV contamination but also to assess the virus molecular dynamics over time, such as the introduction of a new genotype or a specific clustering of HAV strains according to the geographical origin. In Sfax city, (Center-East of Tunisia), only HAV strains of genotype IA are circulating. In rural areas, HAV infection is still highly endemic with probably a water-borne transmission pattern. Nevertheless, the considerable genetic heterogeneity observed in urban areas highlights the changing pattern of hepatitis A epidemiology in these settings. Further molecular studies are strongly needed to better understand HAV epidemiology in Tunisia.

Keywords: hepatitis A virus, epidemiology, Tunisia, incidence, RT-PCR, phylogenetic analysis, genetic relatedness, genotype, outbreak

1. Introduction

Hepatitis A is the most common cause of acute viral hepatitis in the world. This acute necro-inflammatory process of the liver is due to a picornavirus transmitted by the fecal-oral route, which is the hepatitis A virus (HAV). The severity and the clinical outcome of hepatitis A are closely related to the age of infection, with older ages being at risk for symptomatic disease and even acute liver failure. Improvements in socioeconomic and hygienic conditions, during the two last decades, have led to a change in the epidemiology of HAV infection worldwide [1]. This change is associated with a great potential for outbreaks and an increase in the mortality rate due to HAV. Consequently, hepatitis A can currently represent a serious public health problem, especially in regions undergoing this epidemiological change. Thus, it is crucial to recognize this evolution in the HAV epidemiology,

in order to implement adequate preventive measures. Currently, seroprevalence surveys of hepatitis A, in addition to molecular investigation of HAV strains, are very useful tools to assess HAV epidemiology in a given area in the world [2].

2. Endemicity patterns of hepatitis A virus infection

HAV is a small positive-strand RNA virus that is shed in feces as naked nonenveloped virions [2]. Consequently, this virus is characterized by a high resistance in the environment and is primarily transmitted by the fecal-oral route, through direct contact with an infected person or ingestion of contaminated water or food [3].

This transmission explains the fact that the endemicity level of HAV infection, in a particular region in the world, is closely related to socioeconomic indicators and standards of hygiene and sanitation, especially access to clean drinking water. Serological prevalence surveys, based on the detection of total anti-HAV antibodies in serum samples at different ages, are the most useful tool to assess the endemicity of HAV infection. Up to date, four levels of HAV endemicity are defined according to the World Health Organization (WHO) [3]: high (≥90% by age 10 years); intermediate (≥50% by age 15 years, with <90% by age 10 years); low (≥50% by age 30 years, with <50% by age 15); and very low (<50% by age 30 years).

The clinical expression of HAV infection is highly age-dependent, ranging from asymptomatic form, frequently observed in early childhood, to fulminant hepatitis which particularly occurs in older age groups with chronic liver disease [4]. In low-income areas, including sub-Saharan Africa and South Asia, which are characterized by a high level of endemicity, HAV infection is acquired in young children, the age at which infection is often entirely asymptomatic. Thus, in these regions, the burden of hepatitis A is relatively low, and outbreaks are not common [1, 3]. By contrast, in high-income areas, including the United States, Western Europe, and Japan, which are characterized by a very low-endemicity pattern, HAV mainly circulates among specific groups at risk such as men who have sex with men, travelers in highly endemic countries, and intravenous drug users, leading to occasional relatively limited outbreaks [4]. Importantly, in many regions of the world, which are experiencing a deep change in HAV epidemiology such as some parts of Latin America, the Middle East, and Eastern Europe, large-scale community outbreaks are commonly observed. Indeed, improvements in socioeconomic status and hygienic conditions have led to an increase in average age at the time of infection, with adolescents and young adults being the predominant susceptible population, resulting in more symptomatic disease and therefore the occurrence of large epidemics in the community. These hepatitis A epidemics are often very difficult to control and represent a huge public health problem in these countries because of an increase in the incidence of severe illnesses, hospitalizations, and deaths related to this infection. These observations suggest what is known as "the epidemiological transition or shift" [1], which means that the decrease in HAV transmission rate is paradoxically associated to an increase in the incidence of symptomatic hepatitis A.

In Tunisia, HAV infection is still common, but its epidemiology is undergoing a gradual shift. Indeed, improvements in hygiene and socioeconomic conditions have led to changes in the pattern of the age-specific seroprevalence of anti-HAV antibodies; specifically, the prevalence of anti-HAV antibodies in the age group under 10 years declined from 91% in the 1980s [5] to 44% in 2001 [6]. These results suggest that HAV transmission is decreasing among younger children, leading to the occurrence of a larger number of symptomatic cases among adolescents and adults and even more frequent large outbreaks. During the years 2007–2010, community-wide outbreaks of hepatitis A have been recorded in Sfax Governorate

(center east of Tunisia), with the occurrence of severe forms [7]. The increase in the incidence of hepatitis A cases involved nearly all regions of the governorate, including urban and rural areas. During this period, well-delimited outbreaks were observed, especially household and primary school epidemics.

The annual and monthly distribution of hepatitis A cases from 2000 to 2011 showed an endemic circulation of HAV with an increase in the incidence of the disease during the fall and winter season [7]. Importantly, this distribution highlighted similar waves of large outbreaks during 2002–2005, in comparison with those of 2007–2010, suggesting the cyclical trend of HAV infection in Tunisia. The regular evolution of hepatitis A is typical of HAV epidemiological shift; the delay in the exposure to the virus has generated a huge number of susceptible adolescents and adults and significantly increased the average age at infection. As the severity of disease increases with age, this has led to outbreaks of hepatitis A [4]. Consequently, nearly all population will be immunized against HAV until growing cohorts of susceptible young people become predominant after several years, hence leading to new outbreaks.

Among the patients diagnosed during 2007–2010 [7], 35 and 33% belonged to age groups 6–10 years and 11–15 years, respectively, which confirms that susceptibility to HAV is shifting from early age to older children and even adolescents and young adults. However, this shift was more prominent in urban areas than rural ones since the mean age of patients in these regions was 14.8 and 8.5 years, respectively. Two primary school epidemics were reported in rural settings, as well as several household outbreaks. Epidemiological investigation in this study suggested that rural outbreaks may be related to a common source contamination of water. By contrast, in urban areas, the situation was quite different from that observed in rural ones, since the epidemic consisted of many sporadic small outbreaks with no epidemiological link found between HAV confirmed cases.

Indeed, in countries with HAV epidemiological transition, different endemicity patterns simultaneously exist due to differences in socioeconomic development and hygienic practices between regions [1]. Urban areas may benefit the most from improvements in sanitary conditions, especially access to improved water sources and improvements in sewage treatment methods, hence increasing the risk of large outbreaks among adolescents and adults. The heterogeneity in HAV endemicity patterns between rural and urban areas is typical of HAV epidemiological shift.

3. Molecular epidemiology of hepatitis A virus infection

HAV has a single-stranded positive-sense RNA genome of 7.5 kilobases (kb) long [2]. The viral genome has a single open reading frame (ORF), divided into three functional regions, designated P1, P2, and P3. The P1 region encodes capsid polypeptides (VP1, VP2, VP3, and a putative VP4), whereas the P2 and P3 regions encode nonstructural proteins associated with viral replication [8]. Six HAV genotypes are up to now identified; three genotypes (I, II, and III) are of human origin, and three (IV, V, and VI) are of simian origin [8]. When these genotypes are defined by sequence variation within the VP1/P2A junction, there is 15% nucleotide variation between genotypes and 7–7.5% nucleotide variation between subgenotypes. Despite genetic heterogeneity at the nucleotide level, only a single serotype of HAV exists [9].

Although HAV is primarily shed in feces, there is a strong viremic phase during infection which has allowed easy access to virus isolates and the use of molecular markers to determine their genetic relatedness [9]. Currently, molecular epidemiological investigations are widely performed and are considered as a very useful tool

for the identification of HAV transmission patterns and the potential source of the water or food-borne hepatitis A outbreaks. A molecular investigation approach is primarily based on the determination of viral genotype and the genetic relatedness between HAV strains.

Up to date, few investigations on the molecular epidemiology have been performed in Tunisia. In order to characterize HAV strains during the large outbreaks occurred in Sfax, Tunisia, during 2007–2010 [7], a molecular epidemiological study was carried out [10]. Amplification of VP1/2A region of HAV RNA by nested RT-PCR was performed on the serum samples for 159 patients with available epidemiological information [7]. HAV RNA was detected in 80.5% of cases. No relationship was documented between the positivity of HAV RNA and both age and sex ($p = 0.179$ and 0.553, respectively). For HAV RNA-negative cases, the mean delay between onset of symptoms and sampling was 25.5 days (range, 5–47 days), whereas for HAV RNA-positive cases, this delay was significantly lower, with a mean of 10.2 days (range, 1–49 days, $p < 0.001$). These findings suggest, as previously described, that the positivity of HAV RNA is correlated to the sampling time [11–13]. This result can be explained by the short duration of viremia, during natural history of HAV infection. Indeed, viral RNA could be detected on an average of 18 ± 14 days following the onset of clinical symptoms [14]. Another reason for the negativity of HAV RNA is the storage conditions of serum samples (the optimal temperature for the storage of RNAs is −80°C), leading to the degradation of the viral RNA and consequently to the negativity of PCR.

Nucleotide sequencing was performed for positive samples by RT-PCR. Strain genotyping was carried out by the phylogenetic analysis of a 394-nucleotide fragment, encompassing the VP1/2A junction (from nucleotide 2896 to nucleotide 3289, according to HAV strain HM175). Phylogenetic tree was constructed with MEGA software version 6.05, by using Kimura's two-parameter model, with the neighbor-joining algorithm (**Figure 1**). The reliability of the tree was tested by bootstrap resampling of 1000 replications. Nine reference sequences were included in the phylogenetic tree. Nucleotide identity percentages were computed, using the p-distance model included in the MEGA software (**Table 1**).

All clinical HAV strains from different regions of Sfax Governorate belonged to genotype IA [10]. This result is in an agreement with those of other Tunisian studies, which demonstrated that the predominant genotype still continues to be IA [15–17]. In addition, HAV sequences were closer to GBM reference strain (isolated in Germany) than to Asian sequences, suggesting a close genetic relatedness with HAV strains isolated in Mediterranean countries. This concept of related HAV strains according to geographical origin has been previously mentioned in China [18]. Indeed, Asian HAV strains were closer to each other than to the other reported sequences in the United States and Germany.

The mean identity percentage between HAV sequences was 98.1% indicating that clinical HAV strains isolated during 2007–2010 outbreaks were closely related, which confirms the endemic circulation of HAV in Sfax. Nevertheless, phylogenetic analysis evidenced the presence of genetic heterogeneity among HAV strains and identified three different clusters; rural strains clustered together with high bootstrap value (regardless of the outbreak period), suggesting the highly endemic circulation of the same HAV strains in these settings. This close genetic relatedness is most likely related to a common source of contamination [18–20]. Interestingly, the majority of HAV strains isolated during school epidemics shared 100% sequence identity. Of note, the abrupt increase in the number of jaundiced persons, in rural schools, has occurred within a short period of time in two geographically distant settings (Sidi Abdelkefi and Menzel Chaker (Bir Mallouli)). This transmission pattern strongly suggests the presence of a single source for school outbreaks [8].

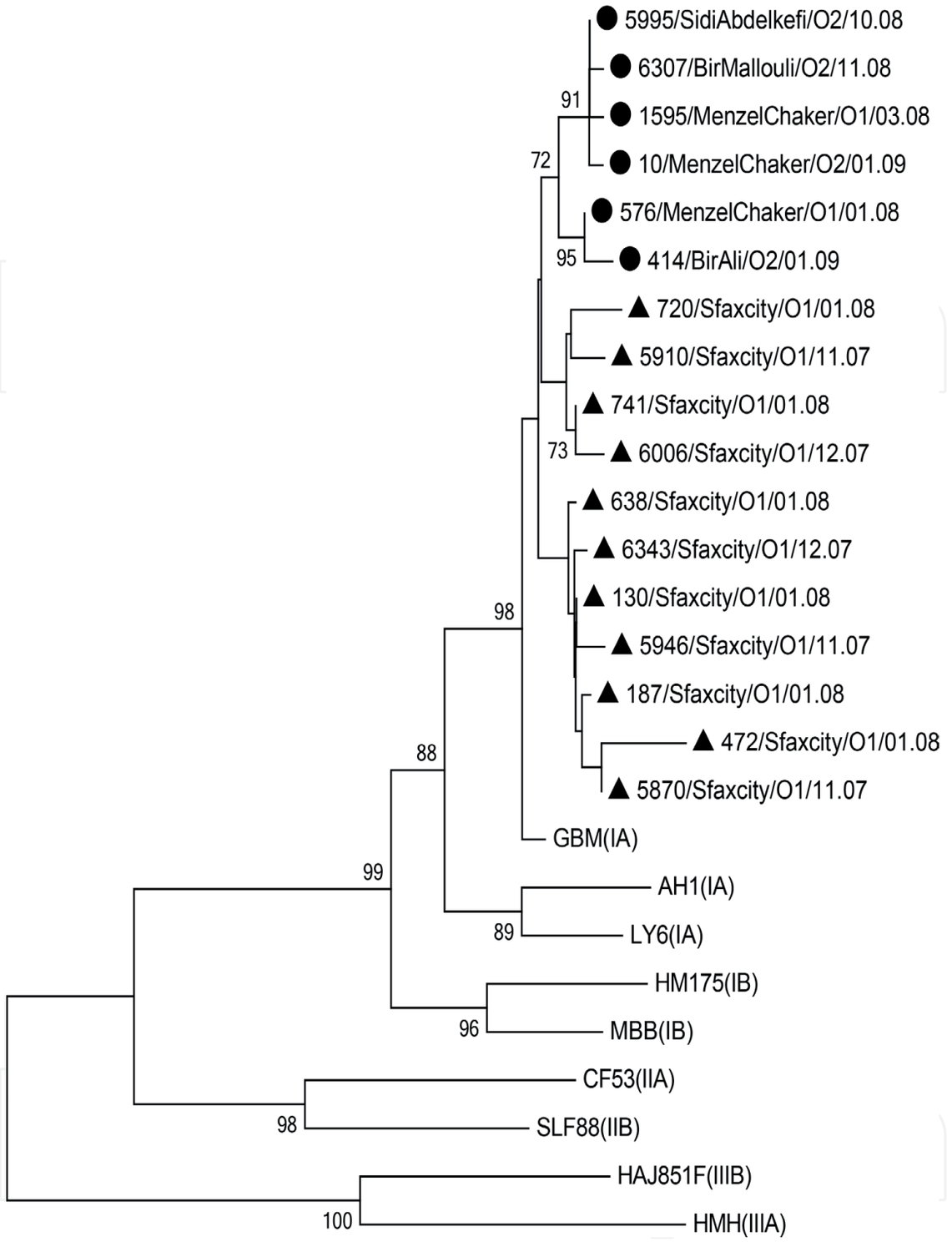

Figure 1.
Phylogenetic tree analysis of VP1/2A junction (394 bp) of HAV genome using Kimura's two-parameter model with the neighbor-joining algorithm. Numbers at tree nodes show bootstrap percentages obtained from 1000 resamplings. Bootstrap values <70% are hidden. Bars indicate genetic distances. Genotypes are shown in parentheses for HAV reference strains (see ***Table 1****). The Tunisian sequences are designated by their lab code followed by their region of origin, outbreak period (O, outbreak), and month and year of isolation. Sequences marked with a black circle indicate HAV strains recovered from rural areas, whereas those marked with a black triangle indicate HAV strains recovered from urban areas. For strains sharing 100% sequence identity, only one representative strain was included in the phylogenetic tree.*

Indeed, the epidemiological investigation highlighted the use by inhabitants of the same source of drinking water (private well) [7]. Inadequate sanitation, evidenced by fecal contamination of drinking water, in addition to the poor hygiene conditions indicates that the mainly route of HAV contamination is water transmission.

	Clinical sequences	
	% Nucleotide identity[a]	(Mean ± SD)[b]
Reference sequences		
GBM (IA)	96.9–98.7	
AH1 (IA)	92.8–94.3	(95.3 ± 0.7)
LY6 (IA)	93.3–94.9	
HM175 (IB)	90.7–92.3	
MBB (IB)	91.5–93.3	(92.3 ± 1.1)
CF53 (IIA)	84.3–86.1	(85.5 ± 1.5)
SLF88 (IIB)	84.3–86.4	
HMH (IIIA)	78.1–80.5	(80.3 ± 1.8)
HAJ85-1F (IIIB)	79.4–81.5	

[a]*Computed using the p-distance model included in the MEGA software.*
[b]*SD: Standard Deviation.*

Table 1.
Nucleotide identity between clinical and reference HAV strains.

Nevertheless, in these rural settings, interhuman transmission of HAV may also play a major role in the endemic circulation of the virus. In closed institutions such as schools, the agglomeration of individuals, sharing of objects, inadequate hygienic conditions, and high proportion of individuals susceptible to hepatitis A facilitate transmission [8].

HAV strains isolated in urban areas showed more genetic variability, since they were grouped into two different clusters, suggesting that urban outbreak may have originated from more than one source. In Sfax city, the epidemic consisted of many sporadic small outbreaks, which made it difficult to carry out a field investigation; thus, the information got from patients might not represent the whole situation in this large area. The genetic diversity of HAV strains was also reported in other regions in the world, where hepatitis A outbreaks observed in urban settings of industrialized countries cannot be linked to one source of contamination [18, 21]. The changing epidemiological pattern in HAV infection throughout Tunisia, particularly in urban areas, may result in more clinical cases in adolescents and adults and greater potential for new outbreaks. This changing pattern seems to be mainly related to improvements in hygiene conditions, since this study confirmed that genotype IA is widely circulating in Tunisia [10]. Thus, urban outbreaks are not linked to the other genotype emerging strains as it was reported by a Korean study [22], which showed that genotype IIIA becomes more prevalent than previously reported and may be the reason for the HAV outbreaks reported in Korea. Nevertheless, the higher genetic variability among HAV strains isolated in urban areas compared to rural ones in Sfax needs to be more evaluated by further molecular studies, in order to increase the understanding of hepatitis A epidemiology in these particular regions.

During urban outbreak, two fulminant hepatitis A cases were reported with fatal outcome, in one case due to an acute liver failure [7]. Unfortunately, only HAV strain from one patient was isolated. Since the time course between onset of symptoms and blood sample collection was very long (47 days), this could possibly explain the disappearance of viremia. It was previously suggested that viral determinants, in addition to host factors, could be involved in HAV disease severity, especially 5'UTR and 2B and 2C nucleotide substitutions [23, 24]. However, no correlation was found between HAV genotype and the different clinical outcomes [25].

Indeed, HAV strain recovered from one patient had 100% sequence identity with two other strains from patients with self-limited acute hepatitis A, indicating that infection with identical HAV strains within VP1/2A junction can result in drastically different clinical outcomes [8].

4. Conclusions

The findings of HAV molecular epidemiology study carried out in both rural and urban settings during large outbreaks in Sfax, Tunisia, in 2007–2010 strongly suggest that HAV infection is still highly endemic in rural settings, mainly related to the use of untreated water from contaminated sources, in addition to person-to-person transmission. However, genetic HAV diversity reported in urban areas, in comparison with rural ones, may reflect the epidemiological shift in these settings. Therefore, a close monitor of molecular HAV epidemiology is needed for a better understanding of HAV epidemiology in Tunisia.

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Conflict of interest

The authors declare no conflict of interest.

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