

Prevalence and effect of occult hepatitis C infection in patients with persistent liver enzyme elevation after achieving 24 weeks of sustained virological response

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Background

Despite achieving sustained virological response (SVR) of chronic hepatitis C infection, some of the treated patients have persistent elevations of transaminases. Occult hepatitis C infection (OCI) could be one of the causes.

Aim

The aim of this study was to detect OCI in peripheral blood mononuclear cells in patients who achieved 24-week SVR with persistent elevations of transaminases.

Methods

We included 998 naïve chronic HCV-infected patients who received treatment at our hospital. Patients with elevated liver enzymes after achieving SVR were determined. HCV RNA PCR in peripheral blood mononuclear cells was done for those patients (group 1) and was compared with a group with normal levels of enzymes, which was matched in age and sex (group 2).

Results

Nine hundred and sixty-five patients achieved SVR (96.69%). Seventy-four (7.7%) patients of them had elevated enzymes. OCI was detected in 14/74 (18.9%) patients of group 1, whereas it was seen in 4/67 (5.9%) in group 2. Cirrhosis, OCI, and obesity were associated with this enzymes elevation ($P=0.005$, 0.024 , and <0.001). By multivariate analysis, none of these three parameters were independently associated with the enzyme elevation. The presence of OCI was not significantly associated with the presence of cirrhosis or obesity.

Conclusion

OCI is not infrequent in patients with persistent transaminase elevations despite obtaining 24 weeks of SVR. Liver cirrhosis, OCI, and obesity could have synergistic effects and should be considered as important risk factors of this persistent enzyme elevation.

Keywords:

directly acting antiviral, hepatitis C, liver diseases, occult hepatitis C infection

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Introduction

Chronic hepatitis C could lead to liver cirrhosis and hepatocellular carcinoma in more than 20% of cases [1]. Occult hepatitis C infection (OCI) is owing to the presence of hepatitis C virus (HCV) RNA in peripheral blood mononuclear cells (PBMCs) or in the liver tissue despite undetectable serum HCV RNA [2]. The gold standard for OCI diagnosis is liver biopsy with detection of HCV RNA in hepatocytes, but diagnosis could be made in 70% of cases through testing HCV RNA in PBMCs [3]. Our aim was to study its prevalence and effect on enzyme elevation after achieving sustained virological response (SVR) with direct-acting antivirals (DAAs).

Patients and methods

This prospective study included all consecutive chronic HCV naïve patients who were treated with DAAs at

our tertiary referral hospital during 2017. Patients with age older than or equal to 18 years and who achieved 24 weeks of SVR after the end of treatment confirmed by undetectable HCV RNA using a quantitative PCR technique with a detection limit of less than 15 IU/ml were included in the study. Patients were excluded from the study if they had signs of hepatocellular carcinoma, advanced co-morbid conditions or uncompensated liver cirrhosis, previous HCV treatment, positive HBs-Ag, anti-HB core IgG, or HIV-Ab. Moreover, patients with pregnancy, history of alcohol intake, or recently received possible hepatotoxic drug were excluded.

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All of our noncirrhotic patients received sofosbuvir 400 mg/day and daclatasvir 60 mg/day orally for 3 months. Patients with cirrhosis were treated with sofosbuvir 400 mg/day and daclatasvir 60 mg/day besides a weight-based ribavirin oral dose for 3 months.

Persistent elevated liver enzymes (alanine or aspartate aminotransferases) are defined as an elevation of liver enzymes above normal level despite achieving SVR, so the elevation persists as it was before starting treatment, either it was continuously elevated after the end of the DAAs therapy or it was normalized for a short period of time and then increased again. The upper limit of transaminases was considered 40 IU/ml for both sex. Written informed consents were obtained from patients before their enrollment as a routine workup before starting their treatment. The study protocol was approved by the local ethics committee.

All patients with elevated transaminases along with 67 patients with normal enzyme levels, matched in age and sex (used as a control group), were tested for HCV RNA in PBMCs. Retrotranscription-PCR of genomic and antigenomic strands of HCV RNA was done using Cobas TaqMan HCV test (Roche Molecular Systems Inc., Branchburg, New Jersey, USA), which is a real-time amplification assay for quantitative detection of HCV RNA in human serum or plasma with a lower limit of detection of 15 IU/ml. Amplification and detection were performed according to the manufacturer instructions.

Statistical methods

Data were statistically analyzed using SPSS, version 20 (SPSS Inc., Chicago, USA), for windows, and a *P* value was considered statistically significant if less than 0.05. Independent samples *t*-test or χ^2 -tests were used to examine the difference between the two groups for continuously distributed or categorical variables, respectively.

Table 1 Demographic data of the patients with persistent elevation of liver enzymes (N=74)

Demographic data	<i>n</i> (%)
Age [mean±SD (range)] (years)	56.4±7.31 (38–70)
Sex (male/female)	49/25 (66.2/33.8)
Diabetes mellitus (yes/no)	20/54 (27)
Cirrhosis (yes/no)	34/40 (45.9)
BMI ≥30 (yes/no) (kg/m ²)	41/33 (55.4)
OCI (positive/negative)	14/60 (18.9)

OCI, occult hepatitis C infection.

Results

Nine hundred ninety-eight naive chronic HCV-infected patients were treated with DAAs; among them, 965 (96.69%) patients achieved SVR. Mean age was 53.2±10.6 (20–75) years, 589 (61%) were males, and approximately one-third of those patients had compensated cirrhosis [302 (31.3%)].

During their follow-up, we found 74 (7.67%) patients with persistent elevation of their liver enzymes despite their virological clearance; their demographic data are illustrated in Table 1.

After performing HCV RNA testing in PBMCs (as described before) in those with elevated enzymes, the results revealed that 14 (18.9%) patients had OCI. We also applied this test for 67 patients with normal enzyme levels, and only four (5.9%) patients with OCI were found (Table 2).

Statistics revealed that liver cirrhosis, OCI, and BMI of at least 30 kg/m² were significantly associated with the occurrence of persistent enzyme elevation (*P*=0.005, 0.024, and <0.001 respectively), whereas diabetes showed no significant relation (Table 2).

By multivariate analysis, none of these three parameters were independently associated with the enzyme elevation.

There was no significant difference between the presence of OCI and the presence of liver cirrhosis, BMI at least 30 kg/m², diabetes, or any other laboratory tested variable (Tables 3 and 4).

Table 2 The relation between persistent elevation of liver enzymes and presence of liver cirrhosis, obesity, diabetes mellitus, and occult hepatitis C infection in patients with sustained virological response (N=965)

	Elevated enzymes (N=74) [<i>n</i> (%)]	No elevated enzymes (N=891) [<i>n</i> (%)]	<i>P</i> value
Cirrhosis (yes/no)	34/40 (46)	268/623 (30)	0.005
BMI ≥30 (yes/no) (kg/m ²)	41/33 (55.4)	321/570 (36)	<0.001
DM (yes/no)	20/54 (27)	215/676 (24.1)	0.23
OCI ^a (positive/negative)	14/60 (18.9)	4/63 (6)	0.024

DM, diabetes mellitus; OCI, occult hepatitis C infection. ^aOCI was tested for all patients with elevated enzymes but only 67 matched patients with normal enzyme levels.

Table 3 The relation between occult hepatitis C and presence of liver cirrhosis, obesity, and diabetes mellitus among patients with persistent elevation of liver enzymes (N=74)

	Positive OCI (N=14) [n (%)]	Negative OCI (N=60) [n (%)]	P value
Cirrhosis (yes/ no)	7/7 (50)	27/33 (45)	0.74
BMI \geq 30 (yes/ no) (kg/m ²)	8/6 (57.1)	33/27 (55)	0.89
DM (yes/no)	4/10 (28.6)	16/44 (26.7)	0.89

DM, diabetes mellitus; OCI, occult hepatitis C infection.

Discussion

The primary goal of hepatitis C therapy is to achieve SVR, as it will lead in most cases to normalization of liver enzymes with fibrosis improvement [4]. Relapses usually occur within weeks after the end of treatment, but it also could occur late, and the reason behind it is still unknown. It was suggested that activation of OCI could be a possible cause [5].

Prevalence of OCI is a matter of debate, and the available results showed great discrepancy from 2.2% in a study on blood donors [6] to 45% among patients on hemodialysis with abnormal liver enzymes [7]. So does OCI have a real association with the elevated liver transaminases and hence has a future potential negative effect on the liver [8] or it is just a noninfective viral particles with no real effect [9]? Studies showed a transaminase elevation with fibrosis progression in some cases, although obtaining a virological cure [10,11].

In our study, the 24-week SVR was 96.69%, taking in consideration that approximately one-third of our treated patients had cirrhosis. We think this is a great success response to the drug regimen sofosbuvir/daclatasvir for noncirrhotic patients and sofosbuvir/daclatasvir/ribavirin for patients with cirrhosis.

The results showed that 74 (7.67%) patients had persistent elevation of their liver enzymes. Although none of the tested parameters was independently associated with the enzyme elevation, yet OCI along with liver cirrhosis and obesity was significantly associated with this elevation. In our opinion, this could be owing to some sort of synergistic effects among the three parameters, and this could raise the possibility of a negative effect of OCI on patients who achieved SVR. We also found no significant difference between the occurrence of OCI and the presence of cirrhosis, obesity, or diabetes mellitus.

Our results goes with Welsch *et al.* [4], who postulated that liver cirrhosis and steatosis could explain the

Table 4 The relation between occult hepatitis C and laboratory investigations in patients with persistent elevation of liver enzymes (N=74)

	Positive OCI (N=14) (mean \pm SD)	Negative OCI (N=60) (mean \pm SD)	Mann-Whitney test	P value
Bilirubin (mg/dl)	0.86 \pm 0.41	1.05 \pm 0.75	0.801	0.423
Albumin (g/ dl)	3.97 \pm 0.22	3.93 \pm 0.48	0.278	0.781
ALT (U/l)	52.9 \pm 16.3	59.2 \pm 18.0	0.809	0.419
AST (U/l)	52.1 \pm 13.1	56.2 \pm 16.7	0.775	0.439
Creatinine (mg/dl)	0.81 \pm 0.17	0.86 \pm 0.23	0.847	0.397
Hemoglobin (g/dl)	13.2 \pm 1.80	13.0 \pm 1.68	0.739	0.460
WBCs ($\times 10^9$ /l)	6.64 \pm 2.19	6.72 \pm 2.16	0.145	0.885
Platelets ($\times 10^9$ /l)	159.7 \pm 71.4	167.1 \pm 62.8	0.642	0.521
INR	1.07 \pm 0.10	1.11 \pm 0.11	1.01	0.310

ALT, alanine aminotransferase; AST, aspartate aminotransferase; INR, international normalized ratio; OCI, occult hepatitis C infection; WBCs, white blood cells.

persistent transaminase elevation in approximately 10% of their treated chronic HCV-infected patients who achieved SVR.

The association between liver cirrhosis and the elevated liver enzymes is understood, as it is already an advanced liver disease. On the contrary, obesity, as a risk factor, could be explained on the basis of its association with hepatic steatosis, which could lead to hepatic inflammation and fibrosis progression [12–14]. Moreover, our results could indicate a possible silent course of OCI, as there was no significant difference regarding liver panel and other biochemical tests among those with OCI and those without.

In agreement with our results, Elmasry *et al.* [15] in their study found that 14 (10%) of 134 treated patients of recurrent hepatitis C after liver transplantation had elevated enzymes, and when they tested only nine patients of them for OCI, they found five (55%) of them were positive.

Our results showed a high prevalence of OCI in the group of patients with elevated transaminases despite the achievement of SVR (18.9%). For this reason, we recommend a strict follow-up for those group of patients to detect whether their acquiescent OCI infection could develop later into active viremia and hence progression of the liver disease or not. This has to be validated in subsequent research studies.

Conclusion

OCI is not rare in patients with persistent elevation of transaminases despite obtaining a SVR after DAAs therapy. It could be considered as a risk factor for this persistent enzyme elevation. Cirrhosis and obesity could also be considered as important risk factors of this enzyme elevation. Future research is needed to determine if there is a long-term effects of OCI and whether retreatment is needed or not.

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Conflicts of interest

There are no conflicts of interest.

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