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Secondary Occult Hepatitis C Virus Infection (HCV) in Chronic HCV Patients after Treatment with Sofosbuvir and Daclatasvir

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A B S T R A C T

This study is a cross-sectional study aimed to assess the rate of occurrence of secondary occult hepatitis C virus (HCV) infection in patients treated with the combination of sofosbuvir and daclatasvir. This study included 40 patients whose serum turned negative for HCV ribonucleic acid (HCV RNA) via real time polymerase chain reaction (RT-PCR) after 3 months of the treatment. Blood samples on EDTA were collected from the patients to detect HCV RNA in peripheral blood mononuclear cells (PBMCs). At the end of the study there were 30 PBMCs HCV RNA negative patients and 10 PBMCs HCV RNA positive patients. Our data revealed the occurrence of secondary occult HCV infection in about 25% of HCV patients treated with the combination of sofosbuvir and daclatasvir.

Introduction

Hepatitis C virus (HCV) is the main cause of chronic liver disease all over the world. HCV affects about 200 million people worldwide. About 350000 deaths per yearare due to HCV infection. HCV is classified as a member of Flaviviridae family and Hepacivirus genus. 30% of infected people resolve their acute infection spontaneously, while about 70% turned to chronic HCV infection (Zaltron *et al.*, 2012). Chronic HCV infection is detected by the persistence of HCV ribonucleic acid (RNA) in the blood for 6 months or more after the onset of acute infection. Many factors can affect the degree of HCV chronicity such as the age at onset of the disease, gender, ethnicity, and jaundice during the acute infection (Chen *et al.*, 2006).

Occult HCV infection (OCI) is identified by the presence of HCV RNA in the liver cells or peripheral blood mononuclear cells (PBMCs) of the patients whose serum samples test negative for HCV RNA by polymerase chain reaction (PCR) assays, with or without

presence of HCV antibodies (Carreno *et al.,* 2006).

OCI can lead to liver cirrhosis and hepatocellular carcinoma (Zaltron *et al.,* 2012). PBMCs might be considered as a long-lived HCV reservoir due to the persistence of viral RNA in the of patients who had cleared their viremia either spontaneously or after antiviral therapy. HCV RNA can be detected in PBMCs instead of liver biopsy in about 70% of patients with an OCI (Carreno *et al.,* 2004).

OCI can be found in some high-risk people like hemodialysis and kidney transplanted patients, cryptogenic liver disease, and immune-deficient patients. But also, some data have been reported about the presence of OCI among healthy people without any liver disease. HCV genotypes 1 and 4 are the most genotypes involved in the OCI (Carreno *et al.,* 2012; Youssef *et al.,* 2012).

OCI has been defined in two different forms: cryptogenic and secondary. Cryptogenic OCI: if the patient has no anti-HCV antibodies but has elevated liver enzymes (Castillo *et al.,* 2004). Secondary OCI: if the patient has anti-HCV antibodies, has normal liver enzymes and had cleared his HCV infection neither spontaneously or after anti HCV therapy (Pham *et al.,* 2004).

In the last few years, the combination of pegylated interferon (PEG-IFN) and ribavirin (RBV) was the only available treatment option for HCV genotype 4 (HCV-4) infection. But later on another treatment options are available such as direct acting antivirals (DAAs) which can used with or without addition of PEG-IFN/RBV. Now, combinations of DAAs are commonly used for the treatment of HCV-4 infection due to higher cure rates, shorter treatment period, a higher genetic barrier, and minimal adverse events (AEs). One of the most effective and commonly used combinations of (DAAs) for the treatment of HCV-4 is the combination of sofosbuvir (SOF) and daclatasvir (DCV) for 3 months (Abdel-Razek *et al.,* 2015). DCV is HCV nonstructural protein NS5Ainhibitor (Yang *et al.,* 2016), while SOF is a nucleotide analogue inhibitor (Bertino *et al.,* 2016).

Results from clinical studies as well as preliminary real-life data showed that the combination of sofosbuvir and daclatasvir, is one of the most successful antiviral therapies with once-daily oral dosing, a low pill burden, good tolerability, limited drug–drug interactions, and also high (90%) sustained virologic response rates. Such a combination has high pangenotypic antiviral potency (Pol *et al.,* 2016).

Materials and Methods

Study design

Cross-sectional interventional study.

Subjects

All subjects included in this study were obtained from outpatient clinic of Alexandria Main University Hospital.

Institutional ethical board approval was taken prior to the study, as well as informed consent was taken from all the participants. A total of 40 adult HCV patients were included in this study (above 18 years of age).

Inclusion criteria were chronic HCV infection and treatment by combination of sofosbuvir (SOF) and daclatasvir (DCV) for three months which suitable for all grades of HCV infection (according to Child Pugh scoring). Exclusion criteria were co-infection with hepatitis B virus (HBV) or human immunodeficiency virus (HIV), schistosomiasis, diabetes

mellitus, hepatotoxic drugs for example methotrexate, alcohol intake and history of hepatocellular carcinoma (HCC).

All the subjects included in the study were subjected for full history taking and clinical examination, Complete blood count, virological profile: HCV Abs, the surface antigen of hepatitis B virus (HBsAg), all liver function tests especially serum glutamate-oxaloacetate transaminase (SGOT), serum glutamate-pyruvate transaminase (SGPT), Alkaline phosphatase, Serum bilirubin (direct and indirect), Serum albumin and Serum prothrombin, tumour marker: α feto protein, thyroid function test: thyroid stimulating hormone (TSH), abdominal ultrasonography to assess state of liver cirrhosis and portal hypertension and upper endoscopy to assess presence of oesophageal and gastric varices and manifestation of portal hypertensive gastropathy.

Serum samples will be collected to detect HCV RNA via real time polymerase chain reaction (RT-PCR) from patients: at the diagnosis and the start of the treatment, after one month of the treatment and after three months of the treatment (at the end of the treatment).

Blood samples on EDTA will be collected from all patients whose serum PCR will turn negative for HCV RNA after one month (early virological response) and those who will turn negative after three months of treatment to detect HCV RNA in PBMCs.

Isolation of serum and PBMC

Serum was separated from whole blood in 2Eppendorf tubes following centrifugation. Isolated serum was immediately stored at -20°C in order to avoid repeat freeze thawing.

Peripheral blood lymphocytes were isolated immediately following blood drawing (5.0 ml) from serum-negative patients. Whole blood was layered over Ficoll-Hypaque (Sigma, USA) density- gradient medium.

HCV RNA quantification

The COBAS AmpliPrepTaqman analyser (CAP-CTM) instrument / COBAS TaqMan Real time PCR HCV Test kit (Roche Molecular Systems, Mannheim, Germany) was used for quantification of viral loads according to the manufacturer's instructions (Fusun *et al.,* 2013).

Statistical analyses

Data were fed to the computer and analyzed using IBM SPSS software package version 20.0**. (**Armonk, NY: IBM Corp**).**Qualitative data were described using number and percent. The Kolmogorov-Smirnov test was used to verify the normality of distribution Quantitative data were described using range (minimum and maximum), mean, standard deviation and median. Significance of the obtained results was judged at the 5% level (Kotz *et al.,* 2006; Kirkpatrick *et al.,* 2013).

The used tests were

Chi-square test

For categorical variables, to compare between different groups

Fisher’s Exact or Monte Carlo correction

Correction for chi-square when more than 20% of the cells have expected count less than 5

Student t-test

For normally distributed quantitative variables, to compare between two studied groups.

Mann Whitney test

For abnormally distributed quantitative variables, to compare between two studied groups

Results and Discussion

This study comprised two groups HCV RNA PBMCs negative patients and HCV RNA PBMCs positive patients.

There were no statistical significant differences between the two groups regarding age (p= 0.244), gender (p= 0.269), presence of risky occupation (p= 1.000), and mode of transmission (p= 1.000). There were no statistical significant differences between the two groups regarding Haemoglobin A1C (p=0.415), Haemoglobin (p=0.208), and total leucocytic count (p=0.310). But there was statistical significant difference between the two groups regarding platelet count (p=0.022).

There were no statistical significant differences between the two groups regarding Serum albumin (p= 0.851), Prothrombin activity (p= 0.356), ESR (p= 0.805), TSH (p= 0.325), and α Feto protein (p= 0.349).

There were no statistical significant differences between the two groups regarding abdominal ultrasonography (p= 0.210) and upper GI endoscopy (p= 1.000).

There were statistical significant differences between the two groups regarding SGOT, SGPT and Alkaline phosphatase (Table 1).

There was no statistical significant difference between the two groups regarding indirect bilirubin but there were statistical significant differences between the two groups regarding direct and total bilirubin (Table 2). Chronic hepatitis C virus (HCV) infection remains a global health threat with the highest prevalence reported in Egypt (Tawhida *et al.*, 2015). In the past few years, a new entity of HCV infection was identified and defined as occult HCV infection (OCI) which is identified by the presence of HCV RNA in the liver cells or peripheral blood mononuclear cells (PBMCs) of the patients whose serum samples test negative for HCV RNA by polymerase chain reaction (PCR) assays, with or without presence of HCV antibodies (Carreno *et al.*, 2006).

OCI has been defined in two different forms: cryptogenic and secondary. Cryptogenic OCI: if the patient has no anti-HCV antibodies but has elevated liver enzymes (Castillo *et al.*, 2004). Secondary OCI: if the patient has anti-HCV antibodies, has normal liver enzymes and had cleared his HCV infection either spontaneously or after anti HCV therapy (Pham *et al.*, 2004).

In our study, HCV RNA was detected in PBMCs of 10out of 40 (25%) chronic HCV patients treated with the combination of sofosbuvir and daclatasvir for 3 months. As we mentioned before, although the detection of HCV RNA in liver biopsy specimens is the gold standard method for diagnosis of OCI, the detection of HCV RNA in PBMCs is an alternative method in the absence of liver biopsy. HCV RNA was detected in the PBMCs of about 70% of patients with occult infection. Therefore, detection of HCV RNA in PBMCs does not detect all OCI cases (Carreno *et al.*, 2004).

Previous studies showed that the age (22–66 years) is the common age of OCI. Our study agreed with that, although we detected OCI in old patients above 66 years. This, together with the fact that male sex is predominant in OCI than female sex which is accordant with previous studies by L´opez-Alcorocho and Castillo *et al.*, (L´opez-Alcorocho *et al.*, 2007; Castillo *et al.*, 2007).

Table.1 Comparison between the two studied groups according to liver enzymes

Liver enzymes		Total (n=40)	Negative HCV RNA (n= 30)	Positive HCV RNA (n= 10)	U	p
SGOT (U/L)	**Before**					
	Min. – Max.	11.0 – 211.0	11.0 – 211.0	31.0 – 132.0	86.0*	0.046*
	Mean ± SD.	59.6 ± 41.0	54.07 ± 43.7	76.4 ± 26.5		
	Median	57.50	36.0	77.5		
	After					
	Min. – Max.	10.0 – 59.0	10.0 – 59.0	14.0 – 39.0	119.5	0.346
	Mean ± SD.	26.9 ± 13.7	26.2 ± 14.8	29.2 ± 10.03		
	Median	26.0	20.50	32.0		
	% of change	**↓44.79 ± 21.62**	**↓39.71 ± 21.75**	**↓60.02 ± 12.57**	0.009*	0.008*
SGPT (U/L)	**Before**					
	Min. – Max.	11.0 – 168.0	11.0 – 168.0	30.0 – 140.0	78.0*	0.024*
	Mean ± SD.	58.40 ± 44.17	51.9 ± 45.92	78.0 ± 32.99		
	Median	52.0	30.0	72.0		
	After					
	Min. – Max.	10.0 – 57.0	10.0 – 57.0	13.0 – 40.0	108.50	0.194
	Mean ± SD.	24.63 ± 12.27	23.73 ± 13.05	27.30 ± 9.65		
	Median	22.50	22.0	27.50		
	% of change	**↓41.10 ± 26.85**	**↓34.12 ± 26.70**	**↓62.04 ± 13.26**	54.0*	0.003*
Alkaline phosphatase(U/L)	**Before**					
	Min. – Max.	19.0 – 193.0	19.0 – 193.0	46.0 – 159.0	149.0	0.975
	Mean ± SD.	87.22 ± 42.86	86.43 ± 44.46	89.60 ± 39.81		
	Median	93.0	94.0	90.50		
	After					
	Min. – Max.	15.0 – 102.0	15.0 – 86.0	42.0 – 102.0	86.0*	0.045*
	Mean ± SD.	57.13 ± 24.42	52.50 ± 23.05	71.0 ± 24.23		
	Median	59.0	59.0	78.0		
	% of change	**↓27.81 ± 19.73**	**↓31.41 ± 20.23**	**↓16.98 ± 13.94**	74.0*	0.018*

U, p: U and p values for Mann Whitney test for comparing between the two groups,
*: Statistically significant at $p \leq 0.05$.
SGOT= serum glutamate-oxaloacetate transaminase, SGPT= serum glutamate-pyruvate transaminase, HCV RNA= Hepatitis C virus ribonucleic acid.

Table.2 Comparison between the two studied groups according to bilirubin

Bilirubin		Total (n=40)	Negative HCV RNA (n= 30)	Positive HCV RNA (n= 10)	U	p
Direct bilirubin(mg/dl)	**Before**					
	Min. – Max.	0.12 – 2.10	0.12 – 2.10	0.30 – 1.30	86.0*	0.044*
	Mean ± SD.	0.64 ± 0.44	0.58 ± 0.46	0.83 ± 0.30		
	Median	0.58	0.32	0.80		
	After					
	Min. – Max.	0.11 – 1.20	0.10 – 0.24	0.10 – 1.20	117.0	0.302
	Mean ± SD.	0.30 ± 0.24	0.20 ± 0.04	0.28 ± 0.21		
	Median	0.24	0.21	0.22		
	% of change	**↓47.37 ± 24.24**	**↓39.58 ± 21.68**	**↓70.74 ± 14.92**	29.50*	<0.001*
Indirect bilirubin(mg/dl)	**Before**					
	Min. – Max.	0.28 – 2.10	0.28 – 2.10	0.30 – 2.0	96.50	0.094
	Mean ± SD.	0.94 ± 0.56	0.87 ± 0.56	1.15 ± 0.56		
	Median	0.79	0.74	1.08		
	After					
	Min. – Max.	0.23 – 1.80	0.23 – 1.80	0.35 – 0.72	139.0	0.731
	Mean ± SD.	0.62 ± 0.30	0.63 ± 0.34	0.59 ± 0.12		
	Median	0.59	0.56	0.63		
	% of change	**↓19.52 ± 36.53**	**↓15.65 ± 34.27**	**↓31.13 ± 42.41**	102.50	0.138
Total bilirubin(mg/dl)	**Before**					
	Min. – Max.	0.51 – 3.30	0.51 – 3.30	0.60 – 3.30	92.50	0.072
	Mean ± SD.	1.59 ± 0.91	1.47 ± 0.91	1.97 ± 0.84		
	Median	1.36	1.07	1.85		
	After					
	Min. – Max.	0.37 – 2.80	0.37 – 2.80	0.45 – 0.95	142.0	0.803
	Mean ± SD.	0.90 ± 0.49	0.93 ± 0.56	0.80 ± 0.15		
	Median	0.84	0.79	0.85		
	% of change	**↓34.05 ± 27.04**	**↓28.91 ± 25.25**	**↓49.48 ± 27.59**	83.0*	0.036*

U, p: U and p values for Mann Whitney test for comparing between the two groups
*: Statistically significant at $p \leq 0.05$
HCV RNA= Hepatitis C virus ribonucleic acid

Several Egyptian studies were conducted to detect the prevalence of occult HCV in different groups of patients, such as in healthy spouses of HCV patients (El Shazly *et al.*, 2015), in patients with chronic lymphoproliferative disorders (Youssef *et al.*, 2012), also in patients with nonalcoholic fatty liver disease (Saad *et al.*, 2011), and hemodialysis patients (Abdelrahim *et al.*, 2016).

Previous study indicated that sexual contact (El Shazly *et al.*, 2015) is one of the most common causes of transmission of OCI. However, in our study, OCI was mainly transmitted by surgery and dental procedures.

In our study, we found that there were statistical significant differences between the two groups regarding liver enzymes as SGOT and SGPT before treatment and in the percent of change (between before and after treatment) as we found that the percent of change decreased significantly in positive group which can be explained by increase rates of liver enzymes significantly before treatment in positive group, while in serum alkaline phosphatase we found that there were statistical significant differences between the two groups after treatment and in the percent of change. Other previous studies revealed an insignificant difference in the prevalence of occult HCV infection between patients with normal or high liver enzymes results (Saad *et al.*, 2011).

Also, we found that there were statistical significant differences between the two groups regarding direct bilirubin before treatment and in the percent of change as we found that the percent of change decreased significantly in positive group which can be explained by increase rates of direct bilirubin significantly before treatment in positive group, while in total bilirubin we found that there were statistical significant differences between the two groups only in the percent of change, also we found that there was no statistical significant difference between the two groups regarding indirect bilirubin.

In conclusion, this study is the first Egyptian study to investigate the prevalence of occult HCV in patients with secondary OCI who had cleared his HCV infection after anti HCV therapy with the combination of sofosbuvir and daclatasvir for 3 months. Despite this, the present study had some limitations, such as absence of confirmed OCI in liver tissue and small sample size. Therefore, further studies with larger sample are recommended and also we recommend some studies be performed to investigate the prevalence of secondary OCI after therapy with new regimens.

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References

Abdelrahim SS, Khairy R, Esmail MA, *et al.*, 2016. Occult hepatitis C virus among Egyptian hemodialysis patients. J Med Virol.

Abdel-Razek W, Waked I. 2015. Optimal therapy in genotype 4 chronic hepatitis C: finally cured? Liver Int Jan., 35 Suppl. 1: 27-34.

Bartolome, J., Lopez-Alcorocho, J.M., Castillo, I., *et al.*, 2007. Ultracentrifugation of serum samples allows detection of hepatitis C virus RNA in patients with occult hepatitis C. *J Virol*, 81(14): 7710-7715.

Bertino G, Ardiri A, Proiti M., *et al.*, 2016. Chronic hepatitis C: This and the new era of treatment. World J Hepatol Jan

18(2): 92–106.

C virus (HCV) and occult HBV and HCV dual infection, *Journal of Medical Virology.*, 79(3): 236–241.

Carreno V, Bartolome J, Castillo I., *et al.*, 2012. New perspectives in occult hepatitis C virus infection. World J Gastroenterol, 18(23): 2887–94.

Carreno, V., Castillo, I., Bartolome, J., *et al.*, 2004. Comparison of hepatitis C virus RNA detection in plasma, whole blood and peripheral blood mononuclear cells of patients with occult hepatitis C virus infection. *J Clin Virol*, 31(4): 312- 313.

Carreno, V., Pardo, M., Lopez-Alcorocho, J.M., *et al.*, 2006. Detection of hepatitis C virus (HCV) RNA in the liver of healthy, anti- HCV antibody-positive, serum HCV RNA-negative patients with normal alanine aminotransferase levels. *J Infect Dis*, 194(1): 53-60.

Castillo I, Rodr´ıguez-Iˇnigo, E, L´opez-Alcorocho, J. M., *et al.*, 2007. "Comparative study on the clinical and virological characteristics among patients with single occult hepatitis B virus (HBV), single occult hepatitis.

Castillo, I, Pardo, M., Bartolome, J *et al.*, 2004. Occult hepatitis C virus infection in patients in whom the etiology of persistently abnormal results of liver-function tests is unknown. *J Infect. Dis.*, 189(1): 7-14.

Chen SL, Morgan TR. 2006. The natural history of hepatitis C virus (HCV) infection. Int J Med Sci., 3(2): 47-52.

El Shazly Y, Hemida K, Rafik M., *et al.*, 2015. Detection of occult hepatitis C virus among healthy spouses of patients with HCV infection. J Med Virol., 87: 424–427.

Fusun Comert, Elif Aktas, H. Agah Terzi., *et al.*, 2013. Evaluation of hepatitis C virus RNA stability in room temperature and multiple freeze–thaw cycles by COBAS AmpliPrep/COBAS TaqMan HCV. Diagnostic Microbiology and Infectious Disease 75: 81–85.

Kirkpatrick LA, Feeney BC. 2013. A simple guide to IBM SPSS statistics for version 20.0. Student ed. Belmont, Calif.: Wadsworth, Cengage Learning.

Kotz S, Balakrishnan N, Read C.B., *et al.*, 2006. Encyclopedia of statistical sciences. 2nd edn. Hoboken, N.J. Wiley-Interscience.

L´opez-Alcorocho, J. M. Rodr´ıguez-Iˇnigo, E. Castillo, I., *et al.*, 2007. The role of genomic and antigenomic HCV-RNA strands as predictive factors of response to pegylated interferon plus ribavirin therapy. *Alimentary Pharmacology and Therapeutics*, 25(10): 1193–1201.

Pham, T.N., Mac Parland, S.A., Mulrooney, P.M., *et al.*, 2004. Hepatitis C virus persistence after spontaneous or treatment-induced resolution of hepatitis C. *J Virol.*, 78(11): 5867-5874.

Pol S, Corouge M, Vallet-Pichard A. 2016. Daclatasvir-sofosbuvir combination therapy with or without ribavirin for hepatitis C virus infection: from the clinical trials to real life. Hepatic Medicine: Evidence and Research, 8: 21-6.

Rezaee-Zavareh MS, Einollahi B. 2014. Treatment of occult hepatitis C virus infection: does it need special attention? Hepat Mon., 14(7): e16665.

Saad Y, Zakaria S, Ramzy I, *et al.*, 2011. Prevalence of occult hepatitis C in Egyptian patients with non-alcoholic fatty liver disease. Open J Intern Med., 1: 33–37.

Tawhida Y Abdel-Ghaffar, Mostafa M Sira, Suzan El Naghi. 2015. Hepatitis C genotype 4: The past, present, and future. World J Hepatol., 7(28): 2792-2810.

Yang SS, Kao JH. 2016. Daclatasvir-containing all-oral regimens for the

treatment of hepatitis C virus infection. Hepatol. Int. Mar., 10(2):258–66.

Youssef SS, Nasr AS, El Zanaty T., *et al.*, 2012. Prevalence of occult hepatitis C virus in Egyptian patients with chronic lymphoproliferative disorders. Hepatitis Research and Treatment, Pp. 1-6.

Zaltron S, Spinetti A, Biasi L., *et al.*, 2012. Chronic HCV infection: epidemiological and clinical relevance. BMC Infect Dis., 12 Suppl 2: S2.

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