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DETECTION OF OCCULT HEPATITIS C VIRUS INFECTION IN PATIENTS WHO ACHIEVED A SUSTAINED VIROLOGIC RESPONSE TO DIRECT-ACTING ANTIVIRAL AGENTS FOR RECURRENT INFECTION AFTER LIVER TRANSPLANTATION

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ABSTRACT:

Background and Aim of the work: The hepatitis C virus (HCV), an enveloped single-stranded RNA virus, was identified in 1989 and it was classified within the Flaviviridae family as a separate genus (Hepacivirus) The virus replicates by the synthesis of the complementary RNA strand (the so-called negative or antigenomic strand. So far, six major genotypes (HCV-1 to HCV-6) have been described, each containing multiple subtypes. We aim to detect occult HCV infection in post liver transplanted patients who received DAAs & achieve SVR12 ,to detect prevalence of this type of infection and its effect on graft dysfunction , fibrosis progression of he graft. **Patients and Methods:** This study was conducted 20 participants who had undergone LDLT for HCV related liver disease, recieved DAAs, achieved & maintained a sustained virologic response 12 weeks after therapy (SVR12) from a peroid of not less than 6 months. They recruited during the period from December 2016 to November 2017, from the Liver Transplantation unit, Faculty of Medicine, Cairo University. **Results:** This study is a cross-sectional prospective case control hospital based study that was done in the liver transplantation unit, El-Manial Specialized hospital, Faculty of Medicine, Cairo University from December 2016 to November 2017. A total 20 patients who had undergone Living donor liver transplantation for HCV related liver disease and they were eligible for HCV treatment and achieved SVR 12;, were included in the study. **Conclusion:** Detection of viral RNA in the peripheral blood mononuclear cells (PBMC) in patients with sustained virologic response following sofosbuvir-based direct acting antiviral treatments revealed detection of HCV RNA in 2 from a total of 20 patients included in our study (10%). Our study has found that occult hepatitis C virus infection is still a significant problem even after the era of direct antiviral agents; We detected the HCV RNA in the Peripheral blood mononuclear cells in 2 out of 20 (10%) among post liver transplanted patiens who maintained a sustained virologic response 12 weeks after therapy (SVR12) with direct-acting antiviral (DAA) agents.

Introduction

The hepatitis C virus (HCV), an enveloped single-stranded RNA virus, was identified in 1989 and it was classified within the Flaviviridae family as a separate genus (Hepacivirus) The virus replicates by the synthesis of the complementary RNA strand (the so-called negative or antigenomic strand. So far, six major genotypes (HCV-1 to HCV-6) have been described, each containing multiple subtypes (Lawrence etal., 2017).

Hepatitis C is a disease with significant global impact. According to WHO updated July 2017 , Globally, an estimated 71 million people have chronic hepatitis C infection. Approximately 399 000 people die each year from hepatitis C, mostly from cirrhosis and hepatocellular carcinoma (WHO 2017).

In 2015, the seroprevalence of HCV infection in Egypt has declined to 6.3% among the studied population with an overall estimated 30%decrease in HCV prevalence in Egypt between 2008 and 2015(Kandeel et al., 2016).

Parenteral exposure to the hepatitis C virus is the most efficient means of transmission, it is a leading cause of chronic liver disease worldwide including cirrhosis and hepatocellular carcinoma (Alberti et al.,1999), (Lawrence etal., 2017).

The liver is the main site of virus replication but it can also replicate at extrahepatic sites such as peripheral blood mononuclear cells (PBMC) (Carreño et al., 2012).

Regarding this infection of PBMC, it has been shown that HCV can propagate in lymphoid cell cultures and that the virus derived is infectious (Carreño et al., 2012).

Hepatitis C recurrence is universal after LT in patients with detectable HCV RNA .Progression of hepatitis C is accelerated after LT and HCV-infected recipients have a reduced graft and patient survival when compared to HCV negative recipients Around one third of HCV-infected LT recipients will suffer an aggressive HCV recurrence after LT and are at risk of clinical decompensation and graft loss . Follow-up of patients with recurrent hepatitis C is usually performed with protocol liver biopsies, which are used to assess the degree of necroinflammation and the fibrosis stage, as well as to exclude other potential causes of graft damage (rejection, drug toxicity) (Carreño et al., 2012) .

The endpoint of therapy is an SVR, defined by undetectable HCV RNA in blood 12 weeks (SVR12) or 24 weeks (SVR24) after the end of therapy, as assessed by a sensitive molecular method with a lower limit of detection ≤15 IU/ml. Both SVR12 and SVR24 have been accepted as endpoints of therapy by regulators in the US and Europe, given that their concordance is >99%. Occult HCV infection seems to be less aggressive than chronic hepatitis C although patients affected by occult HCV may develop liver cirrhosis and even hepatocellular carcinoma (Carreño et al., 2012).

The persistence of very low levels of HCV RNA in serum and in

PBMCs, along with the maintenance of specific T-cell responses against HCV-antigens observed during a long-term follow-up of patients with occult hepatitis C, indicate that occult HCV is a persistent infection that is not spontaneously eradicated (Carreño et al., 2012).

Occult HCV infection has also been described in two other different clinical settings.One of these is in anti-HCV positive, serum HCV-RNA negative subjects with persistent normal values of liver enzymes (asymptomatic HCV carriers), of whom nearly 90% have detectable viral RNA in liver and in peripheral blood mononuclear cells (PBMCs) (Carreño et al., 2012)

In our study we aim to detect occult HCV infection in post liver transplanted patients who recieved DAAs & achieve SVR12 ,to detect prevalence of this type of infection and its effect on graft dysfunction , fibrosis progression of he graft.

Aim of the Work

We aimed to study the possibility of persistence of HCV RNA in peripheral blood mononuclear cells (PBMCs) among post liver transplant patients after successful eradication of HCV RNA from serum by direct acting antiviral agents (DAA) and the impact of different factors on occurrence of such condition.

Patients and Methods

This is a prospective cross-sectional study aimed at detection of Occult Hepatitis C Virus Infection in Patients who achieved a Sustained Virologic Response to Direct-acting Antiviral Agents for Recurrent Infection after Liver Transplantation .

Study population:

This study was conducted 20 participants who had undergone LDLT for HCV related liver disease , recieved DAAs , achieved & maintained a sustained virologic response 12 weeks after therapy (SVR12) from a peroid of not less than 6 months . they recruited during the period from December 2016 to November 2017 ,from the Liver Transplantation unit, Faculty of Medicine, Cairo University,

All patients fulfilled the following criteria :

Inclusion Criteria:

1-Recipients of living donor liver transplantation.

2-Age > 18 year old.

3-Patients who achieved & maintained a sustained virologic response 12 weeks after therapy (SVR12) with direct-acting antiviral (DAA).

4- Patients has no Co-infection i.e. HBs Antigen & anti HIV antibody negative.

Exclusion Criteria:

1-patients who did not achieve SVR 12.
2-A ge < 18 year old.
3-Patient has co-infection i.e. HCV\HBV or HCV\HIV.

Methodology:

Patients who were included in this study underwent the following:

1- Informed consent:

All patients enrolled had signed informed consent. The study was carried out in accordance with good clinical practice (GCP) and Helsinki Declaration (1975).

2- Clinical evaluation:

sex, age, body-mass-index (BMI), time since the liver transplantation, cause of liver failure or end-stage liver disease prior to LDLT, current immunosuppressive drugs and history of rejection (not at the time of pre or post treatment assessment).

3- Laboratory investigations including:

Liver biochemical profile: aspartate aminotranseferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), gamma glutamyletransferase (GGT), total and direct bilirubin, serum albumin. Complete blood count, Prothrombin profile (PT), INR, partial thromboplastin time (PTT). Renal biochemical profile: serum creatinine, blood urea nitrogen (BUN). HCV PCR.

4- Abdominal ultrasonography and Doppler:

Using real time scanning device Siemens ACUSON S3000 Ultrasound System (Siemens AG, Erlangen, Germany) with convex, 6C1 HD transducer, 1.5 – 6.0 MHz, measurements were performed after overnight fasting in the supine, right and left lateral positions. Scanning was done through several lon- gitudinal, oblique and transverse scans. to examine the following:. liver graft texture, presence of intrahepatic biliary radicles dilatation, peak systolic velocity and resistance index (RI) of the hepatic artery and the mean portal vein velocity. Measurements were taken in quiet respiration

5- Assessment of liver fibrosis By :

- Transient elastography (TE) using a FibroScan® device (Echosens, Paris) was performed to measure liver stiffness (LS). TE was performed with a standard M probe, an XL probe (for obese patients).

Table (1) :Cutoff levels of fibroscan for HCV patients (de Lédinghen et al., 2008)

Fibrosis stage Metavir	approximate cut off value in HCV
F0	0 to 5.4 Kpa
F0-F1	5.5 to 5.9 Kpa
F1	6 to 6.9 Kpa
F1-F2	7 to 8.7 Kpa
F2	8.8 to 9.4 Kpa
F3	9.5 to 12.4 Kpa
F3-F4	12.5 to 14.4 Kpa
F4	≥14.5 Kpa

6. Detection Of HCV RNA in Peripheral Mononuclear Cells by Specific RT-PCR

Blood Samples:

Five ml of venous blood was withdrawn on EDTA tubes for all cases, centrifuged, separated for Buffy Coat, and stored at -80°C.

EDTA: (Ethylenediaminetetraacetic acid) a chelating agent that bind calcium and other metals; used as an anticoagulant for preserving blood specimens .

Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR):
RT-PCR using real-time PCR technique:
The principle of Polymerase Chain Reaction (PCR):

Polymerase chain reaction can be defined as selective in vitro amplification of particular DNA region defined by the position of specific primer oligonucleotides.

In the PCR technique, a pair of primers that are complementary to the opposite strands of a DNA duplex is used to flank in between the target region to be amplified and to direct DNA synthesis in opposite and overlapping direction. In each cycle, each of the two strands acts as a template for the generation of two new duplex molecules, this lead to exponential increase in the amount of DNA.

Each cycle is initiated by melting the double strand DNA at (91°C - 95°C) for about one min to obtain single strand templates (denaturation phase). The primer oligonucleotides are then hybridized to their complementary sequence at an optimal temperature that varies from 35°C to 60°C (annealing phase). This is followed by a short pulse of DNA synthesis where the primers extend the DNA (extension phase), this step requires a temperature

of a 72°C and polymerase enzyme for DNA synthesis.
The basic PCR run can be broken into three phases:

I. Exponential phase: in this phase, the exact doubling of the product is accumulating at every cycle; the reaction is very specific and precise.

ii. Linear (high variability) phase: in this phase, the reaction components are being consumed, the reaction is slowing and the PCR product is no longer being doubled at each cycle.

iii. Plateau (endpoint) phase: in this phase, the reaction has stopped, no more products are being made and if left long enough, the PCR products will begin to degrade.

A. RNA Extraction:
HCV RNA was extracted from Buffy-Coat using RNeasy Mini Kit (Qiagen, Santa Clarita, USA) according to the manufacturer's instructions.

B. Molecular Detection of HCV by Real-Time PCR
HCV RNA viral load was performed using the RealTime HCV assay (Abbott Molecular Inc.) according to the manufacturer's specifications. Abbott RealTime HCV assay detects and quantities genotypes 1 to 6. The assay was performed through real-time PCR fluorescence detection on an ABI PRISM® 7500 Real-Time PCR System (Applied Biosystems, Foster City, CA, USA) using fluorescent labeled probes which used for reverse transcription, PCR amplification, and detection/quantitation. The PCR primers and probe-target conserved regions of the 5 untranslated region of the HCV genome. Distilled water was used as a negative PCR control in each amplification. The lower limit of detection is 12 IU/ml, with ≥95% probability. The dynamic range of the assay extends from 12 to 100,000,000 IU/ml.

Statistical analysis:
The collected data were coded, tabulated, and statistically analyzed using SPSS program (Statistical Package for Social Sciences) software version 24.

Descriptive statistics were done for parametric quantitative data by mean, standard deviation and minimum& maximum of the range, and for non-parametric quantitative data by median, while they were done for categorical data by number and percentage.

Analyses were done for parametric quantitative data using paired sample t test, and for non-parametric quantitative data using Wilcoxon signed rank test.

The level of significance was taken at (P value < 0.05)

Results
This study is a cross-sectional prospective case control hospital based study that was done in the liver transplantation unit, El-Manial Specialized hospital, Faculty of Medicine, Cairo University from December 2016 to November 2017. A total 20 patients who had undergone Living donor liver transplantation for HCV related liver disease and they were eligible for HCV treatment and achieved SVR 12;, were included in the study.

Detection of viral RNA in the peripheral blood mononuclear cells (PBMC) in patients with sustained virologic response following sofosbuvir-based direct acting antiviral treatments revealed detection of HCV RNA in 2 from a total of 20 patients included in our study (10%).

Table(2) Demographic characteristics of study subject

	Descriptive statistics (n=20)
Age Range Mean ± SD	(41-61) 52.4±4.9
BMI Range Mean ± SD	(23.8-34.5) 29±3.2
Gender Male Female	19(95%) 1(5%)
DM No Yes	14(70%) 6(30%)
HTN No Yes	18(90%) 2(10%)
Cause of liver transplantation HCV liver cirrhosis MELD >13 HCC on top HCV liver cirrhosis Refractory ascites on top HCV liver cirrhosis	20(100%) 2(10%) 1(5%)
MELD Range Mean ± SD	(11-22) 18±3

Table (2) show The socio-demographic data of the study subjects included age, sex, body mass index (BMI), Diabetes , hypertension and cause of liver transplantationin .The study included 19 males and one female with the mean age of 52.4years and a range from 41 to 61 years. The mean BMI was 29.4 ranging from 23.8to 34.5. The study data indicated that 10%(n=2) of our subjects are hypertensive, 30% are diabetic (n=6).

Table (3): Post treatment laboratory finding of the study subjects:

	Descriptive statistics (n=20)
Hb Range Mean ± SD	(9-17.1) 14±1.9
TLC Range Mean ± SD	(2.8-11) 6.1±2.5
Pl.Count Range Mean ± SD	(89-405) 189±74.2
INR Range Mean ± SD	(0.8-1.3) 1.1±0.1
Urea Range Mean ± SD	(12-49) 31.6±9.1
Creatinine Range Mean ± SD	(0.5-1.4) 0.9±0.2

Table (3) shows the post treatment routine investigation among the study subjects including complete blood count , renal function tests and INR .

Table (4) : comprasion between liver function test pre and post antiviral treatment of the study subjects:

	Pre –treatment	Post-treatment	P value
ALT Range Mean ± SD Median	(9-177) 53±40.5 41	(14-130) 44±39 26	0.533
AST Range Mean ± SD Median	(11-103) 43.1±25.7 36	(14-90) 36.9±22.5 29.5	0.520

ALP Range Mean ± SD Median	(61-686) 235.2±196.9 148	(55-543) 153.4±132.6 109	0.044*
GGT Range Mean ± SD Median	(18-800) 206.4±235.2 113	(12-815) 159±203.6 77.5	0.313
($)Albumin Range Mean ± SD Median	(2.5-5.5) 4.2±0.7 4.2	(3.3-4.8) 4.1±0.4 4.2	0.758
T.BIL Range Mean ± SD Median	(0.3-15) 2.2±3.2 1.4	(0.3-5.4) 1.2±1.2 0.9	0.040*
D.Bil Range Mean ± SD Median	(0.1-11) 1.2±2.5 0.5	(0.1-3.1) 0.5±0.7 0.2	0.076

Wilcoxon signed rank test for non-parametric quantitative data between the two readings Paired samples T test for parametric quantitative data between the two readings Significant level at P value < 0.05

Table(4) & figure (4,5 &6) show comprasion between liver function tests included(ALT,AST,ALP,GGT,Albumin, Total & Direct Bilirubin) ,also show that Alkaline Phosphatase & Total bilirubin were significantly lower in post –treatment values than pre-treatment values P= 0.044 and 0.040 respectively .

Table (5) Lipid Profile of the study subjects :

	Descriptive statistics (n=20)
Cholesreol Range Mean ± SD	(132-263) 163.8±31.7
TG Range Mean ± SD	(54-183) 93.1±32.6
LDL Range Mean ± SD	(54-131) 82.3±22.3
HDL Range Mean ± SD	(30-61) 42.2±7.6

Table (5) shows the lipid profile of all study subjects including serum cholesterol ,triglyceride , LDL and HDL which were in normal range in all patients.

Table(6): Anti viral Treatment Regiment, duration and previous treatment failure of all study subjects.

	Descriptive statistics (n=20)
Antiviral combination SOF/RIBA SOF/DACL SOF/RIBA/DACL SOF/RIBA/INF SOF/SIM SOF/RIBA/LED	7(35%) 4(20%) 4(20%) 2(10%) 2(10%) 1(5%)
Previous treatment failure Yes No	0(0%) 20(100%)
Duration of treatment 3 months 6 months	10(50%) 10(50%)

Table (6) shows that sofosbuvir/Daclatasvir/Ribavirin regimen was used in 20% (n=4), Sofosbuvir/Daclatasvir in 20%(n=4) , Sofosbuvir/Ribavirin in 35%(n=7) of our patients, Sofosbuvir/Ribavirin/ Pegylated Interferon in 10%(n=2) of our patients, Sofosbuvir/Simprevir in 10%(n=2) of our patients and SOF/ Ribavirin /Leadipasvir in 5% (n=1)of our patients. Also, the table shows that 50% of subjects received 12 week (3 months) regiments while 50% received treatment for 24 weeks. Among the 20 patients who received the antiviral treatment (100%) were treatment naïve.

Table(7) : Immunosupressive Therapy recieved by all study subjects.

	Descriptive statistics (n=20)
Immunosuppression Single Double	5(25%) 15(75%)
Immunosuppression1 Tacrolimus Cyclosporine	15(75%) 5(25%)
Immunosuppression2 No mycophenolic acid Everolimus	5(25%) 14(70%) 1(5%)

Table (7) shows 25%(n=5) of our patient recieved monotherapy and 75%(n=15) of our patient recieved dual therapy.

Table(8) : ultra-sonographic data of the study subjects post liver transplantation :

	Descriptive statistics (n=20)
Homogenous echopattern Abnormal echopattern Cirrhosis Ascites Splenomegally HFL	14(70%) 4(20%) 2(10%) 0(0%) 0(0%) 0(0%)

Table (8) shows The ultra-sonographic data of the study subjects regarding liver echopattern, size ,surface, hepatic focal lesions, ascitesand splenomegaly . The liver was cirrhotic in 2 out of 20 subjects (10%), Homogenous echopattern in 14 subjects (70%) and abnormal echopattern in 4 subjects (20).

Table (9): Fibroscan assessment of the study subjects post liver transplantation :

	Descriptive statistics (n=20)
Fibrosis stage F0 F1 F2 F3 F4	7(35%) 7(35%) 2(10%) 2(10%) 2(10%)
Fibroscan Range Mean ± SD	(4-29) 8±5.5

Table (9) shows 7 subjects (35%) were F0 , 7 subjects (35%) were F1 and 6 subjects (30%) were ≥ F2.

Table(10) : Prevalence of HCV RNA in PMNCs of the study subjects:

	Descriptive statistics (n=20)
PCR PMNCs Negative Positive	18(90%) 2(10%)

Table (10) & figure (7) show that only 2 subjects (10%) of the study patients had positive HCV RNA in PMNCs.

Table(11) : Demographic characteristics of occult HCV patients

Patients with +Ve PCR PMNCs	Descriptive statistics (n=2)
Age Range Mean ± SD	(56-57) 56.5±0.7
BMI Range Mean ± SD	(34.4-34.5) 34.5±0.1
Gender Male Female	2(100%) 0(0%)
DM No Yes	2(100%) 0(0%)
HTN No Yes	2(100%) 0(0%)
Cause of liver transplantation -HCV liver cirrhosis MELD >13 -HCC on top HCV liver cirrhosis -Refractory ascites on top HCV liver cirrhosis	2(100%) 0(0%) 0(0%)

Table (11) shows that both Occult HCV patients were male in their 6th decade , have obesity class 1their BMI mean is 34.5± 0.1 , has no diabetes or hypertension.

Table(12): Laborartory investigation of occult HCV patients:

	Descriptive statistics (n=2)
Hb Range Mean ± SD	(11.3-14.3) 12.8±2.1
TLC Range Mean ± SD	(6.3-9.2) 7.8±2.1
Pl.Count Range Mean ± SD	(102-213) 157.5±78.5
INR Range Mean ± SD	(1-1.3) 1.2±0.2
Urea Range Mean ± SD	(30-41) 35.5±7.8
Creatinine Range Mean ± SD	(0.9-1.1) 1±0.2
Cholesreol Range Mean ± SD	(132-183) 157.5±36.1
TG Range Mean ± SD	(71-141) 106±49.5
LDL Range Mean ± SD	(56-104.9) 80.5±34.6
HDL Range Mean ± SD	(49.6-61) 55.3±8.1

Table(12) shows that the routine investigations of the occult HCV patients included CBC , INR ,renal function tests and Lipid profiles were normal except thrombocytopenia in one patient of both , platelets count in this patients was 102.000 cells /microlitre (mcL).

Table(13) : comprasion between Liver function tests of occult HCV patients pre and post antiviral therapy :

Patients with +Ve PCR PMNCs (n=2)	Pre-treatment	Post-treatment	P value
ALT Range Mean ± SD	(28-33) 30.5±3.5	(28-41) 34.5±9.2	0.317
AST Range Mean ± SD	(24-37) 30.5±9.2	(44-66) 55±15.6	0.180
ALP Range Mean ± SD	(61-128) 94.5±47.4	(75-319) 197±172.5	0.180
GGT Range Mean ± SD	(68-121) 94.5±37.5	(64-404) 234±240.4	0.655
Albumin Range Mean ± SD	(4-4.7) 4.4±0.5	(3.8-4.2) 4±0.3	0.655
T.BIL Range Mean ± SD	(2.2-3.1) 2.7±0.6	(1-3.1) 2.1±1.5	0.317
D.Bil Range Mean ± SD	(0.7-0.8) 0.7±0.1	(0.3-0.8) 0.6±0.4	0.317

Wilcoxon signed rank test for non-parametric quantitative data between the two readings
Significant level at P value < 0.05

Table (13) shows that According to liver function tests and statistical data , both occult HCV patients have insignficant elevated Post-treatment AST44 units per liter & 66 units per liter (Range =(44-66), mean±SD =(55±15.6) p value 0.180) , have insignificant elevated pre and post-treatment gamma glutamyl transferase (GGT) post-treatment were 64IU/L & 404 IU/L, (Range=(64-404) mean ±SD = (234±240.4) P value 0.655).

One of both occult HCV patients had insignificant elevated post-trearment ALT (in both patients Range =(28-41), mean±SD =(34.5±9.2) p value 0.317) , the same occult HCV patient had insignificant elevated post-trearment alkaline phosphatase enzyme (ALP)319 IU/L, pre and post treatment total Bilirubin 3.1mg/dL &3.1mg/dL respectively and , pre and post treatment direct bilirubin 0.8 mg/dL&0.8 mg/dL respectively.

(ALP in both patients Range =(75-319), mean±SD =(197±172.5) p value 0.180), (T.Bil in both patients Range =(75-319), mean±SD =(197±172.5) p value 0.180).

Table(14) : Antiviral Treatment regiments, experience and duration of occult HCV patients:

	Descriptive statistics (n=2)

SOF/RIBA SOF/DACL SOF/RIBA/DACL SOF/RIBA/INF SOF/SIM SOF/RIBA/LED	1(50%) 0(0%) 0(0%) 1(50%) 0(0%) 0(0%)
Previous treatment failure Yes No	0(0%) 2 (100%)
Duration of treatment 3 months 6 months	1 (50%) 1 (50%)

Table (14) shows that both occult HCV patients were treatment naïve, one of them (50%) recieved Sofosbuvir/Ribavirin for 24 weeks and another one recieved Sofosbuvir/Ribavirin/ Pegylated Interferon for 12 weeks.

Table(15) : Immunosupressive Therapy recieved by occult HCV patients

	Descriptive statistics (n=2)
Immunosuppression Single Double	1(50%) 1(50%)
Immunosuppression1 Tacrolimus Cyclosporine	1(50%) 1(50%)
Immunosuppression2 mycophenolic acid Everolimus	1(50%) 0(0%)

Table (15) shows that one of occult hcv patients (50%) is on monotherapy of immunosuppressive drug and another one is on dual therapy.

Table (16): Ultra-sonographic data of occult HCV patients post liver transplantation:

	Descriptive statistics (n=2)
U/S Free Abnormal echopattern liver cirrhosis	0(0%) 1(50%) 1(50%)

Table (16) shows that the ultra-sonographic data of the occult HCV patients showed that 50%(n=1) has bright liver and 50% has liver cirrhosis (coarse echopattern).

Table (17): Fibroscan assessment of occult HCV patients post liver transplantation:

	Descriptive statistics (n=2)
Fibrosis stage F0 F1 F2 F3 F4	0(0%) 0(0%) 1(50%) 0(0%) 1(50%)
Fibroscan Range Mean ± SD	(9-13.5) 11.3±3.2

Table (17) shows that One of both patients has fibrosis F2 and another one has fibrosis F4.

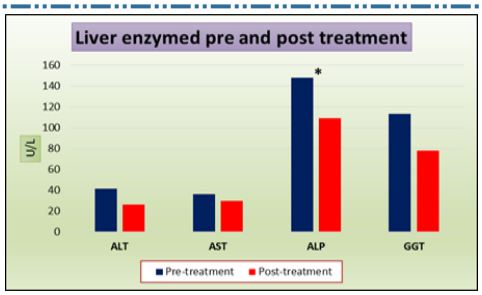

Figure (4) : comprasion of liver enzymes pre & post antiviral treatment among the study subjects.

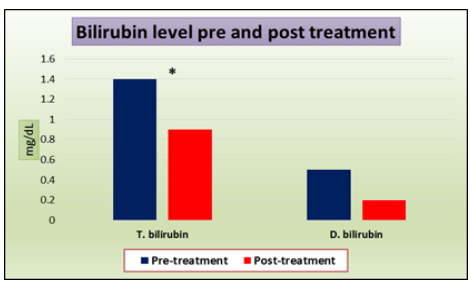

Figure (5) : comprasion of total & direct bilirubin pre & post antiviral treatment among the study subjects.

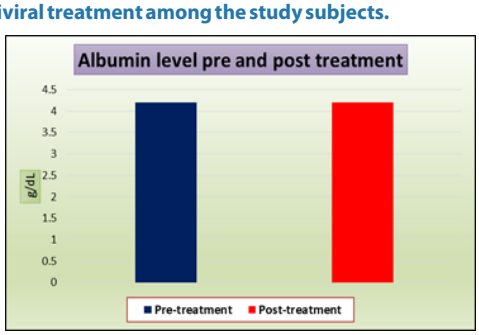

Figure (6) : comprasion of albumin level pre & post antiviral treatment among the study subjects.

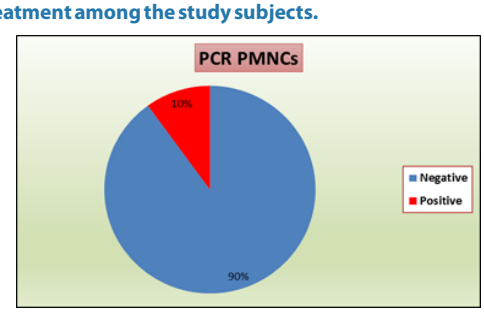

Figure (7) : : Prevalence of HCV RNA in PMNCs of the study subjects

Discussion

In this study, we aimed to study the possibility of persistence of HCV RNA in peripheral blood mononuclear cells(PBMCs) among post liver transplant patients after successful eradication of HCV RNA from serum by direct acting antiviral agents (DAA) and the impact of different factors on occurrence of such condition.

Given the deregulated immunity due to concomitant immunosuppressant , we hypothesized that the post- liver transplantation recipients is susceptible to develop OCI, which may contribute to the abnormal aminotransferases seen after achieving SVR (deLemos et al., 2014).

This study recruited 20 consecutive patients who had undergone Living donor liver transplantation for HCV related liver disease and

they were eligible for HCV treatment and achieved SVR 12; recruitment was done in the liver transplantation unit, El-Manial Specialized hospital, Faculty of Medicine, Cairo University from December 2016 to November 2017.

In our study , all patients underwent Liver transplantation because of decompensated post hepatitic C liver cirrhosis , 2 of them had Hepatocellular carcinoma on top decompensated post hepatitic C liver cirrhosis and one of them has Refractory ascites secondary to decompensated post hepatitic C liver cirrhosis.

When reviewing the demographic data of the current study population, there was a male predominance 19(95%), and the mean age was 52.4 ±4.9 years for the whole study group, and this matches with the known epidemiological features of liver transplant patients who receive antiviral therapy in several studies, EL Masry et al., in 2016 evaluated OCI in Subjects who cleared HCV successfully by DAAs and test positive for HCV Ab and negative for serum HCV RNA and have persistent elevated liver enzymes , the mean of age of their study cohort was 58 and 66.7% were male (ELMasry et al., 2016)

In our study, genomic HCV RNA was detected in PBMCs of 2 out of 20 patients (10%) who cleared HCV successfully by DAAs and test positive for HCV Ab and negative for serum HCV RNA 12 weeks after the end of treatment, this means that the prevalence of occult HCV infection was very low in our patients, and it did not appear to be clinically relevant.

Our result is in accordance with Aboalam et al., 2016 the Prevalence of OCI in their study was 3 out of 25 (12%) in subjects who cleared HCV infection spontanously and test positive for HCV Ab and negative for serum HCV RNA, that is near to our result (Aboalam et al., 2016)

But In El Masry et al., 2016 The results revealed that HCV-RNA was detected in a total of 5 subjects (55%): in the liver tissue of 4 subjects (44%), in the PBMC of two subjects (22%), and one in both (ELMasry et al., 2016).

Occult HCV infection is a mild disease, the current study support this hypothesis by showing no significant association between the detection of occult HCV infection and the main symptoms of the studied patients even existence of the right hypochondrial pain. This was also supported by Pardo et al., in 2007 that compared clinical and pathological characteristics of occult HCV infection patients and untreated chronic HCV patients, and supported by Saad et al., in 2011 whose study aimed to detect the prevalence of OCI among Egyptian patients with non alcoholic fatty liver disease (Pardo et al., 2007),(Saad et al., 2011).

Occult HCV patients confirming the less inflammatory process present in occult HCV provided by the aminotransferase level alanine transaminase (ALT) and aspartate transaminase (AST) which are insignificantly higher (p value = 0.317 & 0.180 respectively) , The relationship between occult HCV infection and serum ALT levels is controversial. While some authors as De Marco et al., 2009 aimed to detect OCI in an unexpected finding in a population unselected for hepatic disease reported normal liver functions in patients with occult HCV infection (De Marco et al., 2009)

The present study revealed an insignificant difference in serum ALT levels between occult HCV patients and the rest of patients in our study. Liver function test results and it is in accordance with EL Masry et al., in 2016 which found occult HCV infection in 5 out of Nine subjects with biochemical abnormality(EL Masry et al., 2016)

So we speculate that the causality of abnormal aminotransferases in the remaining cases remains elusive. Given concomitant immunosuppression, we speculate that other viral pathogens could be responsible for the abnormal liver function.

In identifying the risk factors of Occult HCV infection neither history of diabetus , hypertension nor hyperlipidemia are considered a risk factors of occult HCV

In our study Fibroscan was done for all cases, we noticed that 6 of the patients in our study has Fibrosis ≥ F2 , two of them has positive HCV RNA in PBMCs .

In the present study , seven of our patients recieved sofosbuvir and Ribavirin for 24 weeks ,one of them has occult HCV infection , and two of our patient recieved pegylated interferon , sofosbuvir and Ribavirin for 12 weeks , one of them has occult HCV infection .

El Masry et al., in 2016 showed that 5 of nine patient with abnormal liver chemistry had OCI , two of them recieved Ledipasvir , Sofosbouvir and Ribavirin for 12 weeks, one of the five Occult HCV patient recieved Ledipasvir and Sofosbouvir for 12 weeks, another one recieved only Sofosbuvir and Ribavirin for 20 weeks and the last one recieved simprevir and sofosbuvir for 12 weeks(EL Masry et al., 2016).

According to our study and EL Masry study we did not find any association between the type and duration of antiviral therapy and occurance of occult HCV infection(EL Masry et al., 2016)

This study aimed to address the occurrence of OCI in the immunocompromised host Of note, we did not find any association between the type and dose of immunosuppressant and the onset of OCI and this goes well with EL masry et al., 2016 which evaluated OCI in liver transplant recipients and with Nicot et al., 2010 which evaluated Kidney Transplanted recipients in whom HCV infection was eliminated during henodialysis and showed that administration of immunosuppressive therapy during Kidney transplantation did not cause a relapse, therefore concluding that OCI was not present (Nicot et al., 2010),(EL Masry et al., 2016)

We suggest follow up of both occult HCV patients by real time PCR for HCV RNA in serum every 6 months ,our suggestion is based on Aboalam et al., 2016 which evaluated the prevalence of OCI in subjects who has positive HCV antibody and negative HCV RNA in serum . This study showed 3 patients out of 25 subjects has positive HCV RNA in PMNCs by follow up of these 3 patients one of them became Overt HCV with HCV RNA in serum. This indicates a probability of conversion of occult HCV infection to Overt HCV infection (Aboalam et al., 2016).

We emphasize recommendations of EL Masry et al., 2016 in relation to the necessiaty to exclude other common causes of elevated liver function and fibrosis progression of the graft as graft rejection and screening for other viral pathogens as CMV , EBV ,HBV and herpesvirus.

We suggest an additional course of antiviral therapy can be considered to preserve the longevity of the graft due to possible inflammation caused by occult HCV infection especially in those have elevated liver enzymes,this suggestion need further study.

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

To the best of our knowledge, the current study was the first Egyptian study to investigate the prevalence of occult HCV among post liver transplanted patients who maintained a sustained virologic response 12 weeks after therapy (SVR12) with direct-acting antiviral (DAA).

Despite this, the present study had some limitations, such as

absence of confirmation OCI in liver tissue and small sample size which reduced the statistical significance of the results. Therefore, we recommend a large prospective cohort study to overcome these limitations.

Conclusion

- HCV is a leading cause of chronic liver disease worldwide and can lead to a spectrum of liver diseases from mild inflammation with a relatively indolent course to extensive liver fibrosis and consequent cirrhosis, conferring significant morbidity and mortality to affected individuals. Associated hepatocellular carcinoma is a seriouscomplication of CHC-related cirrhosis with an incidence of5.8% per year in the at-risk population (Burstow et al., 2017)

- Our study has found that occult hepatitis C virus infection is still a significant problem even after the era of direct antiviral agents; We detected the HCV RNA in the Peripheral blood mononuclear cells in 2 out of 20 (10%) among post liver transplanted patiens who maintained a sustained virologic response 12 weeks after therapy (SVR12) with direct-acting antiviral (DAA) agents.

- In identifying the risk factors of Occult HCV infection neither history of diabetus , hypertension nor hyperlipidemia are considered a risk factors of occult HCV.

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