Original Article

Hepatitis C Virus Infection in HIV Positive Attendees of Shiraz Behavioral Diseases Consultation Center in Southern Iran

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ABSTRACT

Objective: To determine the prevalence of HCV co-infection and its correlation with demographic and risk factors among human immunodeficiency virus (HIV)-infected individuals attending Shiraz behavioral diseases consultation (SBDC) Center in southern Iran. **Materials and Methods**: In a cross-sectional study, 226 consecutive HIV-positive patients who referred to SBDC Center from April 2006 to March 2007 were interviewed face-to-face to record demographic data and risk factors of HIV transmission. A 10ml sample of venous blood was drawn from every subject and tested for HCV-antibodies by third generation enzyme linked immunosorbant (ELISA) and recombinant immunoblot assays (RIBA). All samples were also analyzed by qualitative reverse transcriptase polymerase chain reaction (RT-PCR) for detection of HCV-RNA. **Results:** The study population consisted of 214 men (94.7%) and 12 women (5.3%) with a mean age of 35.6 ± 7.9 years. The most prevalent risk factor was imprisonment (88.9%) followed by injecting drug use (79.2%). The prevalence of HCV infection was 88.5% by ELISA and 86.7% by RIBA, while HCV viremia was detected in 26.1% of the patients. HCV-antibody positivity was significantly associated with gender, age, marital status, occupation, injecting drug use, and history of imprisonment. It was inversely related to "having an infected or high risk sexual partner". In the logistic regression model, the predictors of HCV-positivity were injecting drug use (OR = 24.9, $P = 0.004$) and imprisonment (OR = 21.4, $P < 0.001$). **Conclusions:** Prevalence of HCV infection among HIV-positive individuals in our region is very high and there is a need for stricter preventive actions against transmission of HCV among this group of patients.

Keywords: HIV, hepatitis C, iran, prevalence, risk factors

Introduction

Approximately 170 million people are infected with hepatitis C virus (HCV). One of the major health problems today is the viral hepatitis caused by HCV, because this virus has spread worldwide, it has different routes of transmission, and there is no efficient therapy.[1] In 2010, there were approximately 34 million people living with human immunodeficiency virus (HIV).[2] About 14,000 new HIV infections occur globally every day, 95% of which are in developing countries.[3]

It is estimated that among HIV-infected individuals, 4-12 million are co-infected with HCV. The clinical course of chronic HCV infection is accelerated by HIV co-infection which leads to an increased risk of cirrhosis, hepatocellular carcinoma, and decompensated liver disease.[4] As development of highly active antiretroviral therapy has significantly improved the prognosis of HIV, chronic liver diseases have become a prominent cause

Access this article online	
Quick Response Code:	Website: www.ijcm.org.in
	DOI: 10.4103/0970-0218.112437

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Received: 31-08-10, **Accepted:** 13-07-12

of morbidity and mortality in the HIV-HCV co-infected patients.[5]

The prevalence of HCV infection in HIV-positive patients varies from 4.0% to greater than 50.0% in different populations worldwide.[6] Depending on the route of transmission, this prevalence ranges from 3-15% in homosexual/bisexual men, to 80% in injecting drug users (IDUs), to 98% in hemophiliacs. The main determinant of HCV prevalence in HIV-infected cohorts is the proportion of various risk groups present in those cohorts.[7] The high prevalence of HIV/HCV co-infection is due to sharing the same risk factors for transmission, as both viruses are transmitted by blood products, intercourse, and vertical transmission.[8]

Due to differences in the risk factors of HIV/HCV co-infection in various geographical regions in the world, each region should provide its own statistics to create a base for planning the future preventive and therapeutic measures. The aim of this study was to determine the prevalence of HCV co-infection among HIV infected individuals attending Shiraz behavioral diseases consultation (SBDC) Center in Fars Province, southern Iran from April 2006 to March 2007, and to investigate any correlation that HCV co-infection may have with demographic factors and different risk factors.

Materials and Methods

Of 400 consecutive HIV-positive patients who referred to SBDC Center in Fars Province, southern Iran from April 2006 to March 2007, 226 subjects gave informed written consent to participate in this cross-sectional study (refusal rate: 43.5%). After finding out about SBDC center through their physicians or friends, individuals with a high risk for HIV infection voluntarily refer to this center, where consultations and work-ups are done at no cost. Only those with documented HIV infection (by serial ELISA and Western blot) were included in the study. Repeat visitors were excluded.

All subjects were interviewed face-to-face and a form containing questions on demographic data and risk factors of HIV transmission was completed for each patient. The interviews were conducted, by two trained general physicians, in a designated room in SBDC Center where privacy could be provided for each subject. The risk factors under study included history of imprisonment, injecting drug use (IDU), high risk sexual behavior, recipient of blood/blood products, child of an infected mother, and having an infected or high risk sexual partner [Table 1].

A 10 ml sample of venous blood was drawn from every subject; serum was extracted and immediately freezed at −20°C. All tubes were coded and transferred to a −70°C

Table 1: Distribution of demographic factors, risk factors, and HCV-positivity in the population under study

	Prevalence	
	Nos.	%
Demographic factors		
Gender		
Male	214	94.7
Female	12	5.3
Age		
10-19	2	0.9
20-29	49	21.7
30-39	113	50.0
40-49	48	21.2
50+	14	6.2
Marital status		
Married	82	36.3
Single	94	41.6
Divorced	47	20.8
Widowed	3	1.3
Occupation		
Manual[1]	129	57.1
Non-manual[2]	5	2.2
Unemployed	91	40.3
Risk factors		
Injecting drug use		
Yes	179	79.2
No	47	20.8
Received blood or blood product		
Yes	29	12.8
No	197	87.2
High risk sexual behavior[3]		
Yes	100	44.2
No	126	55.8
Infected or high risk sexual partner[4]		
Yes	11	4.9
No	215	95.1
Child of an infected mother		
Yes	0	0
No	226	100.0
Imprisonment		
Yes	201	88.9
No	25	11.1
HCV infection		
ELISA		
Positive	200	88.5
Negative	26	11.5
RIBA		
Positive	196	86.7
Negative	15	6.6
IND	15	6.6
PCR		
Positive	59	26.1
Negative	167	73.9

[1]Subjects with occupations outside an office such as farmers, carpenters, welders, mechanics, painters, drivers, manual workers, and salesmen were considered to have a "manual" occupation, [2]Those who worked in an office-like environment such as bank clerks, government employees, and administrative staff were considered to have a "non-manual" occupation, [3]Subjects with a history of unsafe sexual contact were classified as having "high risk sexual behavior", [4]A patient was labeled as having an "infected or high risk sexual partner" when his/her spouse was either infected with HIV or had a history of unsafe sexual contact, HCV: Hepatitis C virus, ELISA: Enzyme linked immunosorbant, RIBA: Recombinant immunoblot assays, PCR: Polymerase chain reaction

freezer in less than a week. The sera were tested for HCV-Ab by a third generation ELISA (HCV Ultra, China) according to the manufacturer's instructions. Then, a third generation RIBA for HCV antibodies (MP diagnostics, Singapore) was carried out on all samples, followed by a qualitative RT-PCR (Cinnagen Co, Iran) for detection of HCV-RNA.

All data were recorded in a computer database and were analyzed using the STATA software, version 10. Chi-square and Fisher's exact tests were used to evaluate the association of HCV prevalence with demographic and risk factors. Logistic regression analysis was performed to determine the predictors of HCV co-infection. A $P < 0.05$ was considered significant.

The study was approved by the ethical committee of Office of Vice-Chancellor for Research Affairs, Shiraz University of Medical Sciences, Shiraz, Iran.

Results

The study population consisted of 226 HIV-infected patients with the age range 10-57 years and a mean age of 35.6 ± 7.9 years. The majority of the subjects (214) were male (94.7%) while there were only 12 females (5.3%). Distribution of demographic factors, risk factors and HCV positivity among the study population is demonstrated in Table 1. The most prevalent risk factor was a history of imprisonment (201, 88.9%) followed by injecting drug use (179, 79.2%), while none of the patients were "child of an infected mother".

ELISA analysis for HCV antibodies was reactive in 200 subjects (88.5%) and negative in 26 cases (11.5%). Similarly, 196 patients (86.7%) were anti-HCV positive by RIBA analysis, while 15 patients (6.6%) had negative results. In the remaining 15 cases (6.6%), RIBA was indeterminate. RT-PCR analysis for detection of HCV-RNA showed that 59 subjects (26.1%) had HCV viremia whereas 167 patients (73.9%) were clear [Table 1].

Table 2 demonstrates the relationship of HCV seropositivity determined by RIBA in HIV-infected patients with demographic factors as well as with risk factors. HCV infection had a significant association with gender ($P < 0.001$), age ($P = 0.010$), marital status ($P = 0.014$), occupation ($P = 0.003$), IDU ($P < 0.001$), history of imprisonment ($P < 0.001$), and having an infected or high risk sexual partner ($P < 0.001$). The prevalence of HCV infection was higher in men (96.5%), 40-49 age group (97.7%), injecting drug users (99.4%), and in those with manual occupations (96.7%) and a history of imprisonment (98.4%). It was lower in married subjects (85.1%) and in those with an infected or high risk sexual partner (22.2%).

Table 2: Prevalence of HCV co-infection in HIV-positive patients according to demographic and risk factors

	HCV-RIBA nos. (%)		*P* value
	Positive	Negative	
Gender			<0.001
Male	194 (96.5)	7 (3.5)	
Female	2 (20.0)	8 (80.0)	
Age			0.010
10-19	0 (0.0)	2 (100.0)	
20-29	45 (91.8)	4 (8.2)	
30-39	98 (93.8)	7 (6.7)	
40-49	42 (97.7)	1 (2.3)	
50+	11 (91.7)	1 (8.3)	
Marital status			0.014
Married	63 (85.1)	11 (14.9)	
Single	88 (97.8)	2 (2.2)	
Divorced	44 (95.7)	2 (4.3)	
Widowed	1 (100.0)	0 (0.0)	
Occupation			0.003
Manual	119 (96.7)	4 (3.3)	
Non-manual	2 (50.0)	2 (50.0)	
Unemployed	75 (89.3)	9 (10.7)	
Injecting drug use			<0.001
Yes	168 (99.4)	1 (0.6)	
No	28 (66.7)	14 (33.3)	
Received blood or blood product			1.000
Yes	24 (92.3)	2 (7.7)	
No	172 (93.0)	13 (7.0)	
High risk sexual behavior			0.148
Yes	90 (95.7)	4 (4.3)	
No	106 (90.6)	11 (9.4)	
Infected or high risk sexual partner			<0.001
Yes	2 (22.2)	7 (77.8)	
No	194 (96.0)	8 (4.0)	
Imprisonment			<0.001
Yes	187 (98.4)	3 (1.6)	
No	9 (42.9)	12 (57.1)	

HCV: Hepatitis C virus, RIBA: Recombinant immunoblot assays, HIV: Human immunodeficiency virus

Table 3 gives a comparison of ELISA and RIBA tests for HCV-antibody detection in HIV-infected patients. A total of 15 patients who had undetermined (IND) result in RIBA test were excluded from this analysis. Assuming RIBA to be the gold standard test for detection of HCV-antibody, ELISA test gave a false positive result in 2.7% of the cases and a false negative result in 56.5% of the cases. Moreover, sensitivity of ELISA was 93.4% (95% CI = 88.9-96.4) and its specificity was 66.7% (95% CI = 38.4-88.2).

Logistic regression analysis was performed with HCV-positivity based on RIBA, as the dependent variable, and nine demographic and risk factors, as independent variables. It showed that HCV infection was significantly associated with two factors, injecting drug use (OR = 24.9, $P = 0.004$) and imprisonment (OR = 21.4, $P < 0.001$). In other words, in the logistic regression

model, injecting drug use and a history of imprisonment were the only predictors of HCV co-infection among HIV-positive patients [Table 4].

Discussion

The prevalence of HCV co-infection among HIV-positive patients under study, as determined by ELISA method, was 88.5%. This is consistent with the findings of Rahimi-Movaghar and colleagues in Tehran who reported an HCV prevalence of 80.6% in HIV-infected patients.[9] As in our study, they used ELISA for HCV diagnosis, but all their subjects were injecting drug users.

When sera of our HIV-infected patients were tested for HCV-antibodies by RIBA analysis, the prevalence of HCV infection was 86.7%. This is higher than the rates found by other studies from different parts of the world which reported anti-HCV positivity of 8 to 50% based on RIBA.[10-13] Since percutaneous exposure is a very efficient route for HCV transmission,[4] the high positivity rate for RIBA in our study could be accounted for by the large number of injecting drug users (80%) among our subjects.

Using RT-PCR to detect HCV-RNA, we found an HCV viremia of 26.1% among our subjects. This is similar to the findings of Kim and colleagues in the USA who reported an HCV prevalence of 25% by PCR test. In their study of HIV-positive subjects in New York, they first used ELISA to determine HCV serology and then confirmed the positive results by PCR analysis.[14] The observed difference between positivity rates for RIBA and PCR in our study could be due to permanent clearance of hepatitis C virus in some patients and fluctuation of viremia in those with chronic HCV infection.

We observed that there was a highly significant association between male gender and HCV co-infection among HIV-positive subjects. This is in keeping with other studies in USA,[14] Brazil[15] and Gambia.[16] Men are often more likely to engage in high risk behavior in comparison to women and are therefore more prone to common routes of HCV transmission; this is probably a contributing factor to why they have a higher rate of HCV co-infection. However, the low rate of HCV positivity among female participants in our study may not be very reliable, because there were very few HIV positive females in the study.

We found that the rate of HCV positivity increased with increasing age and was highest in the 40-49 age group. This is similar to Tedaldi *et al*., study in the United States.[17] Likewise in Tanzania, the prevalence of HCV infection among HIV-positive patients increased with age with the oldest age group (50+ years) having the highest proportion.[18]

Prevalence of HCV co-infection was highest in single and widowed groups and lowest in married patients. This is as we expected since married people are less likely to practice high risk sexual behavior and are therefore less prone to transmission of HCV via the sexual route. In their study of an HIV-positive population, Nagu *et al*., demonstrated that divorced and widowed subjects had the highest prevalence of HCV co-infection whereas currently married patients had the lowest prevalence.[18]

Similarly, prevalence of HCV co-infection was significantly higher in patients with manual occupations compared to those with non-manual jobs. This is parallel to the findings of previous studies in populations other than HIV-positive subjects. In their community-based study of over 6000 subjects in Taiwan, Wang and colleagues demonstrated that manual occupation was a risk factor for HCV seropositivity.[19] Moreover, occupation as a laborer or agriculture worker was correlated with HCV infection in a group of blood donors in Thailand.[20]

We observed a significant association between IDU and prevalence of HCV co-infection. Many other studies in HIV-positive populations from different regions of the world have consistently declared that the risk of HCV co-infection is higher in injecting drug users. Intravenous drug use was the most frequent risk factor for HCV acquisition in patients with HIV infection in Brazil.[21] Badridze *et al*., also reported that risk of HCV co-infection among injecting drug users was more than three-folds the risk in non-IDUs.[22] The reason for the strong association between IDU and HCV co-infection in HIV-positive patients is that percutaneous exposure is a very efficient route for HCV transmission.

Table 3: Comparison of ELISA and RIBA tests for HCV-antibody detection in HIV-infected patients

	RIBA nos. (%)		Total
	Positive	Negative	
ELISA			
Positive	183 (97.3)	5 (2.7)	188 (100.0)
Negative	13 (56.5)	10 (43.5)	23 (100.0)
Total	196 (92.9)	15 (7.1)	211 (100.0)

HCV: Hepatitis C virus, RIBA: Recombinant immunoblot assays, ELISA: Enzyme linked immunosorbant, HIV: Human immunodeficiency virus

Table 4: Logistic regression model for HCV co-infection based on RIBA test in HIV-positive patients

RIBA	Odds ratio	95% CI	*P* value
Injecting drug use	24.9	2.7-227.3	0.004
Imprisonment	21.4	4.4-103.7	<0.001

HCV: Hepatitis C virus, RIBA: Recombinant immunoblot assays, CI: Confidence interval, HIV: Human immunodeficiency virus

Surprisingly, HCV-antibody positivity was inversely related to "having an infected or high risk sexual partner" which is in disagreement with a number of other studies. Heterosexual contact with a high-risk partner had a significant correlation with HCV infection in a group of HIV patients in the USA.[23] Furthermore, Mendes-Correa *et al*., found that being a sexual partner of an HIV seropositive person was the second largest risk factor for acquisition of HCV.[21] The difference between our finding and that of other authors probably lies within the Iranian culture where AIDS is a social taboo. Besides, high risk practices such as IDU and prostitution are illegal in Iran. It is possible that our subjects were not totally honest in reporting their own risk factors and those of their spouses.

The present study revealed that prevalence of HCV co-infection was significantly higher in patients who had a history of imprisonment. Other studies have also shown that imprisonment is a risk factor for HCV infection in HIV-positive patients as well as in other populations.[24-26] Imprisonment is a constellation of other risk factors for transmission of HCV because of the very large rate of high risk behaviors practiced by inmates such as IV drug abuse, unsafe sex, sharing of needles and blades, tattooing, etc., In addition, most of these high risk behaviors contribute to percutaneous transmission of HCV which is a very effective route of transmission.

Comparing ELISA test results in detection of HCV-antibody to those of RIBA showed that ELISA had a false positive result in 2.7% of cases and a false negative result in 56.5% of the case. The very low rate of false positive result is because the subjects in our study were high risk for acquiring hepatitis C. In fact, the predictive value of ELISA depends on the prevalence of HCV infection in the population under study.[27] Furthermore, we found that sensitivity of ELISA was 93.4% while its specificity was 66.7%. Similarly, other authors stated that the sensitivity of third generation ELISA for detection of HCV-antibody in a high-prevalence population was as high as 97%.[28]

According to logistic regression model, IDU and imprisonment appeared to be predictors of HCV co-infection in HIV-positive subjects. The risk of HCV infection in injecting drug users was 24.9 fold the risk in those with no history of IDU. Likewise, the patients with a history of imprisonment were 21.4 times more likely to have HCV infection. These findings are supported by other authors.[14,24] In Mohsen *et al*., study, a history of IDU was identified as an independent risk factor for HCV infection in multivariate logistic regression analysis (OR = 107.2, 95% CI = 38.5-298.4).[7] Furthermore, a study of a group of injecting drug users in Iran showed that in a multiple regression model, history of prior imprisonment was an independent predictor of HCV infection (adjusted OR = 4.35, 95% CI = 1.88-10.08).[25]

In summary, HCV co-infection is highly prevalent among HIV-positive individuals in our region, and its two predictors are injecting drug use and imprisonment. Therefore, there is a need for stricter preventive actions against transmission of HCV among patients with HIV, such as provision of free disposable syringes to IV drug abusers, designing programs for cessation of IV drug addiction, and establishment of educational centers in prisons.

Acknowledgements

The authors would like to thank the Office of Vice Chancellor for Research Affairs of Shiraz University of Medical Sciences for financial support, and Dr. Ebrahim Ghaderi at the Department of Epidemiology for data analysis. Thanks also to Mr. Saeed Amirizadehfard and Ms. Maryam Nejabat for performing the laboratory tests.

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How cite this article: Davarpanah MA, Khademolhosseini F, Rajaeefard A, Tavassoli A, Yazdanfar SK, Rezaianzadeh A. Hepatitis C virus infection in HIV positive attendees of Shiraz behavioral diseases consultation center in southern Iran. Indian J Community Med 2013;38:86-91.

Source of Support: The funding for this project was provided by the office of Vice Chancellor for Research Affairs, Shiraz University of Medical Sciences, Shiraz, Iran. **Conflict of Interest:** None declared.