Prevalence of Hepatitis B and C Infections and Associated Risk Factors in Pars Cohort Study, Southern Iran

Sanam Hariri†, 1, 2, Sabereh Davari†, 1, 3, Zeinab Malekzadeh 2, 4, Zahra Mohammadi 1, Sahar Masoudi 5, Abdullah Gandomkar 4, Sedigheh Amini-Kafiabad 6, Mahtab Maghsoudloo 6, Shahin Merat 1, 2, Hossein Poustchi 1, Fatemeh Malekzadeh 2-4,*

ABSTRACT

BACKGROUND
Hepatitis B and C virus (HBV and HCV) infections rank among the most frequent infectious diseases with a rising worldwide burden. However, their epidemiology and risk factors are understudied in many regions, including Iran.

METHODS
This study was conducted as part of the Pars Cohort Study (PCS) in Valashahr district, Fars province (2012-2014). Participants received venipuncture for HBsAg and HCV antibody, followed by Polymerase Chain Reaction (PCR) testing. All infected people and their comparison groups completed a risk assessment questionnaire.

RESULTS
Overall, 9,269 people participated in the study; the majority were women and of Fars ethnicity. Prevalence of HBsAg and HCV antibody was 2.3% (n = 215) and 0.3% (n = 26), from whom 23% (n = 47) and 13% (n = 3) had indications for treatment, respectively. During follow-up, among HBsAg-positive individuals who were not on treatment, 62% tested negative for HBsAg, and in 2% HBV DNA had risen to treatment levels. Risk factors for HBV infection were illiteracy [OR = 3.43, 95% CI = 1.1, 10.3], and Turk ethnicity compared to Fars [OR = 1.58, 95% CI = 1.1, 2.3]. History of blood transfusion [OR = 2.00, 95% CI = 1.1, 3.5] and history of drug use [OR = 2.85, 95% CI = 1.1, 7.4] were associated with HCV infection, after adjustment.

CONCLUSION
Further epidemiological studies are needed to identify at-risk populations in different regions. Preventive interventions, including educational programs and transfusion safety strategies, are crucial for reducing the hepatitis burden.

KEYWORDS:
Hepatitis B virus (HBV), Hepatitis C virus (HCV), Prevalence, Risk factors, Treatment, Iran

INTRODUCTION
Viral hepatitis is the fifth leading cause of mortality in the Middle East and...
North Africa (MENA) region with a rising worldwide burden.\textsuperscript{1,2} Hepatitis B and C viruses (HBV and HCV) are the main contributors to chronic liver conditions and liver-related fatalities.\textsuperscript{1} The recent breakthrough in direct-acting antiviral treatments provides an opportunity to achieve elimination targets of the World Health Organization (WHO) by 2030.\textsuperscript{3} Since infected patients are mostly asymptomatic and diagnosed through screening efforts or accidentally, identifying the populations at higher risk is crucial as a preliminary step towards elimination.\textsuperscript{9} However, knowledge of regional epidemiology that is required to guide current control efforts is still inadequate.\textsuperscript{2}

In the MENA region, Iran is hosting the highest proportion of people who inject drugs, which are a major population at risk for acquiring blood-borne infections.\textsuperscript{5} Sexual and mother-to-child transmission are the main routes of HBV spread in Iran, while ongoing HCV transmission seems to be driven by healthcare procedures and injecting drug use.\textsuperscript{6-8} In the recent decade, vaccination programs, transfusion safety strategies, new treatments, and increased public knowledge, have led to a significant reduction in the endemicity.\textsuperscript{9} Therefore, information on the epidemiology and risk factors of viral hepatitis in the Iranian population needs revision.\textsuperscript{10,11} Recently, this country has been classified within the low-prevalent areas for the HBV infection (<2%),\textsuperscript{12,13} the prevalence of HCV is even less, and 0.6% of the general population carry antibodies.\textsuperscript{14} However, more population-based studies are required to replicate the estimates of prior studies in different regions and ethnic groups across Iran.

This study was conducted as part of the Pars Cohort Study (PCS), which investigates the epidemiology of non-communicable diseases in southern Iran.\textsuperscript{15} We designed the current study because of the inconsistency and limited evidence on viral hepatitis epidemiology and risk factors in Iran. Such evidence could help policymakers with budget priorities and developing tailored strategies to meet the elimination targets in middle-income countries.

**MATERIALS AND METHODS**

**Study design**

The main goals of PCS were to assess the prevalence, risk factors, and burden of non-communicable diseases at baseline and a prospective 10-year follow-up in Southern Iran. Enrollment was occurred between 2012 and 2014. All residents aged 40-75 years were invited to participate and were eligible, given they had provided written consent. The protocol of the current study was approved by the National Institute for Medical Research Development [Ethics code: IR.NIMAD.REC.1397.156].

**Study setting**

The study setting was Valashahr district, Fars province, Southern Iran. The district consists of five counties and the city of Valashahr, with 93 villages and more than 40,000 inhabitants, mainly of Fars and Turk ethnicities. During the two years of enrollment, 9,264 people visited the study center for an interview and provided biological samples.

**Study procedures**

For each subject, a trained interviewer collected data by a structured questionnaire and a thorough physical examination. The PCS questionnaire had more than 180 variables, including hepatitis risk assessment questions that were used in the current study. Samples were collected according to the Iranian Blood Transfusion Organization (IBTO) guidelines. Details of the study protocol have been published elsewhere.\textsuperscript{15} All participants received venipuncture, and 500 µl blood was obtained for Enzyme-Linked Immuno-Sorbent Assay (ELISA). Serum was analyzed for the HBV surface antigen (HBsAg) (Murex HBsAg version 3, DiaSorin) and HCV antibody (Siemens, Germany), using available commercial kits. Positive test results were further investigated by Polymerase Chain Reaction (PCR) assay for HBV DNA or HCV RNA. Liver enzymes, including aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), and gamma-glutamyl transferase (GGT) were also measured for all participants.

For people with positive HBsAg or HCV RNA, liver sonography and FibroScan® (502 Touch; Echosens, Paris, France) were performed. The diagnosis and management were according to the European Association for the Study of the Liver (EASL) guidelines. For people with HBV or HCV infection who needed to be treated, treatment was initiated by locally manufactured Tenofovir (Tenobiovir®, Bakhtar Bioshimi, Tehran, Iran) and a
fixed-dose combination of Sofosbuvir 400/Daclatasvir 60 (Sovodak®, Rojan Pharma, Tehran, Iran), respectively. Follow-up appointments, sonography, and laboratory testing were planned for all patients. Sustained Virological Response was evaluated among HCV patients, defined as a PCR testing 12 weeks after treatment completion (SVR12).

People with positive HBsAg or HCV antibody and their comparison groups (with negative test results) filled in a risk assessment questionnaire, including 15 risk factors for hepatitis. We also compared the groups concerning age, sex, marital status, ethnicity, education, and history of cigarette smoking.

**Statistical analysis**

Data are expressed as mean±SD or frequency and percentage, as appropriate. Differences in frequencies were evaluated by simple contingency table analysis (Fisher’s exact test, probability test, and χ² test), and continuous variables were assessed with the t test or Mann–Whitney U test using STATA software, version 12.0 (Stata Corp, College Station, TX, USA). The normality assumption was assessed using the Shapiro-Wick test. Odds ratios (OR) were computed using unconditional logistic regression models, with a 95% confidence interval (CIs). \( p < 0.05 \) were considered to be statistically significant.

**RESULTS**

Overall, 9,269 individuals aged 40-75 years participated in the study; the majority of whom were women (54%), of Fars ethnicity (56%), and married (89%), and almost half were illiterate (49%). Having a history of tobacco, opium, and alcohol use were reported in 21%, 8%, and 2% of all participants, respectively. Characteristics of the PCS participants have been detailed elsewhere.\(^{15}\)

The HbsAg was detected in 215 (2.3%) individuals and 206 participants had also available data on HBV DNA, of whom 47 (23%) needed treatment and all received medication, while 159 (77%) had no indications for treatment. The prevalence of HCV antibody was 0.3% (n = 26), and from the 24 people who had data available on PCR testing, 3 (13%) had positive HCV RNA results. Treatment uptake in both groups of patients was 100%. Characteristics of the infected individuals and the comparison groups are presented in Table 1. Overall, infected people were more likely to be men, married, and illiterate, and most had no history of cigarette smoking or alcohol use.

During follow-up, 40 (85%) patients who were on HBV treatment referred, and all were advised to continue taking medications. Among HBsAg-positive people with no treatment indications who attended the follow-up appointments (n = 95), 59 (62%) tested negative for HBsAg, and for 2 (2%) individuals, HBV DNA had risen to treatment levels. All patients with HCV completed their treatment course and achieved SVR12 (100%).

Overall, all risk factors were more frequent among infected people than non-infected individuals, except cupping (21% vs. 26%, respectively). Among those with positive HCV antibody, history of hospitalization, surgery, accident, and war injury was lower than their comparison group. History of transfusion was associated with having a positive HBsAg (\( p = 0.026 \)), and history of drug use was associated with having a positive HCV antibody test (\( p = 0.023 \)). No significant associations were observed for other factors. The frequency of all risk factors, categorized by HBV and HCV testing results, is shown in Table 2.

A higher prevalence of HBV infection was observed among people of Turk [OR = 1.58, 95% CI = 1.1, 2.3] or other ethnicities [OR = 3.31, 95% CI = 1.4, 7.7], compared to Fars ethnicity. Illiteracy was the other significant risk factor for HBV acquisition, compared to higher education (> 12 years) [OR = 3.43, 95% CI = 1.1, 10.3]. After adjusting for ethnicity and education, we observed that having a history of blood transfusion increases the risk of HBV infection [OR = 2.00, 95% CI = 1.1, 3.5]; this association was significant for Fars ethnicity (\( p = 0.009 \), Table 3).

The mean ± SD age of the participants with positive and negative HCV antibodies was 51 ± 9 and 55 ± 10, respectively. Older age was slightly correlated with a lower risk of HCV [OR = 0.95, 95% CI = 0.9, 1.0]. After adjusting for age, having a history of drug use was associated with an increased risk of HCV infection [OR = 2.85, 95% CI = 1.1, 7.4, Table 3].

The mean of AST was higher among people with positive HBsAg (21 vs. 18) and HCV antibody (22 vs. 19), compared to negatives. For ALT, the mean was higher among those with positive HCV antibody (25 vs. 22) but slightly lower in HBsAg-positive people (22 vs. 23). GGT in both
infected groups was lower compared to the non-infected. No associations were observed in laboratory tests, except for the AST enzyme that was significantly higher among infected participants ($p < 0.0001$, Table 4).

### DISCUSSION

This study investigates the prevalence of HBV and HBC in a semi-urban area in southern Iran and defines...
the role of sociodemographic characteristics and transmission risk factors. The prevalence of HbsAg and HCV antibody were 2.3% and 0.3%, respectively. Risk factors for HBV and HCV acquisition had little in common; Turk ethnicity, illiteracy, and history of blood transfusion showed significant associations with HBV, while having a history of drug use was associated with a higher risk of HCV infection.

Recently, Iran has shifted from low-intermediate to a low prevalence area for HBV.12,13 This changing epidemiology is related to the mass vaccination programs since 1993 among susceptible groups and indicates the efficiency of implemented interventions.16,17 Our observed prevalence is in line with the national estimations and higher compared to some parts of Iran, including Khorasan, Isfahan, Kurdistan, and East Azerbaijan.11,16 However, given that our study population was selected from age cohorts that received no birth dose HBV vaccine, these estimates may not represent the HbsAg prevalence in the general population of Iran.

The prevalence of HCV is half of the national reports and slightly higher compared to Mashhad and Mazandaran.8,14 The same study in Fars province has reported a prevalence of 0.24% for anti-HCV in two villages, which is very similar to our observation in a semi-urban area.18

The mean level of AST was significantly higher among infected people, compared to the comparison group. However, the mean level of ALT in people with positive HbsAg was lower than the negatives. This observation could be related to the higher Body Mass Index (BMI) levels in the healthy group;19 almost 66% of non-infected individuals were overweight or obese, but this

| Table 3: Odds ratio and 95% confidence interval for risk factors significantly associated with having a positive HbsAg or HCV antibody among the Pars Cohort Study (PCS) participants |
|---------------------------------|-----------------|-----------------|-----------------|
| HBV risk factors                | p value         | Odds ratio      | 95% confidence interval |
| Ethnicity                       |                 |                 |                 |
| Turk                            | 0.012           | 1.58            | (1.1, 2.3)      |
| Other                           | 0.006           | 3.31            | (1.4, 7.7)      |
| Education                       |                 |                 |                 |
| University                      | 0.005           |                 |                 |
| Illiterate                      | 0.028           | 3.43            | (1.1, 10.3)     |
| 9-12                            | 0.093           | 2.59            | (0.9, 7.9)      |
| 6-8                             | 0.387           | 1.70            | (0.5, 5.6)      |
| < 5 years                       | 0.649           | 1.33            | (0.4, 4.6)      |
| History of transfusion*         | Yes             | 0.018           | 2.00            | (1.1, 3.5)      |
| HCV risk factors                | p value         | Odds ratio      | 95% confidence interval |
| Age                             |                 | 0.043           | 0.95            |
| History of drug use**           |                 | 0.033           | 2.85            |

* Adjusted for ethnicity and education  ** Adjusted for age

| Table 4: Mean and standard deviation (SD) for laboratory tests in the Pars Cohort Study (PCS), including aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), and gamma-glutamyl transferase (GGT) |
|---------------------------------|----------------|-----------------|-----------------|----------------|----------------|
| Laboratory tests                | HBsAg | HCV antibody |
|                                 | Positive | Negative | Positive | Negative | Positive | Negative |
|                                 | Positive | Negative | Positive | Negative | Positive | Negative |
| Mean                            | 215    | 504      | 26       | 694      | 215    | 504      |
| SD                              | 9.40   | 10.78    | 14.67    | 28.81    | 9.40   | 10.78    |
| Mean                            | 20.73  | 22.76    | 26.00    | 33.26    |
| SD                              | 9.40   | 10.78    | 14.67    | 28.81    |
| ALT                             | 21.58  | 19.27    | 22.11    | 33.26    |
| ALP                             | 283    | 79       | 277      | 323      |
| GGT                             | 25.94  | 49.45    | 28.60    | 43.33    |
rate was 49% in the infected group.

Ethnicity is known to be associated with HCV infection and treatment outcomes, one study has reported a lower prevalence of HCV in Turk and Lor populations, compared to Fars. We found no associations for ethnicity regarding HCV acquisition; however, the prevalence of HBV in people of Turk and other ethnicities was significantly higher than Fars. One explanation could be that Turk and other ethnicities residing in the district are of lower socioeconomic status compared to Fars residents (illiteracy was 44% vs. 56%, respectively). Such findings represent the variations in prevalence, which could be attributed to the diversity in lifestyles and frequency of high-risk behaviors among different ethnic groups residing in Iran.

We observed a significant relationship between having a history of blood transfusion and the risk of HBV infection; this association was significant among the Fars ethnic group ($p = 0.009$). Substantial efforts of the national organization of blood transfusion in recent years have significantly reduced the prevalence rates. However, controlling the transmission via transfusion settings in the southern part of Iran is still of great concern. Also, having a history of drug use was significantly related to an increased risk of HCV infection. This observation is similar to many previous studies, highlighting the importance of HCV screening among people who have a history of drug use.

In line with previous studies, we found that people with higher education (> 12 years) have a remarkably lower risk for HBV compared to the illiterate. Older age was slightly correlated with a lower risk of HCV infection that was in line with the Azar cohort study, where the highest HCV prevalence was observed between ages 40 and 50 years. The positive HCV antibodies were equally distributed between both sexes, similar to the other study in Fars province. For HBV, the prevalence was 3.1% and 1.6% among men and women, respectively; this is also very similar to the reported rates of a recent meta-analysis in Iran. We found no significant difference between sexes regarding the acquisition of neither virus.

**Strengths and limitations**

The large population-representative sample size is the most significant strength of this study. The main limitation was the cross-sectional design, which could not identify the cause and effect relationship. Also, since our sample was limited to the age range of 40 to 75 years, data on the younger population were not available to compare the results.

**CONCLUSION**

The observed prevalence of HBV and HCV infections in a semi-urban area of southern Iran was comparable to the national estimations. Significant associations were found for several factors, including a history of blood transfusion and drug use, and Turk ethnicity. Future interventions should be directed towards controlling transmission in healthcare settings and among people who use drugs. Moreover, immunization should continue with more effort, and screening programs should concentrate on those high-risk ethnic groups for which screening is considered cost-effective. However, further studies among other populations in Iran are required for a better understanding of the epidemiology of viral hepatitis infection in different parts of the country.

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**ETHICAL APPROVAL**

There is nothing to be declared.

**CONFLICT OF INTEREST**

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

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