Iranian J Publ Health, Vol. 37, No.4, 2008, pp.126-130

Original Article

Prevalence of HAV among Healthy Blood Donors Referring to Tehran Transfusion Center

*A Elikaei [1, 2], *Z Sharifi [1], M Mahmoudian Shooshtari [1], M Hosseini [2], Y Maroufi [1]*

[1]Research Center of Iranian Blood Transfusion Organization (IBTO), Tehran, Iran
[2] Dept. of Microbiology, Faculty of Biological Sciences, Shahid Beheshti University, Tehran, Iran

(Received 3 May 2008; accepted 5 Oct 2008)

Abstract

Background: Hepatitis A virus (HAV) is a single strand and non-enveloped RNA virus. It is usually transmitted by the fecal-oral and the blood transfusion routes and causes the hepatitis A disease. Clinically, the hepatitis A is usually mild, particularly in children, in whom it is frequently subclinical. The disease is more serious and prolonged in adults. The objective of this study was to find the prevalence of HAV among blood donors in Tehran, Iran.

Methods: Sera of 407 blood donors in Tehran Blood Transfusion Center who were negative for anti-HIV, HBs Ag and anti-HCV were tested for total anti-HAV antibody, anti-HAV IgM and HAV RNA. Total antibodies (IgG+IgM) and IgM were determined by ELISA using commercial kits. HAV RNA was detected by nested RT-PCR.

Results: The prevalence of total anti-HAV antibodies in blood donors were was 86%, and also the ratio 99% of blood donors was negative for anti-HAV IgM and 1% was equivocal. HAV RNA was not found in any serum samples. The prevalence of total anti-HAV antibodies among blood donors was high; it means most of them were infected in childhood. In spite of, 14% of blood donors were negative for total anti-HAV antibodies; the prevalence of anti-HAV IgM and HAV RNA were very low.

Conclusion: Carefully blood donor selection is performed in Iran.

Key words: *Hepatitis A virus (HAV), Blood donors, Prevalence, Iran*

Introduction

HAV belongs to the family *Picornaviridae* and the new genus *Hepatovirus*. The virion is composed of liner, positive strand RNA, approximately 7.5 kb in length, and capsid containing multiple copies of three or four proteins and measures 27-28 nm in diameter (1, 2). It is non-enveloped virus and very resistant to environmental stress such as heat, drugs and chemical agents (1). HAV infection is acquired usually by the fecal-oral route and by plasma derivates transfusion and causes hepatitis A disease. Infection with this virus leads, after the acute hepatitis, to lifelong protection due to neutralizing antibodies (1). Clinically, the hepatitis A usually is mild, particularly in children, in whom it is frequently subclinical or passed off as gastroenteritis. The disease is more serious and prolonged in adults and can leads to die (3). The most clinically symptoms of HAV infection are dark golden-brown urine, gradual yellowing of the sclera and skin, fever, fatigue, malaise, loss of appetite, nausea and vomiting (3). There is no proof of evolution to chronicity but there are descriptions of fulminate hepatitis in some cases (1).

There are molecular and serological tests to diagnosis of HAV infection, the most important of them is ELISA to detect anti HAV IgG and anti HAV IgM antibody in the patient serum. RT-PCR is used to detect HAV RNA in samples (4, 5).

Small epidemic of HAV have been described in hemophiliac patients treated with factor VIII and IX concentrates, and in cancerous patients whom treated with IL2 (Interleukin 2) and LAK (Lymphokine Activated Killer) concentrates (6-8).

In recent years, concern about the safety of blood in regard to the transmission of blood-borne vi-

***Corresponding author:** Tel/ fax: +98 21 88601599, E-mail: Z.Sharifi@IBTO.ir

ruses has been decreased. Safety has been achieved with a combination of different strategies, such as careful selection of donors, screening for relevant virological markers and viral inactivation/ removal methods. More recently, the implementation of the nucleic acid amplification technologies for the detection of HIV-1, HCV and HBV, has increased safety by reducing the "window period" of the infections. Other viruses, such as Parvovirus B19 (PB19) and HAV, can cause problems for blood safety. These infections could provoke serious complications in some risk groups, such as pregnant women, patients with hematological problems, children and patients with immunodeficiencies (9). So it seems necessary to have appropriate control for this virus presentation in blood banks and in blood transfusion centers.
The objective of this study was determination of the prevalence of HAV in blood donors in Tehran, Iran.

Materials and Methods

In this cross-sectional study, the collection of samples was performed in Tehran Blood Transfusion Center in a period of 6 months, from September 2005 to March 2006. Sera of 407 blood donors who were negative for HIV serum antibody (anti-HIV), hepatitis B surface antigen (HBs Ag) and third generation HCV antibody (anti-HCV) were selected randomly.
IgM anti-HAV was detected by a commercially available ELISA (Enzyme Linked Immunosorbent Assay) (Dia.pro©). Also total anti-HAV antibodies were detected by commercial enzyme immunoassay (Dadebehring©). The ELISA methods were performed according to manufacturer instructions. Nested RT-PCR was used to detect HAV RNA.
HAV RNA was extracted from plasma by "High Pure Nucleic acid Kit" (Roche©). Then from extracted RNA, cDNA was synthesized using "First strand cDNA Synthetic Kit (Roche©). The components used in RT-PCR method were as following: 2 µl of 10 X Reaction Buffer, 4 µl of 25 mM $MgCl_2$, 2 µl of 25 mM deoxynucleoside tri-phosphate, 1.7 µl of each 10 Pmol forward and reverse Primers, 1 µl of 20 U RNAase Inhibitor, 0.8 µl of 20 unit avian myeloblastosis virus (AMV) Reverse transcriptase and 1.8 µl deionized Water. Then 5 µl of extracted nucleic acid was added to 15 µl RT-PCR mixture and reverse transcription was carried out for 1 h at 42º C. The cDNA was amplified by nested-PCR. At first stage these primer were used: HHA1 (5′ TGCAAATTAYAAYCAYTCTGATGA 3′) as forward primer and, HHA2 (5 ′TTTCTGTCCA-TTTYTCATCATTC 3′) as reversed primer. The following components were mixed to make PCR mixture: 2 µl of 10 X PCR Buffer, 0.4 µl of 10 mM dNTP, 1.6 µl of 25 mM $MgCl_2$, 0.2 µl of each 10 Pmol Forward (HHA1) and Reverse (HHA2) primers, 0.3 µl of 5 unit Taq polymerase and 10.3 µl deionised water. Then 15 µl of PCR mixture was added to 5µl of cDNA of HAV genome. PCR amplification was performed for 30 cycles of denaturation for 1min at 95º C, annealing for 1 min at 42º C, and extension for 1 min at 72º C. For the second amplification, 2 µl of the reaction mixture was add to a new bath of 18 µl of PCR mixture containing 10Pmol of each nested primers HHA3 (5′ TTYAGTTGY-TAYTTGTCTGT 3′) as forward primer and HHA4 (5′TCAAGAGTCCACACACTTC 3′) as reversed primer.
For a new PCR amplification cycle has been done as a same procedure.
The PCR products were analyzed by agarose gel electrophoresis using ethidium bromide as a stain sterile distilled water and HAV RNA positive serum sample were used as negative and positive control.

Results

The prevalence of total anti-HAV antibodies (IgG+IgM) were detected in 350 (86%) of the 407 individuals (Fig.1). CI was (83%, 89%).
IgM Anti-HAV was negative in 403 (99%) of serum samples and in 4 samples (1%) was equivocal (Fig. 2).
The electrophoresis of 2% agarose gel showed that all samples were negative for HAV RNA (Fig. 3).

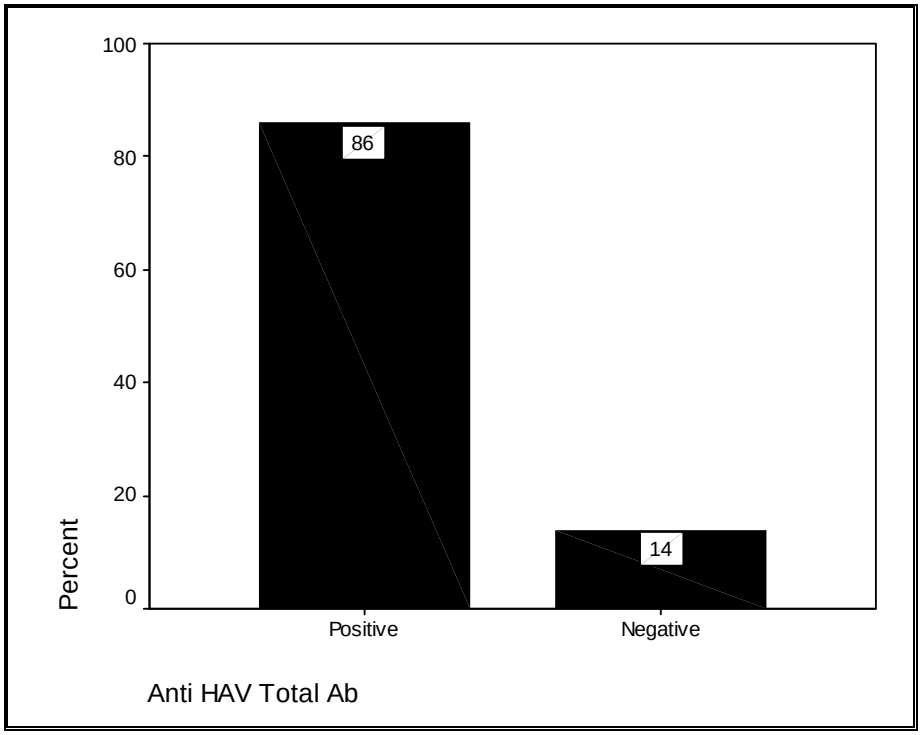

Fig.1: Prevalence of total anti-HAV antibodies in blood donors

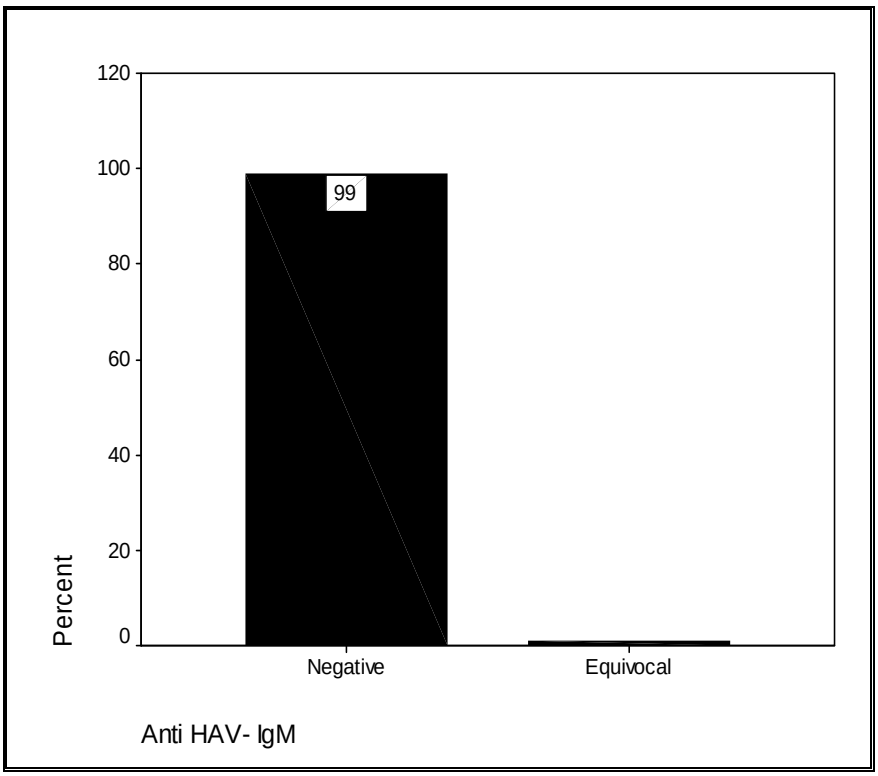

Fig. 2: The frequency of anti-HAV IgM in blood donors

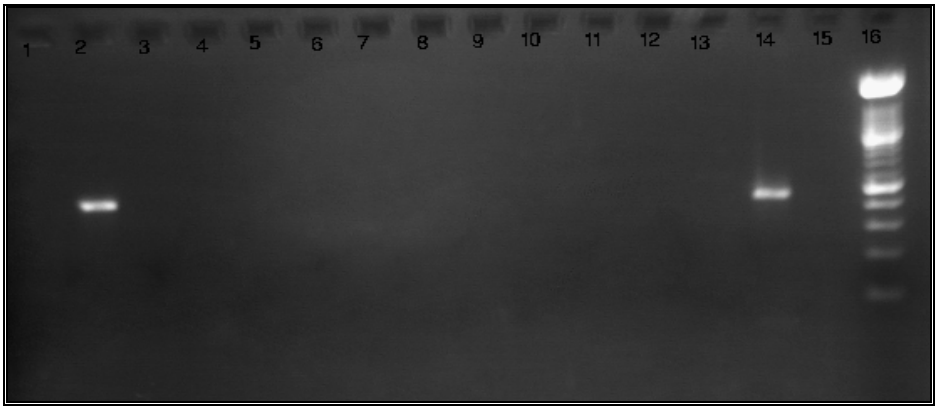

Fig. 3: Electreophorsis of agarose gel of RT nested-PCR product of HAV

Lanes 3-13, PCR product of blood donor serum samples. Lanes 1 and 15, Negative control Lanes 2 and 14 Positive control with 436 base pair length. Lane 16, DNA marker (100bp ladder (Roche©)).

Discussion

There is only one serotype of HAV detected. Infection with this virus brings life-long immunity. Anti-HAV IgM rapidly increases in titer over a period of 4 to 6 wks time and then decline to undetectable level in 3 to 6 month in patients. Anti-HAV IgG can be detected simultaneously within a week or 2 of acute illness, and eventually replaces the IgM antibody, IgG antibody persist for years after infection or vaccination (1).

The results of this study indicate that the prevalence of total anti-HAV antibodies among blood donors is high (86%). At the present time there is no program for anti-HAV vaccination in Iran, so it means that the most of blood donors were infected by HAV in the age of ≤17. In the other words, this research indicates that the percentage of individuals whom are sensitive to the infection is 14%. In spite of this, no positive donations has been found for anti-HAV IgM and HAV RNA. This implies that donor selection is performed carefully in Tehran Blood Transfusion Center.

The prevalence of HAV in blood donors in India was 92.1% positive for anti-HAV antibodies, 1% has been found positive for anti-HAV IgM, and only one positive sample for HAV RNA (10). In Portugal, studies has demonstrated that, above the age of 20 the percentage of individuals immunized was from 72.6% to 88.7% and HAV RNA has been found in non of the blood donors (9,11).

Recently, a transfusion–transmitted HAV case was seen for the first time, demonstrating that this virus can be passed on by labile components and replicate even in an immunocompetent host (10). Nowadays, after years hygienic measures have been increased significantly, so individuals have not been infected in childhood and remained sensitive for HAV and can infected in the future (3). This implies that sensitive people are increasing and it needs some measures to prevent of infection. There is a routine monitoring program in the Iranian Blood Transfusion Organization to control HAV transmission and providing safe blood and plasma derivatives. The careful blood donor selection is the first stage in this way. The use of serological test to exclude viremic is not necessary in blood transfusion centers since HAV antibodies are highly prevalent in the normal population. On the other hand, HAV would not be fully inactivated by procedures that are used for the inactivation of other viruses during the manufacture of plasma derivates, since it is heat-stable and non-enveloped. It consider that testing plasma pools for this virus is performed by a molecular method rather than donor screening

because it is a complementary measure to viral inactivation/removal mechanisms during manufacturing. The polymerase chain reaction (PCR) was used for this purpose. A common strategy is to test donations in minipools and remove all donations with titers more than 10^4 genome equivocal per milliliter (geq/ml) (8, 9, 12). In this study HAV RNA was not detected in 407 pooled samples which indicates that the titer of the genome is lower than 10^4.

Vaccination is just one-way to eliminate HAV from the society. Havrix® and Vaqta® are two most important HAV vaccines which are licensed, and it needs to a full studied program for vaccination, the studies in the other regions of the world have been shown that vaccination decrease the rate of HAV infection significantly (13). In the mass or individual vaccination case, current method of blood monitoring for HAV will not interrupt the blood donor monitoring program which donor history questioned.

Acknowledgements

The authors would like to thank the Research Center of Iranian Blood Transfusion Organization (IBTO) for supporting this study.

The authors declare that they have no conflict of interests.

References

1. Feinstone SM, Gust ID (2000). Hepatitis A virus. In: *Principle and Practice of Infectious Disease* Eds, Mandell GL, Bennet JE and Dolin R. 4th ed. Churchill Livingstone. New York, pp.1920-40.
2. Menick JL (1992). Properties and classification of hepatitis A virus. *Vaccine*, 10 (suppl. 1): 24-6.
3. Koff RS (1992). Clinical manifestations and diagnosis of hepatitis A virus infection. *Vaccine*, 10(suppl. 1): 15-17.
4. Dufour DR (2001). Evaluation of liver function and injury. In: *Clinical Diagnosis and Management by Laboratory Methods.* Ed, Henry JB. 20th ed. WB Sounder. New York, pp. 264-80.
5. Banker DD (2003). Viral Hepatitis (Part-I). *Indian J of Medical Sci,* 57(8): 363-68.
6. Soucie JM, Robertson BH, McCaustland KA, Evatt BL (1998). Hepatitis A virus infections associated with clotting factor concentrate in the United States. *Transfusion*, 38: 573-79.
7. Weisfuse IB, Graham DJ, Will M, Parkinson D, Snydman DR, Atkins M, Karron RA, Feinstone S, Rayner AA, Fisher RI (1990). An outbreak of hepatitis A among cancer patients treated with interleukin-2 and lymphokine-activated killer cells. *J Infect Dis*, 161: 647-52.
8. Tabor E, Epstein JS (2002). NAT screening of blood and plasma donations: evolution and technology and regulatory policy. *Transfusion,* 42: 1230-37.
9. Henriques I, Monteiro F, Meireles E, Cruz A, Tavares G, Ferreira M, Araujo F et al. (2005). Prevalence of Parvovirus B19 and Hepatitis Avirus in Portuguese blood donors. *Transfus Apher Sci*, 33(3): 305-9.
10. Gowland P, Fontana S, Nieden hauser C, Taleghani BM (2004). Molecular and serologic tracing of transfusion- transmited hepatitis A virus. *Transfusion*, 44: 1555-61.
11. Cunha I, Antunes H (2001). Prevalence of hepatitis a virus antibody in a north Portugues population. *Acta Med Port,* 14: 479-82.
12. Hoots WK, Abrams C, Tanakersley D (2001). The food and drug administrations prespective on plasma safety. *Transfus Med Rev,* 15(suppl-1): 20-60.
13. Gadgil PS, Fadnis RS, Joshi MS, Rao PS, Chitambar SD (2007). Sero epidemiology of hepatitis A in voluntary blood donors from pune, western India (2002 and 2004-2005). *Epidemiol Infect,* 7: 1-4.