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EVALUATION FREQUENCY OF OCCULT HEPATITIS C VIRUS INFECTION IN PATIENTS WITH HUMAN IMMUNODEFICIENCY VIRUS INFECTION, KERMAN; IRAN

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ABSTRACT Occult hepatitis C virus infection is a pathological form of chronic hepatitis C virus (HCV) infection. Co-infection human immunodeficiency virus (HIV) and HCV make sever hepatic damage. The aim of this study was to determine the prevalence of occult HCV in Iranian patients with HIV infection. In a cross-sectional study, from Jan 2016 to Jun 2017, 100 Iranian patients with HIV infection were enrolled in this study. After extraction of viral RNA from the plasma and PBMC samples, HCV RNA and HCV genotypes were detected by commercial Real Time PCR kit. Out of the one hundred patients, 22 patients (22%) had co-infection HIV/ HCV infection, nine samples (40.9%) were occult HCV and 13 samples (59.1%) were acute HCV. Genotype 3a in patients with occult HCV and 1a in patients with acute HCV were more abundant. Also, the mean level of liver enzymes in patients with occult HCV higher than that of acute HCV. The study demonstrated that testing only for HCV antibody fails to identify the true prevalence of HCV co-infection among HIV infected patients, also; results revealed the occurrence of occult HCV infection in HIV infected patients. Therefore, it seems that, analysis of Peripheral Blood Mononuclear Cells(PBMCs) for HCV-RNA would be informative for detection of occult HCV.

KEYWORDS

Human Immunodeficiency Virus; Occult HCV; Peripheral Blood Mononuclear Cell; Co-infection; Kerman

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INTRODUCTION:

Hepatitis C virus infection is cause of chronic liver disease around in worldwide, and is a major public health problem that is widespread and has a close relationship with cirrhosis and hepatocellular carcinoma (Abdelrahim et al. 2016, Fazlalipour et al. 2015). An estimated have shown that 170 to 200 million people (2%) of the world's population are infected with the hepatitis C virus(Bartolome et al. 2014). The prevalence of hepatitis C in different countries is from 2-40%. The new Hepatitis C infection is known to be the occult hepatitis C infection, that has been identified to the absence of anti-HCV and HCV-RNA in plasma or serum with abnormal liver enzymes such as ALT (more than 50 IU/ml) and the presence of HCV-RNA in the liver and in Peripheral blood mononuclear cells (PBMC) (Aslan and Altindis 2017, Baid-Agrawal and Berg 2014). Reports have revealed two types of occult hepatitis C: Seronegative occult hepatitis C (negative anti HCV and HCV-RNA) and seropositive hepatitis C (anti HCV-positive and negative serum HCV-RNA)(Bastani et al. 2016). In two types of infections, HCV-RNA is detected in the liver and in the PBMC or in the serum of patients after ultrasound centrifuges(Bokharaei-Salim et al. 2016). There are several differences between patients with hepatitis C Infection Seronegative and Seropositive. Seropositive occult hepatitis C includes patients with anti-HCV-positive and usually normal hepatic enzymes that are likely to be infected (either spontaneously or after antiviral therapy) for most antiviral patients who have received a sustained response(Carreno 2014, Iranmanesh et al. 2015). The person carrying the HCV is asymptomatic since the hepatitis C virus is reproduced in the liver and in the PBMCs of patients with HCV(Castillo et al. 2014). It was thought that low levels of viral load in the blood and HCV-RNA is not detectable by the most sensitive RT-PCR methods(Coppola et al. 2015). HCV-RNA is detected from the serum of patients with HCV after ultrasound- centrifuging and HCV viral transmissible from the patient's to others (El Shazly et al. 2015). The viral particle density in patients with hepatitis C is similar to highly infectious particles in patients with chronic hepatitis C. This suggests that the serum of patients with HCV is potentially infectious(Elmasry et al. 2017). Since the transmission routes for HCV and HIV infection are the same, co-infection with HIV-HCV is relatively common. HIV infection increases the chances of continuing HCV and progressing towards liver fibrosis , liver failure and hepatocellular carcinoma (Farahani et al. 2013). The presence of hepatitis C increases the risk of developing liver toxicity due to the use of retroviral drugs. Treatment of HIV patients with hepatitis C infection prevent from progression of liver disease and cirrhosis or liver cancer, (Laufer et al. 2008). The simultaneous infection of HIV and HCV accelerates HCV related liver disease, which includes a faster change in liver inflammation and hepatocarcinoma in this group of patients. Also, the clinical progression of HIV and the symptoms of AIDS in patients with HIV-HCV co-infection are faster than those who are only infected with HIV.So; in this study frequency of occult HCV in PBMNs of the patients with HIV was determined.

MATERIAL AND METHODS:

Patients:

In this retrospective study, one hundred HIV positive patients referring to the Kerman Behavioral Disorder Counseling Center were enrolled, Before the sampling, the stages of research were explained to the patients, and the patients were signed a satisfactory letter. To each of the patients who entered the study had a code identifier for maintaining the identity of the patients in the next steps of the work. About 5 ml of peripheral blood were collected from each patient into

EDTA-containing vacutainer tubes. Blood buffy coat were isolated from EDTA-treated blood by centrifugation and stored at -70° C for later detection. All patients gave written consent to participate in this study, which conforms to the guidelines of the 1975 Declaration of Helsinki.

Liver Enzyme Test:

All of samples were screened for HIV Ag / Ab, HCV Ab, HBS Ab, using enzyme-linked immunosorbent assay (ELISA) . The positive results of HIV and HCV were controlled by Western Blot and The Recombinant Immunoblot Assay (RIBA). The criteria for selecting the subjects, positive HIV patients. People with autoimmune diseases or genetic disorders, and a history of clinical or biochemical outbreaks of acute hepatitis were excluded. In order to carry out serum liver functional tests, each patient with a 12-hour fasting state was analyzed using specific kits for each test (ALK, AST, ALT) using the Hitachi auto analyzer.

RNA Extraction:

Isolation of PBMCs from whole blood was done by HANK'S method. One ml of HANK'S solution was added to the blood and mixed well. Then centrifuged at room temperature at 12000 rpm. The PBMCs (middle matte layer) were transferred to a 5 ml conical tube containing approximately 2 ml of HANK'S solution (this solution prevents cellular accumulation). The tubes were centrifuged at 1200 rpm for 10 minutes at room temperature. Remove the supernatant and hit the tube until a cell suspension was created. The cells were divided into tubes in 2 ml and stored at -80°C. Extraction of RNA was performed using a RIBO-prep kit from plasma and PBMCs. Evaluation of quantitative the extracted RNA, was performed using spectrophotometric, the concentration and purity of the sample were obtained by using the optical absorbance of the sample at 260 nm (absorption rate of nucleic acids) and 280 nm (absorption and protein contamination).

HCV Real Time PCR and HCV genotyping:

HCV Real Time PCR and HCV Viral Load was performed by HCV-FRT kit (ILS, Russia). To determine of HCV viral load in samples , a standard curve with four standard level of HCV were used. HCV genotyping was performed by HCV genotyping FRT kit (ILS, Russia). HCV genotypes 1 ,2 ,3 ,4 and 6 was detected in this method.

Statistical analysis

The appropriate tables and charts for displaying the results and the mean ± deviation from the criteria, frequency and percent were used to describe the data. Statistical analysis of Pearson chi-square test was performed and P.Value <0.05 was significant (SPSS Version20, Chicago, USA).

RESULTS:

Out of one hundred HIV patients referred to the behavior disorder Kerman Center ,25 patients were female (25%) and 75 patients (75%) were male with a mean age of 47.23 ± 12.65 years old age (Table1). HCV , HIV RNA were extracted from 100 µL of plasma with RIBO-Preb kit (ILS, Russia). For quantification of HBV DNA, real time HBVPlasma of patients was tested with real time-PCR for HCV, HIV-RNA testing, 78 (78%) of the subjects with HIV monoinfection and 22 (22%) had co-infection with HIV / HCV; 9 of whom (40.9) were exposed to hepatitis C and 13 (59.1%) had positive hepatitis C virus (Figure1).Evaluation of hepatitis C virus genotype in 9 patients with latent hepatitis C (11.1%) showed 1 (44.4%) 4 (3%) and (22.2%) 2 cases of genotype 1b(Figure1). The highest percentage of genotype in hepatitis C was detected. Evaluation of genotype in 13 patients with positive hepatitis C in the study group (46.7%) was 7 (1), (33.3%), 5 (3) and (6.7%), 1 (4) (65.7) People 1b and (65.7%) were shown 1 person 3b (Figure2). The highest percentage of genotype was detected in hepatitis C virus positive 3a. In this study, out of 13 patients with hepatitis C, the number of viruses in 10 samples (76.9%) was less than 10^6 viruses in milliliters and in 3 samples (23.1%) more than 10^6 copies per ml (Figure3). Based on statistical results, Genotype 1a has a higher frequency in the population of hepatitis C patients, which has no statistically significant relationship between genotype type and virus load (P = 0.47).The hepatic enzymes ALK, ALT and AST were high in all patients with hepatitis C and positive hepatitis C. In this study, the statistical results showed that there was no significant relationship between hepatic enzymes in patients with hepatitis C and positive hepatitis C patients (P.Value = 0.64). Comparison of patients' data based on Chi-Square Pearson test showed no significant correlation between hepatitis C virus genotype and PBMC in hepatocyte enzymes (P.Value = 0.64).

DISCUSSION:

This study investigated the latent hepatitis C in HIV positive patients in Kerman province, southeast of Iran. The highest percentage of genotypes was detected in hepatitis C virus positive 3a and in latent hepatitis C 1a. The results of our study indicate that the presence of latent hepatitis C is similar to many previous studies(Elmasry et al. 2017). In 2015, Bokharaei and colleagues, studying on 109 patients with HIV, showed that 9.2% of patients were occult HCV. But in this study, the highest percentage of HCV was 3a genotype.(Bokharaei-Salim et al. 2016) Several studies have been done to determine the type of hepatitis C genotype that has been implicated in various diseases(Esmail et al. 2016, Fabris et al. 2003, Farahani et al. 2013). In 2015, Bastani colleagues examined the prevalence of hepatitis C virus infection in patients with µ-thalassemia major. Of the 147 patients 6 (5.7%), were hepatitis C patients. The most hepatitis C genotype was 1a(Bastani et al. 2016) . In 2013, Castilo et al.'s HCV RNA was tested for in the liver samples of 52 patients with chronic HBV infection and 21 (40%) of them were positive for viral RNA (occult HCV infection) (Castillo et al. 2013). In 2016, Mahamud and his colleagues in Pakistan conducted a study to determine the presence of HCV-RNA in liver biopsy from patients with non-native liver enzymes, the most common genotype in this group 3a(Mahmoud et al. 2016) . There was little research on the type of genotype and the burden of the plasma virus. Our study showed that there is no significant relationship between genotype type and virus load (P.Value = 0.47)(Helaly et al. 2017, Jimenez et al. 2017).The results of our study indicate that the hepatic enzymes ALK, ALT and AST are high in all patients with hepatitis C and high hepatitis C infection. In this study, the statistical results showed that there was no significant relationship between hepatic enzymes in patients with hepatitis C and positive hepatitis C patients (P.Value = 0.64)(Koutsoudakis, Perez-del-Pulgar, and Forns 2017). According to the number of patients present in the study, it cannot be said that hepatitis C is one of the reasons for the high levels of liver enzymes. The results of our study indicate that the hepatic enzymes ALK, ALT and AST are high in all patients with hepatitis C and high hepatitis C infection. Also, the statistical results showed that there was no significant relationship between hepatic enzymes in patients with hepatitis C and positive hepatitis C patients (P.Value = 0.64) (Quiroga et al. 2016, Raffa et al. 2007, Rezaee-Zavareh, Ramezani-Binabaj, and Alavian 2015).Many of studies confirm the results of this research and revealed the incidence of hepatitis C in HIV positive patients.

CONCLUSION

Hepatitis C virus is one of the viruses that are transmitted like HIV through blood, transfusion and sexually transmitted infections. Individuals who have HIV, because of the use of antiretroviral drugs or reaction of the immune system, the HCV virus may be hidden and unidentified by routine methods (occult HCV). Therefore, in order to prevent severe liver damage and reduce the risk of hepatocarcinoma, it is suggested that the PBMN of these patients be evaluated for the HCV virus.

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Conflict of interest: The authors declare no conflict(s) of interest.

Table1: Demographic and laboratory data in HIV patients with HCV and Occult HCV

Variables	HIV Patients					
	HCV Negative		HCV Occult		HCV Positive	
	Range	Mean± SD	Range	Mean± SD	Range	Mean±SD
Age	11-65	35.3±12.2	21-54	29.2± 10.2	10-62	35.2±14.2
Female	18	23.07%	2	22.2%	5	38.46%
Male	60	76.92%	7	77.8%	8	61.53%
AST (IU/mL)	12-254	47.7± 34.2	26-586	239.67± 150.38	21-521	180.3±131.4
ALT (IU/ml)	12-352	48.4± 42.8	25-425	274.67± 120.9	32-654	230.2±160.3
ALK (IU/mL)	54-963	292.6± 189.4	321-994	774.1±216.2	354-997	781.4±200.2

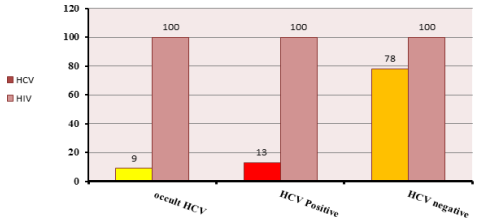

Figure1: Frequency of occult HCV and HCV positive in HIV patients

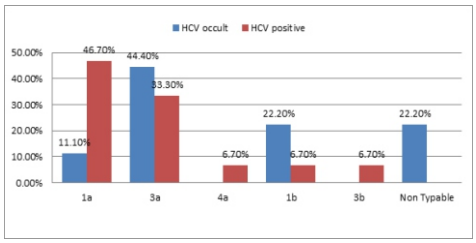

Figure2: Frequency of HCV Genotype in occult HCV vs HCV positive

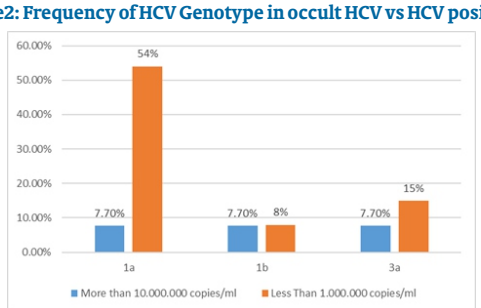

Figure3: Percent mean of HCV Viral load in different Genotypes of HCV

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