

Prevalence of Hepatitis B and C among Patients Looking for Hospital Care; Five Years' Study in Mashhad, Iran

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Abstract- This study was conducted to find out the prevalence, viral load and co-infection of HBV and HCV infection among patients seeking to hospital care in Mashhad, Iran. A total of 402 samples (349 samples for HBV and 53 for HCV) were received and were screened for hepatitis B and C during 2004 to 2014. Viral loads of HBV DNA and HCV RNA were quantified by real-time PCR. Among 349 collected samples, 229 (65.61%) were positive for HBV DNA and 36 (67.92%) for HCV RNA. Among the ones positive for HBV DNA and HCV RNA, HCV was more prevalent (86.11% Vs 58%) ,in male patients, a higher incidence was attributed to HBV than HCV (34.42 Vs 13.88%). The incidence of co-infection of HBV and HCV was in 5 (1.88%) patients. Association of age and load of HBV, HCV showed that higher marginal viral loads found to be more common in the age groups of upper 30 years old ($P=0.064$, $P=0.079$, respectively). The present study provides the preliminary information about high HCV and HBV prevalence. Findings from the current study will be helpful for the better management and control of viral hepatitis among patients looking for hospital care.

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Introduction

Hepatitis B virus (HBV) and Hepatitis C virus (HCV) are small, enveloped DNA viruses called hepadnaviruses (1) and positive-sense RNA virus of the family Flaviviridae, respectively. It is estimated that at least two billion people worldwide have been infected with HBV, and more than three hundred sixty millions of them are infected chronically and at the risk of serious complications as well as early death (2). Clinical manifestations of HBV infection range from in apparent infection to fulminant hepatic failure. Chronic infection develops approximately in 5% of immune-competent HBV-infected adults while up to 100% of infected newborns may become HBV carriers. The long-term consequences of chronic HBV infection include liver cirrhosis and hepatocellular carcinoma (HCC). These life-threatening liver disease complications could affect 15–40% of HBV carriers (3).

Approximately one million people die with chronic HBV and/or HCV infections annually with mostly end-stage liver diseases, namely, decompensated cirrhosis,

liver failure, and hepatocellular carcinoma (HCC). About 70 to 80 percent of HCV infections may persist, and near 30 percent of them will develop end-stage liver diseases including cirrhosis and HCC. Recently mortality rate of liver cirrhosis as a worldwide public health problem have been increased stably (1,4).

Humans are the only reservoir of HBV and the main routes of HBV transmission different in various areas of the world according to their HBV endemicity (5,6).

The prevalence of hepatitis infection varies markedly according to the different geographical areas. This is varies between 0.1 to 2% in United States, Canada, Western Europe, Australia, and New Zealand known as low prevalence areas (7,8), about 2-8% in Mediterranean countries, Japan, central Asia, the Middle East, and Latin and South America, and 8-20% in southern Asia, China, and sub-Saharan Africa as intermediate and high prevalence areas (9). Furthermore, the prevalence of HCV has been reported different in various regions, like 0.4 to 3% of populations in Western Europe and up to 15% in Mediterranean and East European countries.

In developing countries, there is no access to reliable

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information about the prevalence, risk factors and burden of viral hepatitis (10). Iran also is not accepted from this problem. Alavian *et al.*, estimated that the prevalence of HBV infection varies from 2.1 to 2.6% among Iranian males and females, respectively. Some studies show recently the prevalence of HBV is increasing in Iran. Moreover, the majority of studies in Iran (11-14) screened HBV on the specific groups including blood donors, drug abusers not based on the general population. A screening program for hepatitis infection in high risk group was implemented since 1984 in Iran. Therefore, there is not yet published comprehensive screening program in the general population (15). For this reason, the current study was designed to report the epidemiologic features of HBV and HCV in Iranian peoples with positive hepatitis screening test.

Materials and Methods

Study population

In this study, we measured viral loads of HBV and HCV from individuals under any reason who referred to Ghaem Hospital, Mashhad University of Medical Sciences, for detection of HBV and HCV infection, during Jun 2004 to Jun 2014.

Demographic information and clinical data including age, sex, and past medical history were collected. None of them had a history of alcohol consumption, drug-induced hepatitis or other known diseases. The diagnosis of chronic HBV and HCV disease was made by clinical, biochemical, radiological, and endoscopic criteria.

HBV DNA & HCV RNA extraction and Real time PCR

HBV DNA was extracted from a 200 μ L aliquot of serum using a Qiagen mini blood kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Besides HCV RNA extraction was performed from 140 μ L of serum aliquot by Qiagen mini blood kit (QIAamp, Viral RNA minikit, Qiagen, Hilden, Germany).

Viral loads of HBV DNA and HCV RNA were quantified by real-time polymerase chain reaction (real-

time PCR) (HBV RG PCR artus and HCV RG PCR artus, Germany). The claimed lower detection limit of the kit was 100 copy/mL, so lower 100 copy/mL was considered as undetectable HBV DNA and undetectable HCV RNA.

Statistical analysis

Furthermore, SPSS for Windows, version 16 (SPSS Inc., Chicago, IL, USA) was used in all statistical procedures. Data were expressed at mean \pm SD. Normality of data was checked by Kolmogorov-Smirnov test. Differences in proportions were judged by χ^2 test and for quantitative data by T independent test.

Results

There were 349 referral HBV cases among which 120 cases had less than 100 copies of HBV DNA. On the other hand, out of 53 referral cases HCV, only 17 cases had less than 100 copies of HCV (Figure 1). Moreover, co-infection of HBV and HCV was observed in 5 patients (1.88%).

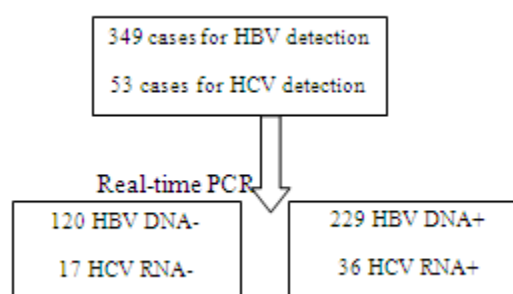


Figure 1. Diagram fellow chart by Real-time assay of studied individuals

Current study results showed that HBV patients (349 cases) were ranged between 8 to 78 years old, and similarly patients with HCV (53 cases) were ranged between 5 to 78 years old. Although mean age of two studied patients groups were significantly different ($P=0.04$; Table 1).

Table 1. Demographic characteristics of studied records

Variables	HBV cases (N= 349)	HCV cases (N=53)
Age, yrs	36.78 \pm 12.80	43.88 \pm 15.81
Gender	Female	13 (24.5%)
	Male	40 (75.5%)
Viral Load	2.38 $\times 10^8 \pm 1.98649 \times 10^8$	3.6470 $\times 10^6 \pm 1.065 \times 10^6$

Male sex was contributed a greater number of

patients than female patients in both HBV and HCV

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disease ($P=0.04$, Table 1). HBV was more prevalent in males, 133 (58%), while female patients had positive incidence of 88 (38.42%). Positivity for HCV RNA in male patients (31 (86.11%)) was more frequent in contrast of HBV DNA for a male. However, this was not observed for female patients as HCV RNA was positive in 5 (13.88%) of female patients. We divided our HBV patients into two groups; more than 30 years old (211 cases; 113 male) and less than 30 years old (138 cases; 88 male). Eight HCV positive patients (5 male and 3 female) been less than 30 years old in and 45 cases patients (10 female and 35 female) were more than 30 years old in. There was statistically significant

difference regarding sex distribution of HBV patients ($P=0.037$) but not HCV patients ($P=0.32$). Association of variables including age and sex with a viral load of HBV and HCV in studied individuals is presented in (Table 2). This Table shows only the difference between age categorization (below and upper than 30 years old) and sex in HBV patients. As a larger number of HBV patients (58%) with more than 30 years old were male and below 30 years old which was attributed a lesser number of HBV patients (42%) were female. Furthermore, association of age and viral load of both HBV and HCV patients were marginal ($P=0.079$, $P=0.064$, respectively).

Table 2. Relation of studied variables with viral load of HBV and HCV

Variables		HBV viral load	P- value	HCV viral load	P- value
Gender	Female	$3.9056 \times 10^8 \pm 2.94603 \times 10^9$	0.0015	$1.5816 \times 10^6 \pm 1.48016 \times 10^6$	0.23
	Male	$1.2759 \times 10^8 \pm 6.9680 \times 10^8$		$4.2305 \times 10^6 \pm 1.8730 \times 10^6$	
Age	<30 years old	$1.2652 \times 10^8 \pm 6.58758 \times 10^8$	0.079	$7.1289 \times 10^7 \pm 1.74789 \times 10^6$	0.064
	>30 years old	$3.1625 \times 10^8 \pm 2.51318 \times 10^8$		$3.0280 \times 10^7 \pm 9.10568 \times 10^6$	

Discussion

Previous reports indicate a wide range of HBV infection prevalence rates of 1.2 and 9.7% in different parts of Iran. Moreover, the other studies among some groups of the adult population represent a decreasing trend in HBsAg prevalence in the general population in Iran (16-19).

According to instruction of Iranian Ministry of Health, hepatitis screening tests are mandatory for many groups including blood donors, health care providers, patients with history of hemodialysis, pregnant women, infants born to HBsAg+ mothers, patients with jaundice or other symptoms of acute hepatitis, first degree relatives of chronic HBV patients, cirrhosis patients, illicit intravenous (IV) drug users, HIV patients, prisoners, and immigrants from countries with high prevalence of hepatitis.

In present study, we found HBV Ag prevalence among referred individuals to our Medical center was 65.61% and was 67.69% about prevalence of HCV, which is similarly in line with previous studies conducted by Raja *et al.*, and Khan *et al.*, reported the overall co-infection rate of about 1.1% and 1.30%, respectively (20-21). However, present study reported a higher rate of co-infection of HBV and HCV (1.88%) compared with the previous studies (20-21).

We found that among those positive for HBV DNA and HCV RNA, HCV was more prevalent among males (86.11% Vs 58%) while for female patients; a higher

incidence was related to HBV DNA than HCV RNA (34.42 Vs 13.88%). While the previous study reported (21) among seropositive male patients, HBV was more prevalent (23.8%) while female patients had high prevalence of HCV (52.2%). These might be due to the different immune response evoked in both male and female patients. Furthermore, it might be due to the difference in measuring tools as we selected more accurate qualitative measurement tools than previous, real time PCR contrast of ELISA, respectively (21). Also, we found significantly increased level of HBV viral load in female patients compared to male patients, this was confirmed earlier by previous studies (22-23).

Regarding the association of age and viral loads, our study results show that age group higher than 30 years old has a higher frequency of HBV and HCV related hepatitis and higher viral loads of HBV and HCV than age less than 30 years old.

Khan *et al.*, reported that both age groups of 21-30 and 40-50 years show the highest frequency of HBV and HCV (21). While other studies similar to current study indicated high prevalence in age group above 30 (24-25). The possible explanation might be the increased chances of infection in the mentioned age groups related to more sexual activity in married patients or unmarried individuals due to these patients are free to indulge in more sexual activity (21-25).

In conclusion, the prevalence of HCV and HBV in Iran is at an increasing rate. Large-scale studies are needed to understand the epidemiology of HCV and

HBV infections. The data of the current study will help in the effective prevention and control measures against HBV and HCV infection in our study area.

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