Original Article

Epidemiology of Hepatitis D Virus and Associated Factors in Patients Referred to Level Three Hepatitis Clinic, Fars Province, Southern Iran

Ali Reza Safarpour[1], PhD; Alireza Shahedi[2], BSc; Mohammad Reza Fattahi[1], MD; Erfan Sadeghi[3], PhD; Majid Akbarzadeh[4], MD; Lida Ahmadi[1], MD; Nika Nikmanesh[1], BSc; Ebrahim Fallahzadeh Abarghooee[4], PhD; Seyedeh Azra Shamsdin[1], PhD; Hassan Akrami[1], PhD; Yousef Nikmanesh[1], PhD

[1]Gastroenterohepatology Research Center, Shiraz University of Medical Sciences, Shiraz, Iran;
[2]Student Research Committee, School of Nursing and Midwifery, Tehran University of Medical Sciences, Tehran, Iran;
[3]Department of Biostatistics, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran;
[4]Department of Internal Medicine, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran

Correspondence:
Yousef Nikmanesh, PhD;
Gastroenterohepatology Research Center, Mohammad Rasulullah Scientific Research Tower, Khalili Ave.,
Postal code: 71936-35899, Shiraz, Iran
Tel: +98 71 36281442
Email: you.nikmanesh@gmail.com
Received: 06 March 2024
Revised: 03 June 2024
Accepted: 28 June 2024

What's Known

- Considering the low hepatitis D virus (HDV) prevalence and its importance in hepatitis B virus (HBV) treatment, this is the first focused prospective study (2001-2023) in the southern region. A cohort study of 137 HBV patients with low viral load and elevated liver enzymes was conducted to screen for HDV. Patients received treatment and consultations.

What's New

- The epidemiology of hepatitis D in South Iran is unclear. This study could help people make health-related decisions. Among 137 participants, 29 (21.1%) were HDV positive. The risk factors include age, single status, low education, underweight, a family history of hepatitis B, blood transfusion, dental procedures, tattooing, multiple partners, IV drug use, smoking, and drinking alcohol. Hepatitis B immunization is protective.

Abstract

Background: Hepatitis D is caused by the hepatitis D virus (HDV) and affects those who have already been infected with the hepatitis B virus (HBV). The epidemiology of hepatitis D in Fars Province, Iran, is poorly understood. This study aimed to investigate the epidemiology of HDV and its associated factors in patients attending Shahid Motahari Clinic, affiliated with Shiraz University of Medical Sciences (Shiraz, Iran).

Methods: This prospective cohort study was conducted in Shiraz, Iran, from 2001 to 2023. This study screened individuals with low HBV viral load and elevated liver enzymes for HDV. Pearson Chi square, Fisher's exact, and Mann-Whitney U tests were used to examine the univariate associations between hepatitis D and various risk factors. Risk factors with $P<0.2$ were analyzed using multiple logistic regression to estimate odds ratios and 95% confidence intervals. $P<0.05$ was considered statistically significant.

Results: The variables were compared between the HDV^+ (29) and HDV^- (108). The variables of age (P=0.002) and using hookah (P=0.040) were statistically significant. The other variables examined in this study were not statistically significant. Increasing age (OR=1.06, 95% CI=[1.019, 1.102], P=0.003) was identified as a risk factor, while dental visits (OR=0.290 95% CI=[0.101, 0.836], P=0.022) were assessed as a protective factor.

Conclusion: Age was a significant risk factor for HDV infection, while a history of dental procedures appeared to be a protective factor. To better understand the epidemiology of HDV, further comprehensive research is necessary, focusing on diverse demographic groups in different regions.

Please cite this article as: Safarpour AR, Shahedi AR, Fattahi MR, Sadeghi E, Akbarzadeh M, Ahmadi L, Nikmanesh N, Fallahzadeh Abarghooee E, Shamsdin SA, Akrami H, Nikmanesh Y. Epidemiology of Hepatitis D Virus and Associated Factors in Patients Referred to Level Three Hepatitis Clinic, Fars Province, Southern Iran. Iran J Med Sci. doi: 10.30476/ijms.2024.101949.3469.

Keywords ● Hepatitis D ● Risk factors ● Epidemiology ● Iran

Introduction

Hepatitis D, caused by the hepatitis D virus (HDV), the smallest known human-infecting virus, is entirely dependent on the hepatitis B virus (HBV) for its life cycle, earning it the classification of a "satellite virus" since infection with hepatitis D is only possible in individuals already infected with hepatitis B.[1-3] This disease is clinically significant as it increases the risk of cirrhosis and liver cancer in patients with hepatitis B

and can manifest as the most severe form of hepatitis.[4] Hepatitis D can present as an acute or chronic infection and can arise in two ways: co-infection, in which an individual acquires both hepatitis B and hepatitis D simultaneously, or superinfection, in which a person who is already chronically infected with HBV becomes infected with HDV.[5] Co-infection in adults rarely proceeds to chronic hepatitis D and usually cures spontaneously (98%), whereas superinfection leads to chronic infection in nearly 90% of cases.[6] Chronic hepatitis delta (CHD) is the most severe form of hepatitis caused by HDV.[7] Vaccination against hepatitis B has proven effective in preventing hepatitis D, and global vaccination efforts have contributed to a reduction in its incidence.[2, 8] Symptoms of hepatitis D include fatigue, nausea, vomiting, fever, loss of appetite, dark urine, pale stools, jaundice, and in severe cases, fulminant hepatitis. These symptoms typically appear 3-7 weeks after infection and are not exclusive to hepatitis D.[4, 9] Hepatitis D is transmitted through the same routes as hepatitis B, primarily through exposure to the blood and body fluids of an infected individual.[10] The diagnosis of the disease involves detecting high levels of immunoglobulin G (IgG) and immunoglobulin M (IgM) in individuals, confirmed by the presence of HDV RNA in serum.[9] Specific populations, including intravenous drug users, individuals with a family history of hepatitis B, migrants residing in high-prevalence areas, hemodialysis patients, men who engage in sexual activity with other men, individuals with a history of oral and dental procedures, blood recipients, and commercial sex workers, are at a higher risk of contracting hepatitis D.[9, 11] The findings of a meta-analysis that reviewed 282 studies from nearly 100 countries indicated that approximately 0.16% of the global population, amounting to about 12 million people, were infected with hepatitis D. They reported that the prevalence of hepatitis D among hepatitis B patients was found to be 4.5%, which corresponded to approximately 0.16% of the global population or around 16 million people affected by the disease.[12] Regions with the highest prevalence of hepatitis D include West Africa, Central Africa, the Pacific Islands, the Middle East, Eastern Europe, South America, and parts of Asia, specifically Central and Northern Asia.[10, 12, 13] Hepatitis D infection exists in Iran and neighboring countries, and the prevalence rate varies across different regions of the country.[14] For example, studies conducted in East-Azerbaijan and Kermanshah Provinces estimated the prevalence of hepatitis D among patients with hepatitis B at 2.17% and 1.7%, respectively.[15, 16] However, a study in Sari reported no cases of hepatitis D among patients with hepatitis B, suggesting that the disease is not endemic in that area.[17] This study aimed to investigate the epidemiology of HDV and its associated factors in the Fars Province, Iran.

Patients and Methods

This prospective cohort study was conducted at Shahid Motahari Clinic, affiliated with Shiraz University of Medical Sciences (Shiraz, Iran), from 2001 to 2023. The study was approved by the Ethics Committee of Shiraz University of Medical Sciences (code: IR.SUMS.REC.1402.375).

Two trained researchers, overseen by a physician, utilized a data gathering form and collected demographic data and risk factors from a database comprising 3787 patients diagnosed with hepatitis B virus (HBV). Written informed consent was obtained from all participants to ensure the confidentiality of the collected information. The inclusion criteria were providing written informed consent and being at least 18 years old. The exclusion criteria were incomplete questionnaires and refusal to participate.

The participants' demographic information was recorded, including age, sex, race, nationality, place of residence, marital status, weight, height, and education. Furthermore, various risk factors were investigated, such as a family history of hepatitis B, multiple sexual partners, hemodialysis and blood transfusion, cupping, dental procedures, cigarette smoking, hookah use, drug abuse (drug injection), alcohol consumption, war injuries, and tattooing.

To detect HDV antibodies, a competitive enzyme immunoassay (ELISA) diagnostic kit (DIA.PRO Diagnostic Bioprobes Srl, Italy) was utilized. These kits were designed to detect antibodies to the HDV in human serum and plasma. The operational principle of the kit involved the following steps:

The study involved the collection of serum or plasma samples from the patients. To determine the presence of HDV antibodies, a competitive assay was conducted. For this purpose, the patient's sample was incubated which labeled polyclonal IgG antibodies specific to HDV. These HDV antibodies competed with a fixed amount of HDV antigen coated on a microplate. Following a washing step, an enzyme-conjugated polyclonal antibody to HDV was added, which bound to the unoccupied HDV antigen on the microplate. To detect enzyme activity, a chromogen/substrate mixture was dispensed into the microplate, and the subsequent color change was measured.

The concentration of the enzyme bound to the solid phase had an inverse correlation with the number of HDV antibodies present in the sample. By comparing the sample's HDV-specific antibody concentration to a predefined cut-off value, the semi-quantitative detection of anti-HDV antibodies was achieved. It had a sensitivity of more than 98%, indicating a strong capacity to accurately identify samples containing HDV antibodies.

All analyses were performed using SPSS software, version 24 (IBM, USA). Descriptive statistics were presented as mean±SD or numbers and percentages. Pearson Chi square, Fisher's exact, and Mann-Whitney U tests were performed to univariately investigate the relationship between hepatitis D and the risk factors. Moreover, those risk factors with P<0.2

Table 1: Demographic information of the patients according to hepatitis D virus status

		Total N=137		HDV⁻ n=108		HDV⁺ n=29		P value
Age		50±13		48±12		57±13		0.002[c]
BMI, (Kg/m^2)	Underweight	50	36.49%	36	72.00%	14	28.00%	0.317[a]
	Normal	43	31.38%	35	81.39%	8	18.61%	
	Overweight & Obesity	44	32.13%	37	84.09%	7	15.91%	
Sex	Male	105	76.64%	85	80.95%	20	19.05%	0.271[a]
	Female	32	23.36%	23	71.87%	9	28.13%	
Marital status	Single	104	75.91%	78	75.00%	26	25.00%	0.051[a]
	Married	33	24.09%	30	90.90%	3	9.10%	
Referred by	Blood Transfusion Centers	46	33.58%	36	78.26%	10	21.74%	0.896[a]
	Hepatitis Clinic	27	19.71%	22	81.48%	5	18.52%	
	Hospital	37	27.00%	30	81.08%	7	18.92%	
	Healthcare Facilities	27	19.71%	20	74.07%	7	25.93%	
Education	High school diploma or higher education	42	34.14%	33	78.57%	9	21.43%	0.955[a]
	Under high school diploma	81	65.86%	64	79.01%	17	20.99%	
Province Category	Fars	116	84.67%	94	81.03%	22	18.97%	0.152[b]
	Other Province	21	15.33%	14	66.67 %	7	33.33%	
Hepatitis B Family History	Yes	53	38.69%	42	79.25%	11	20.75%	0.925[a]
	No	84	61.31%	66	78.57%	18	21.43%	
Blood Transfusion	Yes	12	8.76%	11	91.67%	1	8.33%	0.460[b]
	No	125	91.24%	97	77.60%	28	22.40%	
Dental Procedure	Yes	105	76.64%	86	81.90%	19	18.10%	0.111[a]
	No	32	23.36%	22	68.75%	10	31.25%	
War Injury	Yes	13	9.49%	13	100.00%	0	0.00%	0.070[b]
	No	124	90.51%	95	76.61%	29	23.39%	
Sharp Trauma	Yes	6	4.38%	5	83.33%	1	16.67%	>0.999[b]
	No	131	95.62%	103	78.63%	28	21.37%	
Tattoo	Yes	18	13.14%	15	83.33%	3	16.67%	0.764[b]
	No	119	86.86%	93	78.15%	26	21.85%	
Cupping	Yes	7	5.11%	7	100.00%	0	0.00%	0.345[b]
	No	130	94.89%	101	77.69%	29	22.31%	
Multiple sexual partners	Yes	13	9.49%	12	92.31%	1	7.69%	0.299[b]
	No	124	90.51%	96	77.42%	28	22.58%	
Prison	Yes	8	5.84%	8	100.00%	0	0.00%	0.203[b]
	No	129	94.16%	100	77.52%	29	22.48%	
Cigarette Smoking	Yes	22	16.06%	20	90.90%	2	9.10%	0.162[b]
	No	115	83.94%	88	76.52%	27	23.48%	
Hookah	Yes	15	10.95%	15	100.00%	0	0.00%	0.040[b]
	No	122	89.05%	93	76.23%	29	23.77%	
Drug Abuse Addiction	Yes	19	13.87%	16	84.21%	3	15.79%	0.764[b]
	No	118	86.13%	92	77.97%	26	22.03%	
Alcohol Consumption	Yes	14	10.22%	13	92.86%	1	7.14%	0.300[b]
	No	123	89.78%	95	77.24%	28	22.76%	

HDV⁻: Hepatitis D virus negative; HDV⁺: Hepatitis D virus positive; BMI: Body mass index; Values are expressed as mean±SD or number (%). [a]Pearson Chi square test; [b]Fisher's exact test, [c]Mann-Whitney U test; P<0.05 was considered statistically significant.

Table 2: Multiple logistic regression model

Variables	OR (95% CI)	P value
Age	1.06 (1.019, 1.102)	0.003
Marital status (Single)	0.446 (0.112, 1.769)	0.250
Province (Other provinces)	0.577 (0.190, 1.754)	0.333
Dental Procedure (No)	0.290 (0.101, 0.836)	0.022
Cigarette (No)	0.414 (0.086, 2.001)	0.273

The reference category was indicated within the parenthesis. P<0.2 was considered significant.

were included in a multiple logistic regression model to estimate the odds ratios (OR) and the corresponding 95% confidence intervals (CI). P<0.05 was considered statistically significant.

Results

Out of 3,787 individuals with hepatitis B, 137 (3.6%) cases had a low HBV viral load but elevated liver enzymes and were tested for HDV. Among them, 29 tested positive for HDV, and 108 tested negative. Among the 137 cases, one patient who tested positive for HDV progressed to liver cirrhosis. Additionally, 24 of the 137 individuals had received hepatitis B vaccinations, with five of these testing positive for hepatitis D. None of the 137 participants had a history of diabetes, hypertension, or high cholesterol. Table 1 summarizes the patients' details.

The distribution of age and hookah use was statistically significant between the groups. The mean age in the hepatitis D^+ group was higher than in the hepatitis D^- group. All hookah users were in the hepatitis D^- group. Other factors, including family history of hepatitis B, blood transfusion, dental procedures, war injury, sharp trauma, tattooing, cupping therapy, risky sexual behavior, incarceration, smoking, drug use, and alcohol consumption, were more prevalent in the hepatitis D^- group. However, these differences were not statistically significant.

Most cases in the hepatitis D^+ group were related to the underweight group, accounting for 48.2% of all hepatitis D^+ cases. In contrast, the majority of hepatitis D^- cases (34.2%) were associated with overweight and obesity. Men comprised the majority of the population, in both the hepatitis D^+ and D^- groups.

The multiple logistic regression model shows that for each one-year increase in age, the likelihood of contracting hepatitis D significantly increases by approximately 6% (table 2). Additionally, a history of visiting a dentist significantly reduces the likelihood of contracting the disease by approximately 71%. No statistically significant associations were observed for the variables of marital status, province, and smoking. However, the model showed that married individuals had about a 55% lower likelihood of contracting the disease than single individuals. Furthermore, residents of Fars Province were approximately 42% less likely to contract the disease than residents of other provinces in the country. Additionally, smokers were found to have about 59% lower likelihood of contracting the disease than non-smokers.

Discussion

The findings of this study provided insights into the epidemiology of HDV and its associated factors in the southern region of Iran. In the study population, 21.2% of HBV-infected patients tested positive for HDV (29 out of 137 screened). Age was identified as a significant risk factor for HDV infection. Specifically, the probability of contracting HDV increased by approximately 6% with each one-year increase in age. Dental procedures were found to be a protective factor against HDV infection, with individuals who had a history of dental procedures being about 71% less likely to contract HDV. Other factors investigated in this study, such as sex, race, nationality, place of residence, marital status, family history of hepatitis B, multiple sexual partners, hemodialysis, blood transfusions, cupping therapy, smoking, hookah use, substance abuse, alcohol consumption, war injuries, and tattoos, indicated no statistically significant association with HDV positivity.

The findings of the present study indicated that each one-year increase in age was associated with an elevated risk of HDV infection. This finding was consistent with previous studies conducted by Tahaei and colleagues, Makhlouf and others, and Zi and colleagues, who all reported a higher mean age among individuals with hepatitis D than those with hepatitis B who were not infected with hepatitis D.[14, 18, 19] However, the findings contradicted another study conducted in Nigeria, which found that those who were positive for hepatitis D had a lower mean age than those who were negative for hepatitis D.[20] The underlying reason for the increased risk of hepatitis infection with advancing age might be attributed to immunosenescence, a phenomenon characterized by age-related changes in

immunological functions. Immunosenescence is associated with thymic involution and a decline in the number and activity of T and B cells. While the number of memory T and B cells typically increases, their capacity to respond effectively to new antigens decreases. Moreover, despite their normal or increasing numbers, the granulocytes, monocytes/macrophages, and NK cells could lose functionalities.[21]

In the population of 29 individuals, 19 (65.5%) patients had a history of visiting a dentist and undergoing dental procedures. At first glance, these statistics might suggest that dental visits could increase the risk of infection, which could easily be explained by the mode of transmission of the disease via serum and contaminated dental equipment, consistent with several other studies.[22, 23] However, when this variable was analyzed using the multiple logistic model, it not only did not appear to be a risk factor, but it also resulted in a nearly 71% reduction in the risk of contracting hepatitis D infection. This could be explained by the fact that individuals who visit dentists often have a higher socioeconomic status, which is usually associated with better living conditions, diagnostic, preventive, and medical services, as well as a better lifestyle; all of which could have a protective and preventive effect against Hepatitis D.[24, 25]

Among the population of 29 hepatitis D cases, most individuals were men (68.9%), and they accounted for a larger proportion of those infected with hepatitis D. This finding was consistent with various other studies indicating a greater prevalence of hepatitis D among men.[14, 26] Women typically have more powerful humoral and cell-mediated immune reactions to antigenic stimulation, vaccination, and infection than men.[27] Nevertheless, it is crucial to acknowledge that the elevated risk factors in men, including smoking, alcohol consumption, tattooing, drug injection, and engaging in unsafe sexual practices, contribute to their increased vulnerability to hepatitis D.

Out of 29 individuals with hepatitis D, 26 (89.6%) were unmarried. This finding was also consistent with a previous study, which showed that the proportion of unmarried individuals among those infected with hepatitis D was approximately 70%.[28] However, the findings of the present study differed from those conducted by Kpossou and colleagues in Cotonou, which reported a higher incidence of hepatitis D infection among married individuals.[29] The higher risk in single individuals could potentially be attributed to various factors. Cortisol, a hormone associated with stress levels, tends to be lower in married individuals than in single persons.[30] This is advantageous as elevated cortisol levels can interfere with immune system function. Additionally, married individuals often lead healthier and mentally sound lifestyles than unmarried individuals, engaging in fewer risky behaviors such as substance abuse and alcohol consumption.[30-32] Marriage can also promote health-promoting behaviors.

In both studied populations, the majority of the people had a high school diploma or less. In the group of 108 individuals, 64 (60.0%) had an education level lower than a high school diploma, and in the group of 29 individuals, 17 (58.6%) had an education level lower than a high school diploma. This finding was consistent with the results of a study by Daw and colleagues, which identified low educational level as a risk factor.[28] Additionally, in another study conducted in Mongolia, higher levels of education were associated with a lower prevalence of hepatitis D in the studied population, indicating that education and awareness played a role in disease prevention.[33] One possible explanation for this association was that individuals with higher education levels were likely to have a better understanding of disease prevention methods, risk factors, and health interventions. They might be more knowledgeable about the importance of practicing safe behaviors and taking preventive measures. Furthermore, individuals with higher education levels tend to have a higher occupational status and income in society, which could lead to better living conditions and fewer health-related constraints.

A family history of hepatitis B, blood transfusions, trauma from sharp objects, tattoos, multiple sexual partners, cigarette smoking, and intravenous drug use have all been identified in numerous studies as risk factors for hepatitis D infection.[6, 12, 34, 35] Since hepatitis D is primarily transmitted through contact with contaminated bodily fluids, such as blood or sexual fluids, it is understandable that these risk factors contribute to an increased risk of infection. These factors provide opportunities for the virus to be transmitted through direct contact with infected fluids. However, in the present study, we were unable to find a significant relationship between the aforementioned variables and hepatitis D. Various mechanisms have been proposed in different studies for the aforementioned factors, and the present study has reviewed some of them here. Several studies have mentioned the following factors as to why smoking a single cigarette is a risk factor: Cigarette components possess cytotoxic properties that can activate stellate cells in the liver, leading to fibrosis.[36, 37] Smoking was shown to elevate

the levels of proinflammatory cytokines, including Interleukin-1 (IL-1), Interleukin-6 (IL-6), Interleukin-8 (IL-8), and Tumor necrosis factor-alpha (TNF-α), which can damage liver cells.[36, 38] Additionally, smoking indirectly contributes to secondary polycythemia, characterized by increased carboxyhemoglobin levels that reduce tissue oxygen-carrying capacity, which triggers the release of elevated levels of erythropoietin, and results in an increase in red cell mass. The process of increased red cell destruction, along with excess erythropoietin, promotes additional iron absorption from the intestines, leading to iron accumulation in hepatocytes. This accumulation can cause oxidative stress and contribute to liver injury.[38] Furthermore, nicotine, a component of tobacco smoke, inhibits lymphocyte proliferation, thereby suppressing antibody production.[39] Smoking was found to induce lymphocyte apoptosis, increase cytotoxic T-cell levels, decrease clusters of differentiation 4 (CD4) levels, and impair natural killer cell activity.[40] All of them could reduce the body's defense against infections. Moreover, tobacco smoking was associated with reduced expression of p53, a tumor-suppressing gene involved in various types of neoplasms.[38]

This study had several limitations. Firstly, the population of hepatitis D patients in this study consisted of referred patients from all parts of Fars Province, and due to the lack of a random sampling procedure, they are not representative. The relatively small sample size (primarily due to the rarity of the disease) and the imbalance in sample size between the hepatitis D positive and negative groups resulted in the majority of factors being non-significant in the initial analysis. Consequently, the model identified only two variables as significant contributors. Moreover, the study was confined to Fars Province (Iran), making it challenging to generalize the findings to other regions or provinces due to potential variations in hepatitis D epidemiological characteristics. Additionally, although associations between risk factors and HDV infection were established, the presence of other influencing and confounding factors made it difficult to determine definitive causal relationships. It is worth mentioning that the rarity of this disease limits the number of cases available for analysis and hinders the broader use of multiple regression analysis, which could provide a more comprehensive understanding of the simultaneous effects of various risk factors. Another limitation that could increase the risk of errors in the study related to variables associated with addiction and sexual behavior. Participants might misreport information related to these variables due to reasons such as lack of trust, feelings of shame, concerns about social and legal consequences, and a fear of being judged.

It is noteworthy that out of 108 individuals with negative HDV, similar to the 29 positive HDV cases, all were HBV-infected. Despite HBV treatment, they still had elevated liver enzymes and risk factors for HBV infection. They were compared to HDV-positive individuals in this study.

Conclusion

The prevalence of HDV positivity among individuals infected with HBV in the study population was high, and increasing age was identified as a significant risk factor for HDV infection. In contrast, undergoing dental procedures acted as a protective factor against HDV infection. However, to generalize these results and gain a better understanding of the epidemiology, further comprehensive research is required, focusing on distinct demographic groups in different regions. Therefore, it is suggested that further studies be conducted in this area. Such studies can contribute to better public health strategies, more targeted and cost-effective screening programs, and improved diagnosis, ultimately leading to a reduction in the burden of the disease.

Acknowledgment

We would like to extend our appreciation to Shiraz University of Medical Sciences for their assistance during this research.

Authors' Contribution

AR.S: Study design, monitoring the project, and reviewing the manuscript; A.Sh: Data analysis and interpretation, drafting, and reviewing the manuscript; MR.F: Data analysis and interpretation, drafting, and reviewing the manuscript; E.S: Data analysis and interpretation, drafting, and reviewing the manuscript; M.A: Data gathering, data analysis, and reviewing the manuscript; L.A: Data gathering, data analysis, and reviewing the manuscript; N.N: Data gathering, data analysis, and reviewing the manuscript; E.FA: Data gathering, data analysis, and reviewing the manuscript; SA.Sh: Data gathering, data analysis, and reviewing the manuscript; H.A: Data gathering, data analysis, and reviewing the manuscript; Y.N: Study design, monitoring the project, and reviewing the manuscript.

All authors have read and approved the final manuscript and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Conflict of Interest: None declared.

References

1 Odenwald MA, Paul S. Viral hepatitis: Past, present, and future. World J Gastroenterol. 2022;28:1405-29. doi: 10.3748/wjg.v28.i14.1405. PubMed PMID: 35582678; PubMed Central PMCID: PMCPMC9048475.

2 Lee AU, Lee C. Hepatitis D Review: Challenges for the Resource-Poor Setting. Viruses. 2021;13. doi: 10.3390/v13101912. PubMed PMID: 34696341; PubMed Central PMCID: PMCPMC8538672.

3 Li W, Urban S. Entry of hepatitis B and hepatitis D virus into hepatocytes: Basic insights and clinical implications. J Hepatol. 2016;64:S32-S40. doi: 10.1016/j.jhep.2016.02.011. PubMed PMID: 27084034; PubMed Central PMCID: PMCPMC7114860.

4 Sureau C, Negro F. The hepatitis delta virus: Replication and pathogenesis. J Hepatol. 2016;64:S102-S16. doi: 10.1016/j.jhep.2016.02.013. PubMed PMID: 27084031.

5 Negro F. Hepatitis D virus coinfection and superinfection. Cold Spring Harb Perspect Med. 2014;4:a021550. doi: 10.1101/cshperspect.a021550. PubMed PMID: 25368018; PubMed Central PMCID: PMCPMC4208707.

6 Patel EU, Thio CL, Boon D, Thomas DL, Tobian AAR. Prevalence of Hepatitis B and Hepatitis D Virus Infections in the United States, 2011-2016. Clin Infect Dis. 2019;69:709-12. doi: 10.1093/cid/ciz001. PubMed PMID: 30605508; PubMed Central PMCID: PMCPMC6669285.

7 Dandri M, Volmari A, Lutgehetmann M. The hepatitis delta virus and chronic hepatitis D. J Hepatol. 2022;77:1448-50. doi: 10.1016/j.jhep.2022.05.022. PubMed PMID: 35850738.

8 Taylor JM. Infection by Hepatitis Delta Virus. Viruses. 2020;12. doi: 10.3390/v12060648. PubMed PMID: 32560053; PubMed Central PMCID: PMCPMC7354607.

9 Organization WH [Internet]. Hepatitis D. [cited 12 October 2023]. Available from: https://www.who.int/news-room/fact-sheets/detail/hepatitis-d

10 Pisano MB, Giadans CG, Flichman DM, Re VE, Preciado MV, Valva P. Viral hepatitis update: Progress and perspectives. World J Gastroenterol. 2021;27:4018-44. doi: 10.3748/wjg.v27.i26.4018. PubMed PMID: 34326611; PubMed Central PMCID: PMCPMC8311538.

11 Da BL, Rahman F, Lai WC, Kleiner DE, Heller T, Koh C. Risk Factors for Delta Hepatitis in a North American Cohort: Who Should Be Screened? Am J Gastroenterol. 2021;116:206-9. doi: 10.14309/ajg.0000000000000954. PubMed PMID: 33027083; PubMed Central PMCID: PMCPMC9205618.

12 Stockdale AJ, Kreuels B, Henrion MYR, Giorgi E, Kyomuhangi I, de Martel C, et al. The global prevalence of hepatitis D virus infection: Systematic review and meta-analysis. J Hepatol. 2020;73:523-32. doi: 10.1016/j.jhep.2020.04.008. PubMed PMID: 32335166; PubMed Central PMCID: PMCPMC7438974.

13 Castaneda D, Gonzalez AJ, Alomari M, Tandon K, Zervos XB. From hepatitis A to E: A critical review of viral hepatitis. World J Gastroenterol. 2021;27:1691-715. doi: 10.3748/wjg.v27.i16.1691. PubMed PMID: 33967551; PubMed Central PMCID: PMCPMC8072198.

14 Tahaei SM, Mohebbi SR, Azimzadeh P, Behelgardi A, Sanati A, Mohammadi P, et al. Prevalence of hepatitis D virus in hepatitis B virus infected patients referred to Taleghani hospital, Tehran, Iran. Gastroenterol Hepatol Bed Bench. 2014;7:144-50. PubMed PMID: 25120894; PubMed Central PMCID: PMCPMC4129564.

15 Pouri AA, Ghojazadeh M, Baiaz B, Hamzavi FS, Pourasghari B, Somi MH. Prevalence of hepatitis D virus among HBsAg-positive individuals, 2015-2016: Azar cohort study. Health Promot Perspect. 2020;10:38-42. doi: 10.15171/hpp.2020.07. PubMed PMID: 32104655; PubMed Central PMCID: PMCPMC7036205.

16 Sayad B, Naderi Y, Alavian SM, Najafi F, Janbakhsh A, Mansouri F, et al. Hepatitis D virus infection in Kermanshah, west of Iran: seroprevalence and viremic infections. Gastroenterol Hepatol Bed Bench. 2018;11:145-52. PubMed PMID: 29910856; PubMed Central PMCID: PMCPMC5990919.

17 Torang T, Khanlepour M, Mohammadreza M, Hafez TGF, Iraj M, Khalilian A. Investigation of the prevalence of hepatitis D virus in HBs Ag positive patients and investigation of related factors in Sari city during 2015-2016 (short report). Journal of Mazandaran University of Medical Sciences. 2016;18:102-6. Persian.

18 Makhlouf NA, Morsy KH, Mahmoud AA. Hepatitis D virus infection among hepatitis B virus surface antigen positive individuals in Upper Egypt: Prevalence and clinical features. J Infect Public Health. 2019;12:350-6. doi: 10.1016/j.jiph.2018.12.001. PubMed PMID: 30833193.
19 Zi J, Li YH, Wang XM, Xu HQ, Liu WH, Cui JY, et al. Hepatitis D virus dual-infection among Chinese hepatitis B patient related to hepatitis B surface antigen, hepatitis B virus DNA and age. World J Gastroenterol. 2023;29:5395-405. doi: 10.3748/wjg.v29.i38.5395. PubMed PMID: 37900584; PubMed Central PMCID: PMCPMC10600800.
20 Okonkwo UC, Okpara HC, Inaku K, Aluka TM, Chukwudike ES, Ogarekpe Y, et al. Prevalence and risk factors of Hepatitis D virus antibody among asymptomatic carriers of Hepatitis B virus: a community survey. Afr Health Sci. 2022;22:504-10. doi: 10.4314/ahs.v22i1.59. PubMed PMID: 36032492; PubMed Central PMCID: PMCPMC9382513.
21 Rink L, Wessels I. Immunosenescence. In: Rezaei N, editor. Encyclopedia of Infection and Immunity. Oxford: Elsevier; 2022. p. 259-76.
22 Ziaee M, Azarkar G. Prevalence of hepatitis d virus infection among patients with chronic hepatitis B attending birjand hepatitis clinic (East of iran) in 2012. Hepat Mon. 2013;13:e11168. doi: 10.5812/hepatmon.11168. PubMed PMID: 24171009; PubMed Central PMCID: PMCPMC3800676.
23 Su CW, Ochirkhuree B, Namdag B, Badamnachin B, Ganbold S, Gidaagaya S, et al. Risk factors associated with hepatitis D virus infection and preventive strategies in Mongolia. J Chin Med Assoc. 2024;87:480-7. doi: 10.1097/JCMA.0000000000001073. PubMed PMID: 38417133.
24 Wang J, Geng L. Effects of Socioeconomic Status on Physical and Psychological Health: Lifestyle as a Mediator. Int J Environ Res Public Health. 2019;16. doi: 10.3390/ijerph16020281. PubMed PMID: 30669511; PubMed Central PMCID: PMCPMC6352250.
25 Najafi E, Amini-Rarani M, Moeeni M. Inequality in dental care expenditure in Iranian households: analysis of income quintiles and educational levels. BMC Oral Health. 2021;21:550. doi: 10.1186/s12903-021-01912-6. PubMed PMID: 34702242; PubMed Central PMCID: PMCPMC8549140.
26 Osiowy C, Swidinsky K, Haylock-Jacobs S, Sadler MD, Fung S, Wong D, et al. Molecular epidemiology and clinical characteristics of hepatitis D virus infection in Canada. JHEP Rep. 2022;4:100461. doi: 10.1016/j.jhepr.2022.100461. PubMed PMID: 35360523; PubMed Central PMCID: PMCPMC8961228.
27 Klein SL, Morgan R. The impact of sex and gender on immunotherapy outcomes. Biol Sex Differ. 2020;11:24. doi: 10.1186/s13293-020-00301-y. PubMed PMID: 32366281; PubMed Central PMCID: PMCPMC7197158.
28 Daw MA, Daw AM, Sifennasr NEM, Draha A, Daw A, Daw A, et al. The epidemiological characterization and geographic distribution of hepatitis D virus infection in Libya. Pan Afr Med J. 2020;35:120. doi: 10.11604/pamj.2020.35.120.20055. PubMed PMID: 32637018; PubMed Central PMCID: PMCPMC7320781.
29 Kpossou AR, Sogbo F, Zomahoun AMO, Alassan KS, Vignon RK, Sokpon CNdM, et al. Hepatitis D in patients infected with Hepatitis B Virus in cotonou: Characteristics and risk factors. Open Journal of Gastroenterology. 2020;10:31-44. doi: 10.4236/ojgas.2020.102004.
30 Chin B, Murphy MLM, Janicki-Deverts D, Cohen S. Marital status as a predictor of diurnal salivary cortisol levels and slopes in a community sample of healthy adults. Psychoneuroendocrinology. 2017;78:68-75. doi: 10.1016/j.psyneuen.2017.01.016. PubMed PMID: 28171850; PubMed Central PMCID: PMCPMC5365082.
31 Salvatore JE, Gardner CO, Kendler KS. Marriage and reductions in men's alcohol, tobacco, and cannabis use. Psychol Med. 2020;50:2634-40. doi: 10.1017/S0033291719002964. PubMed PMID: 31685061; PubMed Central PMCID: PMCPMC7198324.
32 Schoeppe S, Vandelanotte C, Rebar AL, Hayman M, Duncan MJ, Alley SJ. Do singles or couples live healthier lifestyles? Trends in Queensland between 2005-2014. PLoS One. 2018;13:e0192584. doi: 10.1371/journal.pone.0192584. PubMed PMID: 29489832; PubMed Central PMCID: PMCPMC5830314.
33 Dambadarjaa D, Mukhtar Y, Tsogzolbaatar EO, Khuyag SO, Dayan A, Oyunbileg NE, et al. Hepatitis B, C, and D Virus Infections and AFP Tumor Marker Prevalence Among the Elderly Population in Mongolia: A Nationwide Survey. J Prev Med Public Health. 2022;55:263-72. doi: 10.3961/jpmph.21.573. PubMed PMID: 35678000; PubMed Central PMCID: PMCPMC9201085.
34 Rizzetto M. Hepatitis D Virus: Introduction and Epidemiology. Cold Spring Harb Perspect Med. 2015;5:a021576. doi:

10.1101/cshperspect.a021576. PubMed PMID: 26134842; PubMed Central PMCID: PMCPMC4484953.

35 Chen HY, Shen DT, Ji DZ, Han PC, Zhang WM, Ma JF, et al. Prevalence and burden of hepatitis D virus infection in the global population: a systematic review and meta-analysis. Gut. 2019;68:512-21. doi: 10.1136/gutjnl-2018-316601. PubMed PMID: 30228220.

36 Battah KA, Badranc DH, Shraideh ZA. Effect of Cigarette Smoking on the Structure of Hepatocytes: TEM Study. International Journal of Morphology. 2016;34. doi: 10.4067/S0717-95022016000400011.

37 Wen J, Zhou Y, Wang J, Chen J, Yan W, Wu J, et al. Interactions between Th1 cells and Tregs affect regulation of hepatic fibrosis in biliary atresia through the IFN-gamma/STAT1 pathway. Cell Death Differ. 2017;24:997-1006. doi: 10.1038/cdd.2017.31. PubMed PMID: 28304404; PubMed Central PMCID: PMCPMC5442468.

38 Rutledge SM, Asgharpour A. Smoking and Liver Disease. Gastroenterol Hepatol (N Y). 2020;16:617-25. PubMed PMID: 34035697; PubMed Central PMCID: PMCPMC8132692.

39 Mahmoudzadeh L, Abtahi Froushani SM, Ajami M, Mahmoudzadeh M. Effect of Nicotine on Immune System Function. Adv Pharm Bull. 2023;13:69-78. doi: 10.34172/apb.2023.008. PubMed PMID: 36721811; PubMed Central PMCID: PMCPMC9871277.

40 Qiu F, Liang CL, Liu H, Zeng YQ, Hou S, Huang S, et al. Impacts of cigarette smoking on immune responsiveness: Up and down or upside down? Oncotarget. 2017;8:268-84. doi: 10.18632/oncotarget.13613. PubMed PMID: 27902485; PubMed Central PMCID: PMCPMC5352117.