Epidemiologic profile of viral hepatitis B and C in North of Iran: Results from PERSIAN Guilan Cohort Study (PGCS)

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Research note

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Abstract

Objective: Hepatitis B (HBV) and C (HCV) viruses are two severe infectious diseases with a high global health impact. This study aimed to evaluate the prevalence of HBV and HCV in the Prospective Epidemiological Research Studies of the Iranian Adults (PERSIAN) Guilan Cohort Study through immunological and molecular methods. Results: The blood samples were obtained from 10520 enrolled participants. Complete biochemical and hematological assessments plus urine analysis were done. The presence of HBsAg, anti-HBs, anti-HBc, and anti-HCV antibodies for all participants and HBeAg and anti-HBe antibody for HBV positive patients were evaluated. HBV genomic DNA and HCV genomic RNA were extracted from positive serum samples. The real-time PCR assay was done to quantify HBV and HCV genomes. HCV genotyping was also performed. The HBV and HCV prevalence was 0.24% (95% CI, 0.16 % to 0.35%) and 0.11% (95% CI, 0.06 % to 0.19%), respectively. Rural participants were significantly more HBV positive than urban peoples (P=0.045) while male individuals were significantly more HCV positive than female participants (P=0.013). Our detected HBV and HCV prevalence were lower than other cities/provinces of Iran, which may be due to lifestyle or other unknown reasons.

Introduction

The hepatitis B (HB) is a viral infection whose target tissue is the liver tissue and can cause acute and chronic illnesses [1]. According to the 2016 World Health Organization (WHO) statistics, 240 million people who are positive for at least six months of HBsAg are reported as HB positive individuals [2]. Based on one modeling study in 2016, the global prevalence of HBsAg was 3.9% (95% uncertainty interval [UI] 3.4-4.6) [20]. Finally, the highest rates of hepatitis B are found in Africa and East Asia [3-6].

The hepatitis C (HC) is the leading cause of chronic liver disease, leading to chronic hepatocellular carcinoma with a high economic burden [7-13]. It has silent epidemiology and, at the same time, is a primary blood-borne infection worldwide [14-16]. According to the latest global health statistics, 130-150 million people are infected with HCV [17], and 700,000 people die every year [18]. Between 1990 and 2013, global viral hepatitis deaths increased from 0.89 million (95% uncertainty interval [UI] 0.86–0.94) to 1.45 million (1.38–1.54) [19].

Therefore, Iran is classified as a low to intermediate prevalence areas [21]. The last updated meta-analysis about HB prevalence in Iran shows that among the general population, approximately 2.2% in 2016 [22].

Although specific population sub-groups such as hemophiliacs and hemodialysis are more susceptible to HB and HC [23], evaluation of the prevalence of these two viruses in the general population also is critical. Considering the importance of hepatitis B and C in this study, the prevalence of these viruses in people referring to the cohort of Guilan province will be discussed.

Methods
Participants

The PERSIAN Guilan Cohort Study (PGCS) is a part of Prospective Epidemiological Research Studies of the Iranian Adults (PERSIAN) cohort study, started in September 2014 in Someh’ E Sara (GPS coordinator Latitude: 37.308003 & Longitude: 49.315022), Guilan, Northern of Iran and recruited both men and women aged 35-70 years, with the aim of subsequent follow for ten years [24,25].

This study was a cross-sectional study conducted on 10520 people who had complete baseline information to diagnose HB and HC.

Sampling and biochemical assessments

The aseptic blood samples were collected from the cubital vein into vacutainers. The total number of WBC, RBC, platelet, lymphocyte, monocyte, and granulocytes were counted. The serum sample was harvested and stored at -20 °C until use for complete biochemical assessment. The Hb concentration and level of Hct, MCV, MCH, MCHC, RDWCV, RDWSD, plateletcrit, MPV, PDW were also evaluated. A urine sample was collected and used immediately for measuring the specific gravity (SG), pH, and creatinine levels.

Virological assessments

The presence of HBsAg, anti-HBs and anti-HCV antibodies was determined by Electrochemiluminescence (Cobas e 411, Roche, Germany). For positive patients, these four tests plus the presence of HBeAg and anti-HBe antibodies were measured again. HB genomic DNA was extracted from positive serum samples by viral DNA extraction kit (QIAGEN, Germany). HC genomic RNA was also extracted from positive serum samples by viral RNA extraction kit (Roche, Germany). The qPCR assay was carried out using a TaqMan-based commercial available kit (QIAGEN, Germany) to quantify HB and HC genomes based on the manufacturer's instructions. HC genotyping was done using the HC Genotype plus Real-TM kit (Sacace Biotechnologies, Italy).

Ethical statement

This study was confirmed by the Ethics Committee of Guilan University of Medical Sciences (Ethics code: IR.GUMS.REC. 1396.254).

Statistical analysis

Qualitative data were expressed as frequency and percentage, and their association with HB and HC statuses were analyzed using the Chi-square test. Quantitative data were presented as mean ± SD, and the differences between HB/HC positive and negative groups were analyzed using two independent sample t-test. All statistical analysis was performed using SPSS version 23. The P-value of \( \leq 0.05 \) was considered statistically significant.
Results

The demographic characteristics of our patients and the statuses of HB and HC infection are presented in Table 1. Most of our participants were female (53.5%), rural (56.1%), married (97.2%) with primary education (< 12 years) (72.1%) without smoking (75.2%) or alcohol consumption (85.3%). Besides, most of them had a history of surgery (63.3%) and hospitalization (80.6%) and had no transfusion (89.5%) or genital aphthous (98.8%).

Table 1. Mean and SD of quantitative variables plus frequency and percentage of qualitative variables in total participants and based on HB and HC statuses.

| Variables                  | HB                  |                   |          |          | P value | HB                  |                   |          |          | P value |
|----------------------------|---------------------|-------------------|----------|----------|---------|---------------------|-------------------|----------|----------|---------|
| Age (year)                 | 51.52±8.90          | 54.48±5.04        | 51.51±8.90 | 0.095    | 54.08±10.79 | 51.51±8.89          | 0.317    |          |          |         |
| BMI (kg/m²)                | 28.15±5.82          | 26.32±4.00        | 28.16±5.82 | 0.114    | 26.49±3.66  | 28.15±5.83          | 0.523    |          |          |         |
| Gender                     |                     |                   |          |          |         |                     |                   |          |          |         |
| Male                       | 4887 (46.8)         | 16 (0.3)          | 4871 (99.7) | 0.078    | 9 (0.2)  | 4878 (99.8)         | 0.013    |          |          |         |
| Female                     | 5633 (53.5)         | 9 (0.2)           | 5624 (99.8) | 3 (0.1)  | 5630 (99.9) |                     |          |          |          |         |
| Habitat                    |                     |                   |          |          |         |                     |                   |          |          |         |
| Urban                      | 4619 (43.9)         | 6 (0.1)           | 4613 (99.9) | 0.045    | 7 (0.1)  | 4612 (99.9)         | 0.108    |          |          |         |
| Rural                      | 5901 (56.1)         | 19 (0.3)          | 5882 (99.7) | 5 (0.1)  | 5896 (99.9) |                     |          |          |          |         |
| Marital status             |                     |                   |          |          |         |                     |                   |          |          |         |
| Married                    | 10224 (97.2)        | 25 (0.2)          | 10199 (99.8) | 0.490    | 12 (0.1) | 10212 (99.9)        | 0.647    |          |          |         |
| Single                     | 296 (2.8)           | 0 (0)             | 296 (100)  |          | 0 (0)    | 296 (100)           |          |          |          |         |
| Education                  |                     |                   |          |          |         |                     |                   |          |          |         |
| Primary (<12 years)        | 7590 (72.1)         | 21 (0.3)          | 7569 (99.7) | 0.301    | 10 (0.1) | 7580 (99.9)         | 0.673    |          |          |         |
| Diploma (12 years)         | 1284 (12.1)         | 4 (0.2)           | 2280 (99.8) | 2 (0.1)  | 2282 (99.9) |                     |          |          |          |         |
| Academic (>12 years)       | 646 (6.1)           | 0 (0)             | 646 (100)  |          | 0 (0)    | 646 (100)           |          |          |          |         |
| Smoking                    |                     |                   |          |          |         |                     |                   |          |          |         |
| Yes                        | 2577 (24.5)         | 5 (0.2)           | 2572 (99.8) | 0.861    | 3 (0.1)  | 2574 (99.9)         | 0.943    |          |          |         |
| No                         | 7908 (75.2)         | 20 (0.3)          | 7888 (99.7) | 9 (0.1)  | 7899 (99.9) |                     |          |          |          |         |
| Not-cure                   | 7 (0.1)             | 0 (0)             | 7 (100)    |          | 0 (0)    | 7 (100)             |          |          |          |         |
| Alcohol consumption        |                     |                   |          |          |         |                     |                   |          |          |         |
| Yes                        | 1515 (14.4)         | 4 (0.3)           | 1511 (99.7) | 0.497    | 2 (0.1)  | 1513 (99.9)         | 0.900    |          |          |         |
| No                         | 8977 (85.6)         | 21 (0.2)          | 8956 (99.8) | 10 (0.1) | 8967 (99.9) |                     |          |          |          |         |
| Surgery                    |                     |                   |          |          |         |                     |                   |          |          |         |
| Yes                        | 6637 (63.3)         | 16 (0.2)          | 6621 (99.8) | 0.539    | 9 (0.1)  | 6628 (99.9)         | 0.512    |          |          |         |
| No                         | 3855 (36.7)         | 9 (0.2)           | 3848 (99.8) | 3 (0.1)  | 3852 (99.9) |                     |          |          |          |         |
| Hospitalization            |                     |                   |          |          |         |                     |                   |          |          |         |
| Yes                        | 8456 (80.6)         | 19 (0.2)          | 8437 (99.8) | 0.561    | 10 (0.1) | 8446 (99.9)         | 0.653    |          |          |         |
| No                         | 2036 (19.4)         | 6 (0.3)           | 2030 (99.7) | 2 (0.1)  | 2034 (99.9) |                     |          |          |          |         |
| Transfusion                 |                     |                   |          |          |         |                     |                   |          |          |         |
| Yes                        | 1001 (9.5)          | 4 (0.4)           | 997 (99.6)  | 0.703    | 1 (0.1)  | 1000 (99.9)         | 0.970    |          |          |         |
| No                         | 9395 (89.5)         | 21 (0.2)          | 9374 (99.8) | 11 (0.1) | 9384 (1)  |                     |          |          |          |         |
| Not know                   | 95 (0.9)            | 0 (0)             | 95 (100)    |          | 0 (0)    | 95 (100)            |          |          |          |         |
| Genital aphthous           |                     |                   |          |          |         |                     |                   |          |          |         |
| Yes                        | 130 (1.2)           | 0 (0)             | 130 (100)   | 0.732    | 0 (0)    | 130 (100)           | 0.828    |          |          |         |
| No                         | 10362 (98.8)        | 25 (0.2)          | 10337 (98.8) | 12 (0.1) | 10350 (99.9) |                     |          |          |          |         |

In the primary screening, participants with positive results of HBsAg (n = 36) and anti-HCV antibodies (n = 36) were detected. Among these patients, the overall prevalence of HB and HC was 0.24% (95% CI, 0.16%
to 0.36\%) (n = 25) and 0.11\% (95\% CI, 0.06 \% to 0.19 \%) (n = 12), respectively according to the qPCR assay. The geographic distribution of HB positive and HC positive patients based on gender is presented in Figure 1. The prevalence of HB and HC in Tulmat (0.38 and 0.14\%, respectively) are higher than Central part (0.06 and 0.08\%, respectively) of Someh’ E Sara. Rural participants were significantly more HB positive than urban people (P=0.045), while male individuals were significantly more HC positive than female participants (P=0.013). No other significant associations were detected between other evaluated demographic variables with HB and HC prevalence.

The complete blood and urine analyses of our participants are presented in Table 2. HB positive patients had significantly lower platelet count (P=0.043), RDWCV (P=0.023), cholesterol (P=0.033), LDL (P=0.043), and LDL: HDL ratio (P=0.002) compared to HB negative participants. HC positive patients had significant higher MCH (P=0.036), MCHC (P=0.047), AST (P=0.032), ALT (P=0.030) and HDL (P=0.039) and significant lower LDL (P=0.028) and LDL: HDL ratio (P=0.001) compared to HC negative individuals.
Most HB positive patients (52%) had less than 300 copies/ml of HB DNA. While most HC positive patients (58.4%) had $10^5$-$10^6$/ml copies of HC RNA. The most detected HC genotype was 2a (58.33%) compared to 1a (25.00%) and 1a & 2a (16.67%). First-degree relatives of all HC positive patients were also checked for HC infection by qPCR. Just a child of one patient had HC infection with genotype similar to her mother as 1a.

**Discussion**

In the present study, the prevalence of HB and HC among participants of the PERSIAN Guilan Cohort Study (PGCS) were 0.24% and 0.11%, respectively. Moreover, rural participants were significantly more HB positive, while male individuals were significantly more HC positive. HB positive patients had significantly lower platelet count, RDWCV, cholesterol, LDL, and LDL: HDL ratio and HC positive patients had
significantly higher MCH, MCHC, AST, ALT, and HDL, and significant lower LDL and LDL: HDL ratio compared to related negative individuals.

The prevalence of HB and HC is very different worldwide, related to geographical region and demographic factors. In 2015, it has been reported that HB seroprevalence was 8.83% (0.48 - 22.38) in African region, 0.81% (0.20 - 13.55) in Americas region, 3.01% (0.67 - 14.77) in Eastern Mediterranean region, 2.06% (0.01 - 10.32) in European region, 1.90% (0.82 - 6.42) in South East Asian region, and 5.26% (0.37 - 22.70) in Western Pacific region [26]. Also, there is much diversity in HB prevalence between different states/provinces of each country. Since 2006 when the national vaccination program for Iranian people born after 1993 was started and continued, an obvious decrease in the HB prevalence was seen [27]. Therefore, Iran is classified as a low to intermediate prevalence areas [21]. Although our detected HB infection rate is lower than the reported pooled prevalence of HB in Iran among the general population (2.2%) in 2016 [22]. It is approximately similar to our previous report about volunteer blood donors (0.45 - 0.48%) [28] and to reported rates from Karaj (0.4%) [29], Kermanshah (0.7%) [30] and Kurdistan (0.8%) [31]. Also, our reported HB infection rate is lower than those reported from Birjand (1.6%) [32], Tehran, Golestan, and Hormozgan (2.6% [33]), and Nahavand (2.3%) [34]. In addition, some population sub-groups are more likely susceptible to have HB. For instance, in Guilan province, 71.3% of hemophiliacs [35], and 0.38 - 3.8% of hemodialysis patients [36-38] were HB positive. We found that men are more HB positive than women (16 vs. 9 cases), which is similar to previous reports from Iran about a higher prevalence of HB infection in men [39,22]. Furthermore, the prevalence of HB and HC in Tulemat is higher than in other geographical areas of Someh’ E Sara. It might be related to the high number of drug abusers in this area compared to other areas. Based on the subgroup analysis, the prevalence of HB is higher among people who are drug abuser than those are not (p<0.001).

The pooled HC prevalence of 0.3%, 6.2%, and 32.1% was reported for general, intermediate- and high-risk Iranian populations, respectively [40]. Again, diversities between different cities/provinces and subgroups are seen. It has been reported that all healthy adults of Isfahan and Mashhad, blood donors of Tehran, Ardabil, and Ahvaz, infertile male of Tehran, and male blood donors of Tabriz were HC negative [41]. Our detected HC prevalence (0.1%) is lower than the pooled prevalence of HC among the general population of Iran (0.3%) [40] and is differed from the previous report from Rasht (0.03%) and Guilan (0.32%) [28]. Also, our detected HC prevalence is lower than other reported prevalence from the Northern provinces of Iran. The prevalence of HC was 0.48% in Babol and 0.18 to 1% in Golestan. However, Zamani et al. reported a similar HC prevalence (0.08%) in the general population of Mazandaran province. Higher male HC positivity, as seen in our study, was also reported previously from Kerman, Zahedan, and Kavar. However, in opposite to our study, Ghadir et al. reported that females were more HC positive compared to males in the general population of Golestan [41,42]. The finding of one woman who her daughter also was HC positive and both had the same HC genotype highlighted the precise role of interfamilial HC transmission and confirmed the significant role of close relatives, which was reported previously [43].

Although we detected no significant associations between demographic variables and prevalence of HB and HC, it seems that different demographic features of the population in different regions are the most
important reasons for these differences in HB and HC prevalence. Based on Baig's study, the male to female ratio of HB increased during the reproductive years. There may be an influence of estrogen in the protection of hepatocytes against the development of chronic liver disease [44]. In Zeng et al. study, married people had the highest prevalence of HBsAg [45]. On the other hand, Ataei et al. demonstrated no statistical difference observed in terms of marital status in Isfahan province, but males (OR= 3.79) had a higher prevalence of HB than women [46].

Regarding biochemical analysis, we found some significant differences. A decrease in LDL level and subsequently LDL: HDL ratio in HB and HC positive patients is interesting. These are in line with those reported recently as significant hypolipidemia in patients with HB [47] and HC [48]. Lower platelet count in HB positive, as we found in this study, also reported previously [49]. It can be said that both HB and HC influenced the liver tissue, and the changes in biochemical and hematological parameters can be related to these changes in the hepatic functions.

**Conclusion**

In summary, we found lower HB and HC prevalence compared to other regions of Iran. Compared to previous reports from our province, Guilan, the HB and HC prevalence also decreased. It may be due to the preventive strategy or increase of the medical knowledge of peoples, which must be evaluated in further studies.

**Limitation**

The limitation of our investigation was the study of a specific age group and did not include the high-risk population, such as young people, sex workers, and intravenous drug abusers.

**Abbreviations**

HB: Hepatitis B, HC: Hepatitis C, HBcAg: Hepatitis Core Antigen, HBsAg: Hepatitis B Surface Antigen

**Declarations**

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**Author contributions**

Study conception and design: F.J, M.N, and F.M

Acquisition of data: M.S and S.Y
Statistical analysis: M.S, M.N and S.H
Interpretation of results: F.J, F.M, M.F and S.H
Drafting of the manuscript: F.J, S.Y, M.F and S.H

All authors approved the final version of the article, including the authorship list.

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**Availability of data and materials**

The study protocol and the datasets analyzed are available from the corresponding author upon request.

**Ethics approval and consent to participate**

Written consent was taken after informing the purpose and importance of the study to each participant. To ensure confidentiality of participant's information, codes were used whereby the name of the participant and any identifier of participants was not written on the questionnaire. This study was confirmed by the Ethics Committee of Guilan University of Medical Sciences (Ethics code: IR.GUMS.REC. 1396.254).

**Consent for publication**

Not applicable

**Competing interests**

The authors declare that they have no competing interests in this work.

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Figures

Figure 1

Geographic distribution of HB and HC positive patients. (The map depicted in figure is our own work)