Middle East J Dig Dis. July 2023;15(3):196-202

doi 10.34172/mejdd.2023.344 http://mejdd.org

Middle East Journal of Digestive Diseases MEJDD

Original Article

The Seroprevalence of Hepatitis A in Patients with Positive Human Immunodeficiency Virus

Navid Omidifar[1], Kamran Bagheri Lankarani[2], Mir Behrad Aghazadeh Ghadim[3], Nika Khoshdel[4], Hassan Joulaei[5], Parisa Keshani[5], Seyyed Amirreza Saghi[6,7],Yousef Nikmanesh[8*]

[1]Biotechnology Research Center and Department of Pathology, Medical School, Shiraz University of Medical Sciences, Shiraz, Iran
[2]Department of Internal Medicine, School of Medicine, Health Policy Research Center, Shiraz University of Medical Sciences, Shiraz, Iran
[3]Department of Clinical Biochemistry, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran
[4]Department of Medicine, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran
[5]HIV/AIDS Research Center, Institute of Health, Shiraz University of Medical Sciences, Shiraz, Iran
[6]Cellular and Molecular Biology Research Center, Larestan University of Medical Sciences, Larestan, Iran
[7]Student Research Committee, Larestan University of Medical Sciences, Larestan, Iran
[8]Gastroenterohepatology Research Center, Shiraz University of Medical Sciences, Shiraz, Iran

Abstract

Background: Hepatitis A virus (HAV) can have severe manifestations in adult patients with other liver diseases, particularly in those infected with human immunodeficiency virus (HIV). This study aimed to measure immunity against HAV in HIV-positive individuals to determine the necessity of vaccination against HAV in this population.

Methods: This cross-sectional study investigated 171 HIV-positive patients aged 18 years or older who were tested for serum IgG anti-viral hepatitis A antibody. The prevalence and its determinants were analyzed based on patient data.

Results: The average age of the patients was 44.2 years old. The prevalence of HAV antibody positivity was 97.7%. The prevalence was higher in patients older than 30 years. There was a close association between hepatitis C virus (HCV) infection ($P=0.002$). There were no significant correlations between antibody levels and sex, marital status, employment status, education level, economic status, smoking status, drug use status, and physical activity level. The mean and median $CD4^+$counts in patients with positive (reactive) antibody (Ab) levels were 458 and 404 ± 294, respectively, while the mean and median $CD4^+$counts in patients with non-reactive antibody levels were 806 and 737 ± 137, respectively, in those who tested negative for anti-HAV Ab ($P=0.05$).

Conclusion: The prevalence of anti-hepatitis A IgG antibodies in people with HIV was very high in Shiraz. There is an increasing trend in the number of older patients and those with HCV infections. The negative association with CD4 was borderline in this study, which needs to be confirmed in larger groups.

Keywords: HAV, HIV, Seroprevalence, IgG

Cite this article as: Omidifar N, Bagheri Lankarani K, Aghazadeh Ghadim MB, Khoshdel N, Joulaei H, Keshani P, et al. The seroprevalence of Hepatitis A in patients with positive human immunodeficiency virus. *Middle East J Dig Dis* 2023;15(3):196-202. doi: 10.34172/mejdd.2023.344.

Received: March 4, 2023, **Accepted:** June 20, 2023, **ePublished:** July 30, 2023

Introduction

Among viral hepatitis, hepatitis A is mostly observed in developing countries, with the incidence rate recently reaching 100% up to the age of 5 years,[1] however, according to statistics published in 2018, the infection rate today is 3.8 people in up to 100 000 people.[2] This disease is highly prevalent in countries such as Iran, similar to its prevalence in other Asian and Middle Eastern countries.[3] In addition, hepatitis A virus (HAV) can have severe manifestations in adult patients with other liver diseases, particularly in those with human immune deficiency virus (HIV) infection. In a study conducted 14 years ago by the Iranian Blood Transfusion Organization, 95% of blood donors had antibodies against this pathogen.[4] Historically, most people in developing countries acquired the necessary immunity to this pathogen through exposure to it in childhood and were immune to it in adulthood. Along with the increase in personal health status and the change in the way of life in these regions, changes in the epidemiology of the disease were observed, and the age of infection increased. As older people are more likely to contract cancer, many societies use immunization methods to reduce the risk of death caused by cancer.[5]

HAV is resistant to bile and detergents because it does not have a coating similar to that of other viruses. This virus is found in the environment and sewage but is inactivated by chlorine and formalin. The disease is transmitted by the fecal-oral route and often through contaminated drinking water.[6-8] Several outbreaks of this virus have been reported through contaminated food consumption and polluted water.[9,10] Another way to contract this virus is through sexual intercourse or close contact with an already

*Corresponding Author: Yousef Nikmanesh, Emails: nikmanesh_y@sums.ac.ir and you.nikmanesh@gmail.com

infected person. It is highly contagious.[11,12] After entering the body, the virus multiplies in the digestive system and liver cells and then dies off. The cells are then removed from them. Cell death is caused by an immune response in the body.[13] The disease is often asymptomatic in children. As the age of an individual increases, the probability of symptoms increases. The probability of death is also the same.[14] On the one hand, there has been a recent increase in the level of health in different parts of Iran, which is expected to lead to an increase in the number of sensitive people in society. The incubation period typically lasts between 15 and 50 days. There is no specific treatment, and the symptoms will disappear within 2 months. It is believed that the best way to learn is to engage in hands-on activities.[14] The clinical manifestations of this virus can range from asymptomatic to acute liver failure, but typically it does not become chronic. The risk of fulminant hepatitis in this type of hepatitis A is higher than that in other types of hepatitis.[15,16] Little evidence suggests that hepatitis A can lead to chronic liver disease or even death, although it is believed that it may increase the severity and mortality of such conditions in patients with chronic hepatitis. Hepatitis A can be caused by liver failure due to autoimmune hepatitis.[17,18]

Ten days before the onset of symptoms and the appearance of antibodies in the serum, the amount of virus in the feces is high. The virus is present in the blood even before the person develops the symptoms of the disease. The most common diagnosis of hepatitis A is based on serological tests. The level of the enzyme alanine transferase increases at the same time as the level of antibodies against hepatitis A is measured. A positive IgM antibody against hepatitis A is a sign of recent infection with the virus. Normally, there is no need to use antibody detection methods if the disease is expected to last for less than 6 months. The simultaneous presence of a positive titer of IgG anti-HAV antibody and a negative titer of IgM anti-HAV antibody indicates a history of past diseases and is not currently a disease.[19-21]

Hepatitis A is a common infectious disease that can be prevented through vaccination. Currently, effective vaccines are available to protect against hepatitis A infection. These vaccines provide long-term protection against infections. However, there is no consensus worldwide on the duration of protection or when to give a booster shot or booster vaccine in the future. Studies have shown that after a full initial course of vaccination, levels of protective antibodies remain in the body of healthy individuals for more than 10 years, and there is no evidence that accepts hepatitis A vaccination is curative after a primary course of vaccination against the virus.[22,23] The general recommendation is that people with chronic liver diseases, coagulation factor disorders, and people who are HIV positive should be vaccinated against hepatitis A because of the potential risk of infection and severe disease.[22,24]

Acquired immunodeficiency syndrome (AIDS) is a major social and economic problem affecting human health. Currently, AIDS is the fourth leading cause of death in all age groups.[25] Although mortality from HIV infection is decreasing, hepatitis viruses are still an important factor in mortality from this disease.[26] 36.7 million people are infected with HIV worldwide. 1.2 million of these people live in the United States. According to UNAIDS statistics released in 2016, 66 000 people in Iran were infected with HIV. According to statistics from this organization, since 2010, the number of new HIV cases has increased by approximately 21%. The death rate from this infection has decreased by 14%. In Europe and the United States, approximately 8 to 16% of patients with HIV are simultaneously infected with hepatitis B virus (HBV).[27-30]

It is possible that patients can be simultaneously infected with two or all three viruses at the same time.[31] Hepatitis A is a common infectious agent of acute hepatitis, which is mainly transmitted through the fecal-oral route from an infected person to a healthy person. The prevalence of hepatitis A is higher in areas of poor health and socioeconomic status. Most HIV-positive patients have other health problems that may increase their risk of liver failure and death from hepatitis A. Chronic hepatitis C virus (HCV) infection greatly increases the risk of fulminant hepatitis and death from HAV.[32] Vaccination against the HAV is not routinely carried out in Iran. This study aimed to measure immunity against HAV in HIV-positive individuals to determine the necessity of vaccination against HAV in this population. If the antibody titer is low in a high percentage of HIV-positive people, routine vaccination of HIV-infected people may help reduce mortality and morbidity in these people.

Method and Materials

Sampling

This cross-sectional study was designed at the Shiraz University of Medical Sciences in the southwest of Iran. It included 200 HIV-positive patients from all our available trial population who were aged 18 years or older and were tested for serum IgG anti-virus hepatitis A antibody. All the patients provided informed consent before participating in the study. Age, sex, and number of $CD4^+$ cells were determined from patient records before blood samples were collected. Most patients were using anti-*HIV* drugs and sometimes antibiotics, which do not interfere with the HAV antibody titer measurement protocol. A blood sample of 5 cc was collected from each patient. The serum was maintained at a temperature below 20 °C until use. The quantitative luminescent laboratory technique was used to measure the amount of anti-hepatitis A antibody (IgG) in serum. Index values greater than 1.1 were considered positive, while index values less than 0.9 were considered negative. Items between 0.9 and 1.1 were retested again.

Inclusion criteria

HIV-positive patients who were 18 years or older and who were registered at the AIDS Research Center in 2019-2020

were included in the study using the census method.

Exclusion Criteria

Lysed, severely lipemic, and icteric samples, samples with distorted patient characteristics and no name, and samples that were sent to the laboratory late were considered as poor quality samples and excluded from our study. Only people who had complete files and had been sampled for antibody testing were included in the study. There were 10 patients with unknown antibody titers due to inadequate serum volume and 19 patients with incomplete files or a lack of access to blood sampling.

Data Collection Tools and Process

Sampling was performed after collecting data from the files. Five milliliters of venous blood was drawn from each patient, and blood coagulation was performed for 3 hours. The samples (blood serum) were separated by centrifugation at 3600 rpm (revolutions per minute) for 5 minutes. For this purpose, a dedicated kit for anti-hepatitis A antibody was purchased and tested according to the protocol after blood collection and serum preparation.

Calculation of Test

The number of samples that could be read by the device from the signal was recorded for each sample. The cut-off point for the desired result is determined by adding the positive control and the negative control divided by three [(positive control + negative control) ÷ 3]. If the number of cut-off points is more than 1.1, the sample will be named positive, and below 0.9 will be named negative, and if it is between 0.9 and 1.1, the sample should be repeated. For each serum sample, the above assay was performed twice, and relevant information was recorded for 181 samples (10 patients whose antibody titers were not determined were excluded).

Statistical Analysis Method

Statistical analysis was done using the SPSS software (version 18). The Mann-Whitney test, Fisher's exact test, and Spearman's coefficient of correlation were used to determine the relationship between seropositivity and sociodemographic or blood information of patients. A 95% confidence interval and two-sided *P* value less than 0.05 were used in all analyses.

Results

The study found that hepatitis A is commonly present in HIV-positive patients and that IgG titration is common in patients with the disease. The average age of the patients was 44.2 years. The antibody titer ranged from 0 to 18, with a mean of 9.2 ± 3.6. Four (2.3%) patients had no antibodies (non-reactive). These four cases were men. This means that the antibody level was positive in 97.7% of the patients (Figure 1).

Table 1 displays the antibody levels according to the demographic variables. Fisher's exact test did not show any significant relationship between antibody levels and the variables of sex, marital status, employment status, education level, economic status, smoking status, drug addiction status, hepatitis B and C status, and patients' levels of physical activity ($P > 0.05$).

The mean antibody titers of the hepatitis C patients (IgG and IgM positive) were 10.08 ± 3.46, with a median of 9.98, and the mean antibody titers of the hepatitis C-negative group were 8.4 ± 3.67, with a median of 11.18. The Mann-Whitney test showed a significant difference in antibody titers between the two groups ($P = 0.05$). Antibody titers in the hepatitis B group (HBV positive) were 10.49 ± 3.29 with a median of 12.19, and in the hepatitis B negative group, with a mean of 9.11 ± 3.96 with a median of 8.65. The Mann-Whitney test showed no significant difference in the antibody titers of the two groups ($P = 0.184$).

The mean and median age of cases with positive antibody levels were 44.6 ± 9.1 and 43 years, respectively, and the mean and median age of negative cases were 29.5 ± 5.5 and 30 years, respectively. The Mann-Whitney test showed a significant relationship between antibody levels and age ($P = 0.002$). On the other hand, the Spearman coefficient of correlation between age and antibody titer was r = 0.167 ($P = 0.03$), indicating a positive and significant correlation between age and antibody level. In addition, there was a positive and significant relationship between the duration of the disease and the level of antibodies; therefore, the Spearman correlation coefficient of the duration of the disease and the level of antibodies was r = 0.305 ($P < 0.001$). The mean and median $CD4^+$ counts in patients with positive (reactive) antibody levels were 458 and 404 ± 294, respectively, and the mean and median $CD4^+$ counts in patients with non-reactive antibody levels were 806 and 737 ± 137, respectively, in those who tested negative for Ani HAV Ab. The Mann-Whitney test showed a slightly significant difference between the $CD4^+$ counts of patients with positive and negative antibody levels ($P = 0.05$), although the Spearman correlation coefficient test did not show a relationship between the $CD4^+$ count and the antibody titer (r = 0.032; $P = 0.68$).

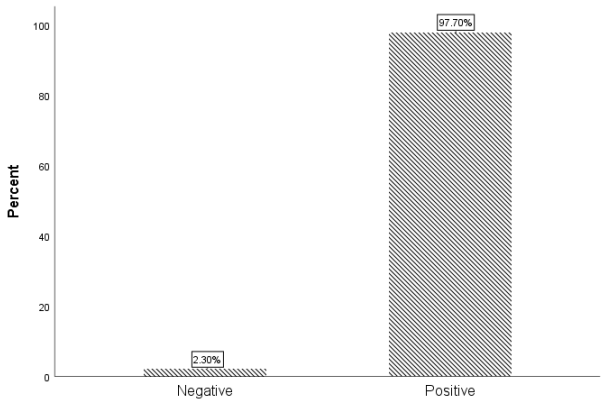

Figure 1. There is a high prevalence of HAV seroprevalence in HIV-positive patients. The study found that 97.7% of the studied HIV patients had anti-hepatitis A IgG antibodies, and 2.3% of the patients had no (non-reactive) antibodies.

Table 1. Anti-HAV IgG antibody levels in HIV-positive patients according to demographic variables and *P* value level based on Fisher's exact test.

Antibody		Positive		Negative		*P* value[a]
Variable	**Level**	**Number**	**Percent**	**Number**	**Percent**	
Sex	Male	93	95.9	4	4.1	0.134
	Female	74	100	0	0	
Marital status	Single	36	92.3	3	7.7	0.063
	Married	83	98.8	1	1.2	
	Widow	48	100	0	0	
Employment status	Employed	72	97.3	2	2.7	1
	Unemployed	95	97.9	2	2.1	
Level of Education	Illiterate	6	100	0	0	0.441
	Elementary	61	100	0	0	
	Middle School degree	51	96.2	2	3.8	
	Diploma	40	95.2	2	4.8	
	Bachelor's degree	8	100	0	0	
	Master's degree	1	100	0	0	
Economic status	1	121	98.4	2	1.6	0.378
	2	37	94.9	2	5.1	
	3	9	100	0	0	
Smoking status	Yes	80	98.8	1	1.2	0.623
	No	87	96.7	3	3.3	
Drug addiction status	Yes	37	100	0	0	0.578
	No	130	97	4	3	
Hepatitis C status	Yes	80	97.6	2	2.4	1
	No	87	97.8	2	2.2	
Hepatitis B status	Yes	12	100	0	0	1
	No	155	97.5	4	2.5	
Physical activity level	1	44	97.8	1	2.2	1
	2	74	97.4	2	2.6	
	3	40	97.6	1	2.4	
	4	9	100	0	0	

[a] Fisher's exact test.

Discussion

In this study, 171 patients with HIV were tested for HAV antibodies. The study found that HAV is common among patients with HIV and that IgG titration is often useful for diagnosing HAV infection. Considering that stable immunity is explained by IgG titration and that IgG is the major factor and more important than IgM in stable immunity, IgM examination is used to screen the early stages of the disease, and IgG examination is specialized for stable immunity. The age of the patients ranged from 23 to 70 years, with 44.2 ± 9.3 years being the average age. The patients' weights ranged from 42 to 100 kg, with an average of 64.8 ± 11.6 kg. The disease ranged from 1 to 25 years, with an average of 7.7 ± 5.2 years.

The patients' files included their demographic information, such as sex, age, marital status, employment status, economic status, educational level, smoking status, drug addiction status, and physical activity status. The study found that 97.7% of patients with HIV had anti-hepatitis A IgG. This suggests that they were likely to have been previously exposed to HAV. In this study, the highest relative frequency was found among married people, unemployed people, people with primary education, and people with low economic levels.

Since hepatitis A is most commonly transmitted through the fecal-oral route, the prevalence of this virus is likely higher in people with poorer health.[33] A study conducted by Taghavi et al found that the prevalence of hepatitis A antibody (IgG) positivity was 88.2%.[34] Also, another study conducted by Bagheri Lankarani et al. found that a high intake of fruits and vegetables is beneficial for a person's health. The study found that the prevalence of anti-HAV IgG was 66.5%.[35] Meanwhile, the study found that 97.7% of the 171 HIV patients studied had positive antibody responses to the virus.[17] Considering the country's increasing health levels, it can be seen that the majority of the patients studied had positive antibody titers, which may indicate a lower level of health. This case itself may explain the background and circumstances of the patient's HIV infection. Therefore, it can be concluded that the

prevalence of HAV is high in the research group of HIV patients whose most common method of infection is fecal-oral, as mentioned above. As a result, HIV infection in these individuals is an important contributor to infection. The health of these people is likely to be poor. It is suggested that a study should be conducted that considers the causes of this disease and its relationship with HAV.

In a study by Moisseeva et al, the prevalence of hepatitis A in Ukraine was investigated. They studied whether people living in Kyiv have anti-hepatitis A antibodies. In 1001 people, they found that older people were more likely to have antibodies. The average prevalence of this antibody was 31.9%, which increased with age. Among people over 51 years of age, the rate was 81.7%.[36] In the present study, the mean and median ages of antibody-positive patients were 44.6 ± 9.1 and 43 years, respectively, and the mean and median ages of antibody-negative patients were 29.5 ± 5.5 and 30 years, respectively. The Mann-Whitney test showed a significant relationship between antibody levels and age ($P = 0.002$). On the other hand, Spearman's correlation coefficient between age and antibody titer was $r = 0.167$ and $P = 0.03$, showing a positive and significant correlation Between age and antibody levels. With increasing age, the prevalence of positive antibody titers increased, which was associated with greater exposure. Given the prevalence of nearly 100% positive antibody titers in infected people, there is no need for vaccination, but this prevalence may vary from city to city. With improved hygiene in recent years, this prevalence of positive titers is expected to increase in the coming years.

Miri et al conducted a study to determine the effects of a new product on customer satisfaction. The results showed that the new product successfully increased customer satisfaction. The use of this new product showed a high prevalence of hepatitis A in Mashhad. This study was conducted on 1563 randomly chosen individuals from Mashhad's general population over two months in 2009. Serum samples were tested for antibodies against hepatitis A using ELISA. The results were analyzed using the SPSS software, and chi-square and Student's *t* tests were used to analyze the data. The prevalence of hepatitis A infection was about 69.6% in both men and women so the prevalence of hepatitis A infection did not differ between men and women populations. The prevalence of hepatitis A antibody positivity has increased from 9.4% in people aged < 5 years to 100% in people over 65 years of age.[37]

In a study by Karimi et al in Shahrekord, the prevalence of hepatitis A was approximately 90.8%. The results showed that 455 of 501 (90.8%) serum samples, including 211 males and 290 females, were positive for HAV IgG antibodies. Educational level, age, marital status, and race were related to the level of serum IgG antibodies against the HAV.[38] There was no significant relationship between antibody level and sex, marital status, employment status, education level, economic status, smoking status, drug use status, and physical activity level of the patients in our study. The study found that the p-value was greater than 0.05, which suggests that the relationship between sex and hepatitis A is real. There was no significant relationship between the prevalence of hepatitis A and the level of education or marital status, which was not equivalent to the results of Karimi et al. The median $CD4^+$ count in patients with positive antibody levels was 458 and 404 ± 294; the median $CD4^+$ count in patients with non-reactive antibody levels was 806 and 737 ± 137 in those who tested negative for Anti HAV Ab. The Mann-Whitney test showed a slight difference in the number of $CD4^+$ cells in patients with positive and negative antibody levels ($P = 0.05$), while the Spearman correlation coefficient test showed There was no significant relationship between the number of $CD4^+$ cells and IgG antibody level against HAV ($P = 0.68$).

Weissman et al found that patients who adhere to a strict diet are more likely to experience positive results than patients who do not adhere to a strict diet. In 138 HIV-positive patients who received a hepatitis A vaccine, the number of female patients and $CD4^+$ cells at the time of vaccination were both found to be associated with an improved response to the vaccine.[39] Patients with higher $CD4^+$ cell counts after HIV infection had higher antibody levels after vaccination than patients with fewer $CD4^+$ cells. This was statistically significant ($P = 0.001$). (458 ± 294 and 404 compared to 737 ± 137 and 806)

The small sample size was one of the limitations of this study, and it is clear that if the sample size is increased, the results obtained would be more reliable. Some limitations of this research include the difficulty in evaluating laboratory results, as well as the lack of cooperation from several patients. This has made it difficult to collect information from them.

Conclusion

It seems that the prevalence of anti-hepatitis A IgG antibodies in people with HIV in Shiraz is high. There is an increasing trend in the number of older patients and those with HCV infections. The transmission routes of HCV and HBV are almost similar, but due to this important situation in which HCV infection does not have a vaccination protocol, and so many people do not know about their infection, the population of people infected with HCV is more than that of those infected with HBV, resulting in an incredibly larger HCV-positive population, which is preparing a more relevant relationship between the study variables. The negative association with CD4 was borderline in this study which needs to be confirmed in larger groups.

The prevalence of anti-hepatitis A IgG antibody increases with age, but this is not related to sex or $CD4^+$ cell level., Due to the possibility of sexual transmission of the HAV, as well as the ability of poor health in people with hepatitis C, the likelihood of meeting the HAV in these people increases (Mann-Whitney test). Also, It is proposed to carry out this study on a larger population of patients with HIV, taking into account the causes of the disease.

Authors' Contribution

Conceptualization: Navid Omidifar.
Data curation: Navid Omidifar.
Formal analysis: Kamran Bagheri Lankarani.
Funding acquisition: Yousef Nikmanesh.
Investigation: Nika Khoshdel.
Methodology: Mir Behrad Aghazadeh Ghadim.
Project administration: Yousef Nikmanesh.
Resources: Hassan Joulaei, Parisa Keshani.
Software: Seyyed Amirreza Saghi.
Supervision: Yousef Nikmanesh.
Validation: Navid Omidifar.
Visualization: Seyyed Amirreza Saghi.
Writing–original draft: Navid Omidifar.
Writing–review & editing: Seyyed Amirreza Saghi.

Competing Interests

The authors declare no conflict of interest related to this work.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon online request.

Ethical Approval

This study was approved by the Shiraz University of Medical Sciences (the ethical approval code: IR.SUMS.MED.REC.1399.435).

References

1. Lemon SM, Ott JJ, Van Damme P, Shouval D. Type A viral hepatitis: a summary and update on the molecular virology, epidemiology, pathogenesis and prevention. *J Hepatol* 2018;68(1):167-84. doi: 10.1016/j.jhep.2017.08.034
2. Rosenberg ES, Rosenthal EM, Hall EW, Barker L, Hofmeister MG, Sullivan PS, et al. Prevalence of hepatitis C virus infection in US states and the district of Columbia, 2013 to 2016. *JAMA Netw Open* 2018;1(8):e186371. doi: 10.1001/jamanetworkopen.2018.6371
3. Farhood B, Geraily G, Alizadeh A. Incidence and mortality of various cancers in Iran and compare to other countries: a review article. *Iran J Public Health* 2018;47(3):309-16.
4. Hosseini SA, Golchin E, Sharif M, Sarvi S, Ahmadpour E, Rostamian A, et al. A serological investigation and genotyping of Toxoplasma gondii among Iranian blood donors indicates threat to health of blood recipients. *Transfus Apher Sci* 2020;59(3):102723. doi: 10.1016/j.transci.2020.102723
5. Smith RA, Andrews KS, Brooks D, Fedewa SA, Manassaram-Baptiste D, Saslow D, et al. Cancer screening in the United States, 2018: a review of current American Cancer Society guidelines and current issues in cancer screening. *CA Cancer J Clin* 2018;68(4):297-316. doi: 10.3322/caac.21446
6. Gargano JW, Adam EA, Collier SA, Fullerton KE, Feinman SJ, Beach MJ. Mortality from selected diseases that can be transmitted by water – United States, 2003-2009. *J Water Health* 2017;15(3):438-50. doi: 10.2166/wh.2017.301
7. Sarangi G, Dash M, Mahapatra D, Paty BP, Mohanty DP, Chayani N. Fecal-oral-transmitted hepatitis A and E prevalence in Eastern India: a 3-year retrospective study. *J Med Soc* 2019;33(2):86-90. doi: 10.4103/jms.jms_67_17
8. Shouval D. Hepatitis A virus. In: Kaslow RA, Stanberry LS, LeDuc JW, eds. *Viral Infections of Humans*. New York: Springer; 2014. p. 417-38.
9. Najar S, Naghavi NS. Molecular detection of hepatitis A virus in urban sewage in Isfahan, Iran. *Med Lab J* 2018;12(4):22-8. doi: 10.29252/mlj.12.4.22
10. Rodríguez-Lázaro D, Cook N, Ruggeri FM, Sellwood J, Nasser A, Nascimento MS, et al. Virus hazards from food, water and other contaminated environments. *FEMS Microbiol Rev* 2012;36(4):786-814. doi: 10.1111/j.1574-6976.2011.00306.x
11. Ashour HM, Elkhatib WF, Rahman MM, Elshabrawy HA. Insights into the recent 2019 novel coronavirus (SARS-CoV-2) in light of past human coronavirus outbreaks. *Pathogens* 2020;9(3):186. doi: 10.3390/pathogens9030186
12. Correia G, Rodrigues L, Gameiro da Silva M, Gonçalves T. Airborne route and bad use of ventilation systems as non-negligible factors in SARS-CoV-2 transmission. *Med Hypotheses* 2020;141:109781. doi: 10.1016/j.mehy.2020.109781
13. Cuthbert JA. Hepatitis A: old and new. *Clin Microbiol Rev* 2001;14(1):38-58. doi: 10.1128/cmr.14.1.38-58.2001
14. Kemmer NM, Miskovsky EP. Hepatitis A. *Infect Dis Clin North Am* 2000;14(3):605-15. doi: 10.1016/s0891-5520(05)70123-9
15. Shin EC, Jeong SH. Natural history, clinical manifestations, and pathogenesis of hepatitis A. *Cold Spring Harb Perspect Med* 2018;8(9):a031708. doi: 10.1101/cshperspect.a031708
16. Debing Y, Neyts J, Thibaut HJ. Molecular biology and inhibitors of hepatitis A virus. *Med Res Rev* 2014;34(5):895-917. doi: 10.1002/med.21292
17. Amado Garzón SB, Villanueva Ortega MM, Botero Bahamón JD. Chronic diarrhea: a case of Waldenström macroglobulinemia. *Rev Gastroenterol Mex (Engl Ed)* 2018;83(4):467-9. doi: 10.1016/j.rgmx.2017.08.003
18. Carbone M, Neuberger JM. Autoimmune liver disease, autoimmunity and liver transplantation. *J Hepatol* 2014;60(1):210-23. doi: 10.1016/j.jhep.2013.09.020
19. Prasidthrathsint K, Stapleton JT. Laboratory diagnosis and monitoring of viral hepatitis. *Gastroenterol Clin North Am* 2019;48(2):259-79. doi: 10.1016/j.gtc.2019.02.007
20. Okano H, Nakano T, Ito R, Tanaka A, Hoshi Y, Matsubayashi K, et al. The spontaneous clearance of hepatitis E virus (HEV) and emergence of HEV antibodies in a transfusion-transmitted chronic hepatitis E case after completion of chemotherapy for acute myeloid leukemia. *Clin J Gastroenterol* 2020;13(2):252-9. doi: 10.1007/s12328-019-01024-3
21. Pondé RA. Atypical serological profiles in hepatitis B virus infection. *Eur J Clin Microbiol Infect Dis* 2013;32(4):461-76. doi: 10.1007/s10096-012-1781-9
22. Van Damme P, Banatvala J, Fay O, Iwarson S, McMahon B, Van Herck K, et al. Hepatitis A booster vaccination: is there a need? *Lancet* 2003;362(9389):1065-71. doi: 10.1016/s0140-6736(03)14418-2
23. Van Damme P. Long-term protection after hepatitis B vaccine. *J Infect Dis* 2016;214(1):1-3. doi: 10.1093/infdis/jiv750
24. Peto TJ, Mendy ME, Lowe Y, Webb EL, Whittle HC, Hall AJ. Efficacy and effectiveness of infant vaccination against chronic hepatitis B in the Gambia Hepatitis Intervention Study (1986-90) and in the nationwide immunisation program. *BMC Infect Dis* 2014;14:7. doi: 10.1186/1471-2334-14-7
25. Mokdad AH, Forouzanfar MH, Daoud F, Mokdad AA, El Bcheraoui C, Moradi-Lakeh M, et al. Global burden of diseases, injuries, and risk factors for young people's health during 1990-2013: a systematic analysis for the Global Burden of Disease Study 2013. *Lancet* 2016;387(10036):2383-401. doi: 10.1016/s0140-6736(16)00648-6
26. Kassaian N, Adibi P, Kafashaian A, Yaran M, Nokhodian Z, Shoaei P, et al. Hepatitis C virus and associated risk factors among prison inmates with history of drug injection in Isfahan, Iran. *Int J Prev Med* 2012;3(Suppl 1):S156-61.
27. Park JS, Saraf N, Dieterich DT. HBV plus HCV, HCV plus HIV, HBV plus HIV. *Curr Gastroenterol Rep* 2006;8(1):67-74. doi: 10.1007/s11894-006-0066-9
28. Sohn AH, Hazra R. The changing epidemiology of the global paediatric HIV epidemic: keeping track of perinatally HIV-infected adolescents. *J Int AIDS Soc* 2013;16(1):18555. doi: 10.7448/ias.16.1.1855518555

29. Alter MJ. Epidemiology of viral hepatitis and HIV co-infection. *J Hepatol* 2006;44(1 Suppl):S6-9. doi: 10.1016/j.jhep.2005.11.004
30. Spradling PR, Richardson JT, Buchacz K, Moorman AC, Brooks JT. Prevalence of chronic hepatitis B virus infection among patients in the HIV Outpatient Study, 1996-2007. *J Viral Hepat* 2010;17(12):879-86. doi: 10.1111/j.1365-2893.2009.01249.x
31. Zhang F, Zhu H, Wu Y, Dou Z, Zhang Y, Kleinman N, et al. HIV, hepatitis B virus, and hepatitis C virus co-infection in patients in the China National Free Antiretroviral Treatment Program, 2010-12: a retrospective observational cohort study. *Lancet Infect Dis* 2014;14(11):1065-72. doi: 10.1016/s1473-3099(14)70946-6
32. Lin KY, Chen GJ, Lee YL, Huang YC, Cheng A, Sun HY, et al. Hepatitis A virus infection and hepatitis A vaccination in human immunodeficiency virus-positive patients: a review. *World J Gastroenterol* 2017;23(20):3589-606. doi: 10.3748/wjg.v23.i20.3589
33. Farajzadegan Z, Ghaffari Hoseini S, Kelishadi R, Jamshidi F, Nokhodian Z, Noori R, et al. Systematic review and meta-analysis on the age-specific seroprevalence of hepatitis A in Iran. *J Res Med Sci* 2014;19(Suppl 1):S56-63.
34. Taghavi SA, Hosseini Asl MK, Talebzadeh M, Eshraghian A. Seroprevalence study of hepatitis A virus in Fars province, southern Iran. *Hepat Mon* 2011;11(4):285-8.
35. Bagheri Lankarani K, Honarvar B, Molavi Vardanjani H, Kharmandar A, Gouya MM, Zahraei SM, et al. Immunity to hepatitis-A virus: a nationwide population-based seroprevalence study from Iran. *Vaccine* 2020;38(45):7100-7. doi: 10.1016/j.vaccine.2020.08.071
36. Moisseeva AV, Marichev IL, Biloschitchkay NA, Pavlenko KI, Novik LV, Kovinko LV, et al. Hepatitis A seroprevalence in children and adults in Kiev city, Ukraine. *J Viral Hepat* 2008;15 Suppl 2:43-6. doi: 10.1111/j.1365-2893.2008.01028.x
37. Miri R, Ahmadi Ghezeldasht S, Hedayati-Moghadam MR, Fathimoghadam F, Bidkhori H, Shamsian SA, et al. Seropositivity of antibodies against hepatitis A virus in Mashhad, northeast of Iran, 2009: a cross-sectional community-based study. *Razavi Int J Med* 2015;3(2):31-4. doi: 10.30483/rijm.2015.118383
38. Karimi A, Imani-Rastabi R, Moezzi M, Moradi M. Hepatitis A seroprevalence and associated risk factors: a community-based cross-sectional study in Shahrekord, Iran. *Arch Clin Infect Dis* 2016;11(1):e32288. doi: 10.5812/archcid.32288
39. Weissman S, Feucht C, Moore BA. Response to hepatitis A vaccine in HIV-positive patients. *J Viral Hepat* 2006;13(2):81-6. doi: 10.1111/j.1365-2893.2005.00658.x