Chapter from the book *Viral Hepatitis - Selected Issues of Pathogenesis and Diagnostics*

Hepatitis A: Clinical, Epidemiological and Molecular Characteristics

Zahid Hussain
Centre of Excellence in Biotechnology Research, King Saud University, Riyadh, Saudi Arabia

1. Introduction

Hepatitis A virus (HAV) is a member of the Hepatovirus genus of Picornaviridae family. HAV is a non-enveloped (naked), linear, single stranded RNA virus of an icosahedral symmetry measuring 27-32 nm in diameter (Feinstone, 1973). The infectious particle consists of capsid protein and RNA genome (Fig. 1). The buoyant density of the mature particle is 1.33g/cm$^3$ in CsCl solutions and the sedimentation coefficient is 160S in sucrose solutions (Ticehurst, 1983).

HAV causes an acute self limited illness. It does not lead to chronic hepatitis or a carrier state and only rarely leads to fulminant hepatic failure. HAV interferes with liver function and sparks an immune response that leads to liver inflammation (Koff, 1998). Natural infection with virus results from ingestion of fecally contaminated food and water. Virions apparently reach the liver through blood or systemic circulation and are taken up by hepatocytes (Siegl, 1988). The virus in the liver is recognized by receptor sites on the hepatocyte membrane and engulfed by the cell (Fig. 2) (Anderson, 1988). Inside the cell the virus uncoats, releases viral RNA and begins transcription (Teixeira, 1982). Once HAV completes replication in the liver, it excretes in bile and finally shed in stool.

Like all picornaviral genomes, HAV is divided into three parts: (i) 5’ non-coding region (NCR) that comprises approximately 10% of the genome (ii) single open reading frame...
(ORF) of 2227 amino acids, that encode all the viral proteins, with regions designated as P1 for capsid proteins, P2 and P3 for non-structural proteins and (iii) short 3' non-coding region (Fig. 3). HAV RNA genomes lack the cap assembly found at the 5' end of mRNA species that normally guides the ribosomal complex to the translation start site (Najarian, 1985). Instead, an internal ribosome entry site (IRES) formed by the 5'NCR functions to initiate translations in HAV including other picornaviruses (Borman, 1997; Totsuka, 1999). However, unlike other picornavirus IRESes, the HAV IRES requires an intact eukaryotic initiation factor 4G for its optimal activity (Totsuka, 1999). Several other host proteins are found to be associated with synthetic RNAs representing segments of the 5' NCR (Chang, 1993). The viral capsid protein (P1) is further divided into VP4, VP2, VP3 and VP1 regions. The non-structural P2 and P3 polyproteins are divided into 2A, 2B, 2C and 3A, 3B, 3C, 3D respectively (Fig. 3). HAV polyprotein is processed into precursor intermediates and mature proteins by the proteolytic activities of encoded viral proteins. HAV 2A, 2B, 2C protein encodes 45, 251 and 335 amino acids respectively. The 2A and 3C are identified as

Fig. 2. Diagrammatic representation of life cycle and replicative phase of hepatitis A virus. (Adapted from: Anderson, 1988)

Fig. 3. Genomic structure of hepatitis A virus. HAV genome is divided into a 5' non-coding region (5' NCR), a giant open reading frame, and a non-coding region (3' NCR). The coding region is subdivided into regions P1, P2 and P3. (Adapted from: Totsuka and Moritsugu, 1999)
processing enzyme in hepatitis A virus. The translated 2A regions function as intermediary, partially located on the surface (VP1) and some are assembled inside the virion (Totsuka, 1999). Both 2B and 2C proteins play an important role in the replication of the viral RNA. P3 polyproteins encodes 3A, 3B, 3C and 3D proteins with 74, 23, 219 and 489 amino acids respectively. 3C protein acts as sole protease for HAV protein processing, while 3D is the RNA dependent RNA polymerase (Schultheiss, 1994).

2. Clinical and biochemical features

Persistent infection of HAV takes four clinical phases. First phase is incubation period that varies from 15-45 days (mean 30 days) (Ciocca, 2000). HAV excretion in the faeces continued for 1-2 weeks before the onset of illness and at least 1 week afterward. The prodromal period corresponds to second phase and characterized by nonspecific symptoms followed by gastrointestinal symptoms such as nausea (loss of appetite), fatigue, abdominal pain, malaise, anorexia, fever, vomiting and flu like complaints (Lemon, 1997). These symptoms are usually short lived and followed by complete recovery. Third stage is mostly characterized by increase in bilirubin level. Jaundice becomes clinically apparent when the total bilirubin exceeds 2.0-4.0 mg/dL (Fig. 4). In half of the hepatitis A patients clinical signs such as hepatomegaly and hepatic tenderness are prominent. The final phase is a convalescent period during which the patient recovers. Signs and symptoms usually lasts for less than 2 months, although 10-15 percent of symptomatic persons have prolonged or relapsing illness lasting up to 6 months (Hussain, 2005). The increase of serum aminotransferases, bilirubin (both total and direct), and alkaline phosphatase is the most striking laboratory findings of hepatitis A. In most of the hepatitis A cases level of serum aminotransferases increases mildly, but in severe cases it may be elevated to significantly high level that ranges from 1,000-1,500 IU/liter (Hussain, 2005).

2.1 Diagnostic features

Hepatitis A cannot be differentiated from other types of viral hepatitis on the basis of clinical or epidemiologic features alone. Diagnosis of acute hepatitis A is based upon the detection of anti-HAV IgM antibodies or presence of HAV RNA in serum or faeces. In the majority of persons, serum anti-HAV IgM becomes detectable 5-10 days before onset of symptoms. IgM antibodies are detectable soon after infection and can remain detectable for about 6 months (Fig. 4). The anti-HAV IgG appears early in the course of infection and remain detectable for the person's lifetime and provides lifelong protection against the disease. Total anti-HAV testing is used in epidemiologic studies to measure the prevalence of previous infection or by clinicians to determine whether a person with an indication for pre-exposure prophylaxis is already immune. HAV RNA can be detected in the blood and stool of the majority of persons during the acute phase of infection by using nucleic acid amplification methods, and sequencing is used to determine the relatedness of HAV isolates for epidemiologic investigations. HAV RNA can also be detected in blood during the incubation period, acute phase, and 18-30 days after the onset of illness (Kwon, 2000; Hussain, 2006). However recent study suggests presence of HAV RNA for an average of 95 days and viremia persisted longer after the onsets of symptoms (average, 79 days) (Bower, 2000; Normann, 2004).
3. Epidemiological characteristics

Hepatitis A is an enterically transmitted viral infection of public health problem all over the world. Faecally contaminated food and water is the common source of HAV infection. Consumption of contaminated food is a leading cause of large number of outbreaks in the past that affect hundreds and thousands of people (Koff, 1998). The source of most reported foodborne hepatitis A outbreaks has been HAV-infected food handlers. Waterborne transmission predominates in developing countries and is responsible for infection at early age. Therefore, it is responsible for endemicity rather than clinical outbreaks. In contrast, waterborne transmission in developed countries accounts for a very small proportion of HAV infections. Ninety percent of infections in children are subclinical or asymptomatic whereas exposure of adults and adolescents mainly leads to clinical form. Asymptomatic or unrecognized infections in children play an important role in HAV transmission and serve as a primary source of infections to others. The special groups of adult population such as men who have sex with men (MSM) or intravenous drug users (IVDUs) sustained the risk of HAV infection (Cotter, 2003; Vong, 2005). The blood borne transmission is also responsible for number of outbreaks of hepatitis A (Peerlinck, 1998; Ridolfo, 2000).

HAV Infection is hyperendemic in vast areas of the world, with approximately 1.5 million clinical cases per year (Fig. 5) (WHO, 2000). The worldwide distribution is uneven and is based on determinants such as socioeconomic conditions and geographic factors (Craig, 2004; Wasley, 2006; Jacobsen, 2010). In developing countries, the incidence of disease in adults is relatively low because of exposure to the virus in childhood. Most adults in these areas show prevalence of antibodies against hepatitis A. In developed world endemicity is usually very low and clinical cases occur almost exclusively in adults (Feinstone, 1996; Marinho, 1997). The variable age distribution among hepatitis A patients in developing and developed countries is a consequence of differing standards of hygiene and sanitation. In many developing countries, improved hygiene standards and socio-economic conditions have led to a reduction in exposure to HAV in childhood and hence large non-immune
adult population in the community. This leads to a shift or transition from asymptomatic childhood infections to an increased incidence of symptomatic or clinical disease in adults (Hussain, 2006). The persistence of circulating HAV may lead to hepatitis A outbreaks in susceptible non-immune adult population (Arankalle, 2001; Hussain, 2006).

Fig. 5. The global prevalence of hepatitis A virus infection based on presence of anti-bodies to hepatitis A virus i.e. anti-HAV. (Adapted from: Wasley, 2006)

4. Molecular epidemiological characteristics

Genetic heterogeneity of hepatitis A has been revealed by sequencing different genome regions, including VP3 carboxyl terminus, the VP1 amino terminus and the VP1/2A junction (Cohen, 1987; Arauz-Ruiz, 2001; Costa-Mattioli, 2002) (fig. 6). The VP3 C-terminal region is relatively conserved, the VP1 amino acid terminus presents an intermediate variability, while VP1/2A junction is more variable and is used to distinguish one strain from another (Costa-Mattioli, 2002). The genetic variability observed within the putative VP1/2A junction (168 nucleotides) initially defined seven (I-VII) genotypes (Khanna, 1992; Robertson, 1992; Ching, 2002). However, recently new classification of HAV has been done based on the complete sequences of the 900 nucleotides of VP1 region (Costa-Mattioli, 2002) (Fig. 6). The phylogenetic analyses of VP1 sequences identified six genotypes (I-VI) that differ among themselves 15-25%. Three isolated from humans (I-III) and three from a simian origin (IV-VI). The genotypes I, II and III were further subdivided into subgenotypes A and B, which differ in approximately 7.5% of base positions. The worldwide genotype distribution showed genotype I and III comprise the vast majority of human strains within the studied population (Fig. 7). Sub-genotype IA comprises the majority of the human strains studied and constitutes major virus population in North and South America, China, Japan, Russia and Thailand. The sub-genotype IB contains strains from Jordan, North Africa, Australia, Europe, Japan and South America. Most of the remaining human HAV strains segregate into genotype III that is further divided into two sub-genotypes, IIIA, and IIIB (Cohen, 1987; Jansen, 1990; Robertson, 1992). The sub-genotype IIIA have been subsequently identified in specimens collected from humans with hepatitis A in India, Sri

Fig. 6. The genomic organization of VP3 C-terminal, the VP1 amino acid terminal and VP1/2A junction region of hepatitis A virus. The complete sequence of the 900 nucleotides of the VP1 gene has been used for new classification of HAV. (Adapted from: Costa-Mattioli, 2003)

In contrast, genotype II has rarely been reported worldwide. Recently, former genotype VII (SLF 88 isolate) has been reclassified within the genotype IIB (Costa-Mattioli, 2002; Lu, 2004) (Fig. 8 & 9). Similarly, sub-genotype II (CF-53/Berne isolate) has been defined as IIA in new HAV classification (Fig. 8 & 9).
Fig. 8. Neighbour joining phylogenetic tree of the VP1/P2A region using the two-parameter model of Kimura. Genotypes and sub-genotypes are shown along with strains name. The strains (SLF-88 and 9F94) in bold has close genetic relationship and hence in latest nomenclature genotype VII has been reclassified in genotype IIB. (Adapted from: Costa-Mattioli, 2002)

The three simians genotypes were defined by unique nucleotide sequences from the P1 regions of HAV strains. In addition, all simian HAVs have a distinct signature sequence at the VP3/VP1 junction which distinguishes these strains from human HAVs.

Fig. 9. The phylogenetic tree based upon the nucleotide sequence of the structural proteins showing 3 different genotypes of human isolates and 2 simian isolates. (Adapted from: Costa-Mattioli, 2002)
4.1 Application of molecular technique in deciphering outbreaks
Molecular techniques provide an important tool in decoding the epidemiological outbreaks of hepatitis A. These techniques have been successfully applied for the detection numerous HAV outbreaks linked to various sources such as clinical specimens and environmental samples such as faecally contaminated water, food (Hutin, 1999). There are several examples where molecular techniques such as RT-PCR and sequencing have been used effectively in outbreaks to definitely relate the source of infection (De Serres, 1999; Sanchez, 2002; Chironna, 2004; Arankalle, 2006; Tallon, 2008). The molecular epidemiological techniques also helped to report several outbreaks in major European cities among men who have sex with men (MSM) (Reintjes, 1999; Bell, 2001; Stene-Johansen, 2002) as well as in intravenous drug users (IVDUs) (Davidkin, 2007).

4.2 Mode of evolution in HAV
HAV replicates as complex dynamic mutant distributions and quasispecies. The evolutionary study of HAV over time also suggests continuous generation of variants or quasispecies that coexist in time and in different environment (Costa-Mattioli, 2003; Sanchez, 2003a). These findings suggest that beyond mutations and genetic recombination, HAV exploits variation strategy in dominance to promote and ensure its survival. Despite some degree of nucleotide heterogeneity at the capsid region of HAV, there is not an equivalent degree of amino acid variation (Aragonès, 2008; Pérez-Sautu, 2011). Severe structural constraints in the hepatitis A virus capsid prevent more extensive substitutions necessary for the emergence of new serotype, and hence existence of single serotype of human HAV (Bosch, 1998).

5. Complications related to hepatitis A
Fulminant hepatitis A is a rare complication, with reported incidence of 0.015 to 0.9% of overall cases worldwide (O’Gardy, 1993). The fulminant hepatitis A is occasional phenomenon during which more expensive necrosis of the liver occurs that follows impairment of hepatic synthetic processes, excretory functions and detoxifying mechanism. It occurs during the first 4-6 weeks of illness which is characterized by sudden onset of high fever, marked abdominal pain, vomiting and jaundice followed by development of hepatic encephalopathy associated with deep coma and seizures (O’Gardy, 1989; Takahashi, 1991). Mortality is highly correlated with increasing age, survival being rare over the age 45 years (Acharya, 1996). Clinical signs indicating liver failure include a rapid decrease in size of the liver, prolongation of the prothrombin time and decrease in the aminotransferase level as the bilirubin level continues to rise (Evangelos, 1989; Vento, 1998).

5.1 Mortality rate
The vast majority of hepatitis A patients make a full recovery and fatality rate is low. The estimated mortality rate is 0.1% for children less than 15 years old, 0.3% for adults ages 15 to 39, and 2.1% for adults ages 40 and old (Hollinger, 1996; Debray, 1997). The acute HAV super infection with chronic liver disease is also associated with severity and high mortality (Keffe, 1995; Vento, 1998).
5.2 Treatment
Specific treatment is not available for acute hepatitis A virus infection. As in most cases the infection is self-limiting and is followed by complete recovery without chronic sequelae, and no specific interventions are required. Patients with hepatic failure are highly recommended to transfer to centre capable of performing liver transplants.

6. HAV control and prevention strategies
HAV control and prevention strategies vary, depending on the country. The current WHO position paper (2000) includes specific positions for countries, depending on their HAV endemicity. In highly endemic countries, almost all persons are asymptotically infected with HAV in childhood, which effectively prevents clinical hepatitis A in adolescents and adults. In these countries, large-scale vaccination program are not recommended. In countries of intermediate endemicity where a relatively large proportion of the adult population is susceptible to HAV, and where hepatitis A represents a significant public health burden, large-scale childhood vaccination may be considered as a supplement to health education and improved sanitation. In regions of low endemicity, vaccination against hepatitis A is indicated for individuals with increased risk of contracting the infection, such as travelers to areas of intermediate or high endemicity. Other high-risk persons include MSM and IVDUs communities (Francis, 1984; Widell, 1983). Persons with underlying chronic liver disease from any cause, particularly if they are older than 45-50, are at increased risk of fulminant hepatitis A and should be immunized. Immunization of foodhandlers could prevent common source outbreaks of hepatitis, but the cost effectiveness of such a strategy is not known (Lemon and Shapiro, 1994).

6.1 Vaccine and immune response
HAV vaccines from various manufacturers are widely available in the market throughout the world. These vaccines have tremendous response with observed anti-antibody among children (91-100%) and young healthy adults (96%) which persists for up to 15-20 years after completion of vaccination schedule (WHO, 2000; Hammitt, 2008).

7. Conclusion
HAV remain an important cause of hepatitis outbreak and is a major public health problem worldwide especially in developing countries. It is mostly reported from poor sanitary and unhygienic surroundings, which emphasizes the need for improving the public health measures to prevent epidemics of hepatitis A. The changes in epidemiological pattern would increase the disease burden, may cause large community outbreaks and lead to increased healthcare cost. The emergence of new serotype is highly unlikely, although new variants can emerge if virus population is forced to severe immune selective pressure. Since hepatitis A exists as a single serotype and human is the only host, it is possible to eradicate by selective vaccination against individuals who are susceptible and sero-negative for HAV-IgM.
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9. References


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There are a lot of important issues related to viral hepatitis studies: molecular biology of viruses, laboratory diagnostics, epidemiology, treatment etc. However, there is a number of special textbooks and monographs on the subject. Considering this fact and rather fast progress in our understanding of the problem this book focuses on the important sections of the problem immune pathogenesis of parenterally transmitted viral hepatitis and some aspects of hepatitis diagnostics. Seven chapters were prepared by several groups of researchers to share information and results of studies with specialists working in the field and persons who are interested to learn about the viral hepatitis issue. The Nobel Prize Committee (the field of physiology and medicine, 2011) awarded Bruce A. Beutler and Jules A. Hoffmann for their discoveries concerning the activation of innate immunity whilst Ralph M. Steinman was awarded for his discovery of the dendritic cell and its role in adaptive immunity. We are proud to say that our book is in line with these discoveries, because 3 chapters cover the problems of innate and adaptive immune response in case of viral hepatitis.

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