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Pilot Study of Occult Hepatitis C Virus among Egyptian Blood Donors

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A B S T R A C T

Hepatitis C virus (HCV) represents a major health problem in Egypt. The screening of blood donors is one method to reduce the spread of HCV. However, there is an emergence of a condition known as occult HCV infection (OCI) which is defined as the presence of HCV-RNA in peripheral mononuclear blood cells (PBMCs). Therefore, the aim of the present study was to study the presence of OCI among blood donors by real time polymerase chain reaction. The study included 100 randomly selected blood donors. The volunteer's blood donors' were subjected to full clinical data history and blood samples were obtained for serological studies of HCV, hepatitis B s antigen (HBsAG) and HIV1/2 antibodies by enzyme linked immnosorbent assay (ELISA) and liver transaminases. Then the presence of HCV-RNA was detected in plasma and PBMCs by real time polymerase chain reaction. The mean age± SD of the studied blood donors was 34.8± 10.1 years they were 69% males and 31% females. The residence of most of them was urban 77%. All blood dobnors wre negative for HIV by ELISA, HCV IgG was positive in 10% of them and HBsAg was present in one blood donor. Isolated HCV-RNA in PBMCs was detected in 5 donors (5%), HCV-RNA was present in plasma of 7 blood donors with positive HCV-IgG. There was significant association between HCV IgG and HCV-RNA in plasma (P=0.0001). Moreover, there was significant increase in alanine aminotransferase in the donors positive for HCV-RNA in PBMCs and in HCV-RNA positive in plasma (55.0± 4.9, 55.0 ± 13.4 respectively) compared to negative blood donors (28.0± 10.0), P=0.0001. Also, aspartate aminotransferase had significantly elevated level in the donors positive for HCV-RNA in PBMCs and in HCV-RNA positive in plasma (47.8± 6.6, 47.6 ± 14.7, respectively) compared to negative blood donors (24.8± 9.7), P=0.0001. The present study highlights the prevalence of occult hepatitis C virus among blood donors. This pilot study demonstrated the presence of occult hepatitis C associated with elevated liver transaminases enzymes that can be used as a clue for such condition. There is a need for nucleic acid amplification technology in blood banks for complete assurance of blood transfusion safety. There is a need to large population studies for proper determination of occult hepatitis C in Egyptian blood donors.

Introduction

Occult hepatitis C infection (OCI) has emerged in the last decade as the presence of HCV RNA in hepatocytes and/or peripheral blood mononuclear cells (PBMCs) with no detectable HCV RNA in the serum (Ocana *et al.*, 2011). There are two types of occult hepatitis C types, seronegative OCI with negative antibody for HCV and seropositive OCI with positive antibody for HCV (Carreño *et al.*, 2012). OCI have been claimed to be associated with 10% of cryptogenic liver diseases. Cryptogenic liver diseases are

defined as group of liver diseases are diagnosed without obvious etiology. Moreover, it is recognized in general population (Bokharaei-Salim *et al.,* 2011; Keyvani *et al.,* 2013).

There have been many reports about the prevalence of seronegative OCI in normal population that determined the prevalence of OCI to range from 2.2 up to 3.3% (De Marco *et al.,* 2009; Lin *et al.,* 2016)

The presence of seronegative OCI was found to be associated with positive hepatitis B virus s antigen and/or elevated alanine aminotransferase (ALT) (Lin *et al.,* 2016).

The common proposed theory for the presence of OCI is the occurrence of mutation in the virus encapsidation capacity or the formation and release of virions into blood circulation leading to low levels of viremia that are below the detection limit for some molecular assay (Carreño, 2006).

In cryptogeneic liver diseases, detection of OCI by determination of HCV RNA in hepatocytes is the gold standard technique for diagnosis. However, it is hampered by presence of several risks such as bleedings and evading of other organs. The use of identification of HCV RNA in peripheral blood mononuclear cells (PBMCs) provides safe and easily accessible sample for diagnosis. However, it lacks sensitivity and may diagnose about 70% of patients with increase its diagnostic sensitivity by multiple sampling (Ocana *et al.,* 2011; Castillo *et al.,* 2011).

OCI represents a hidden thread in blood transfusions practice. The protocol of blood screening of the donors by the presence of HCV antibodies or even the detection of HCV RNA in serum samples plus determination of aminotransferases encymes may do not exclude the presence of occult HCV infection (Quiroga and Carreño, 2005).

There are few reports about the prevalence of OCI in Egyptian blood donors.

Therefore, the aim of the present study was to study the presence of OCI among blood donors by real time polymerase chain reaction.

Materials and Methods

The study is a cross-sectional prospective study. The study included random selected 100 apparently healthy blood donors from Emergency Hospital blood bank, Mansoura University, Egypt during December-Febraury 2016. The study was approved by Mansoura faculty of medicine ethical committee and informed consent was obtained from each subject. The volunteer's blood donors' were subjected to full clinical data history about medical conditions such as hypertension, Diabetes mellitus and history of previous jaundice. Donors with previous history of jaundice, hepatic or renal diseases were excluded from the study.

Blood Sample

From each subject ten milliliter blood samples were obtained. Blood sample was divided to three tubes one without anticoagulant for sera separation and the other two with EDTA for PBMCs separation and plasma separation. Serum sample for each subject was used for measurement of liver enzymes including alanine aminotransferase (ALT) aspartate aminotransferase (AST) and for bilirubin and albumin measurement by autoanalyzer (Dialab 450 system). Also serum was used for serological studies for hepatitis C virus antibodies - IgG (HCVIgG) and hepatitis B virus s antigen (HBsAg) and HIV1/2 antibodies by enzyme linked immunosorbant

assay (Dia-Pro, ITALY). The separated plasma were kept frozen at -70° C for further molecular study for hepatitis C virus RNA detection by real time PCR. PBMC were immediately prepared from the blood samples by the standard density gradient centrifugation using Leucosep tubes (Greiner Bio One GmbH, Germany). The isolated PBMCs were washed as per the manufacturer's instructions. The cells were then counted using a hemocytometer (Neubauer chamber). Aliquots of approximately 2.5 million cells were stored at -70° C.

Detection of HCV RNA by Real Time PCR

Real Time PCR for amplification of HCV-RNA

Extraction of HCV-RNA from Plasma

HCV-RNA was extracted from plasma by the use of Qiagen (Qiagen-Germany) extraction kit for RNA according to the manufacturer protocol.

Extraction of RNA from PBMCs

HCV-RNA was extracted from PBMCs by lysis of the cells pellets at first by the use of lysis buffer included in Qiagen extraction kit (Qiagen-Germany). The extraction was completed by the use of the extraction kit according to the manufacturer protocol.

Amplification of HCV-RNA by Real Time PCR

Amplification was carried by the use of the amplication kit provided from Qiagen and by the use of Applied Biosystems 7500 Fast Real-Time PCR Thermal cycler (Life Technologies, Alexander City, AL). Negative control was used from healthy volunteers and positive samples were used from patients with chronic active HCV. Positive results were considered after two positive results in two different amplification processes.

Definition of occult HCV

The sample that was positive for HCV-RNA in PBMCs and negative for HCV antibodies by ELISA was defined as occult HCV.

Statistical Analysis

Data were collected, revised, coded and entered to the statistical package for social science (SPSS) version 24. The quantitative data were presented as mean, standard deviations and ranges. The qualitative data were presented as number and percentages and comparison was performed by chi-square. The comparison between the studied groups were done by using One Way Analysis of Variance (ANOVA). P was considered significant if it was$\leq$ 0.05.

Results and Discussion

The study included 100 blood donors. Their mean age± SD was 34.8± 10.1 years they were 69% males and 31% females. The residence of most of them was urban 77%. All blood dobnors wre negative for HIV by ELISA, HCV IgG was positive in 10% of them and HBsAg was present in one blood donor. Isolated HCV-RNA in PBMCs was detected in 5 donors (5%), table 1.

HCV-RNA was present in plasma of 7 blood donors with positive HCV-IgG. There was significant association between HCV IgG and HCV-RNA in plasma (P=0.0001), with three blood donor with positive HCV IgG and negative of HCV-RNA, table 2

In the comparison between different groups of the blood donors according to the presence of HCV-RNA either in plasma or isolated in

PBMCs, there was significant increase in ALT in the donors positive for HCV-RNA in PBMCs and in HCV-RNA positive in plasma (55.0± 4.9, 55.0 ± 13.4 respectively) compared to negative blood donors (28.0± 10.0), P=0.0001. Also, AST had significantly elevated level in the donors positive for HCV-RNA in PBMCs and in HCV-RNA positive in plasma (47.8± 6.6, 47.6 ± 14.7, respectively) compared to negative blood donors (24.8± 9.7), P=0.0001. Moreover, bilirubin had statistically elevated bilirubin in the blood donors with HCV-RNA in PBMCs (1.04± 0.5) compared to those with HCV-RNA in plasma and negative blood donors (0.7± 0.3 for both), P=0.001. HCV-RNA quantity had significant elevated level in PBMCs (9.2 $\pm 0.710^6$ IU/ml) compared to HCV-RNA level in plasma (5.4± 0.5), P=0.0001, table 3.

Infection due to hepatitis C virus is a global thread to the health with serious sequels on the liver leading to cirrhosis and hepatocellular carcinoma (WHO, 2011). In Egypt, hepatitis C virus represents a major health problem with high prevalence rates that may approach 24% among certain age groups with predominant genotype 4 (Abdel-Aziz, 2000; Tanaka *et al.,* 2004).

In the present study the sroprevalence of antibodies to HCV was 10%. The seroprevalence rates for HCV among blood donors varied among different studies depending upon the number of screened donors, their age, geographical region and the method of screening. The ranges were from 9.02% up to 19.4% (E1-Gohary *et al.,* 1995; Kamel *et al.,* 1992; Saeed *et al.,* 1991; Khattab *et al.,* 2010). The blood donors represent community HCV infection state.

Among the seropositive blood donors, 7% had positive HCV-RNA in the plasma samples. In total population study, According to the Egyptian ministry of health survey, 2015 (Ministry of Health and Population Egypt, 2015), 6.3% of population within age 1–59 years have HCV antibodies, and 4.4% have HCV RNA.

The rates differ according to the geographic location of the study. The finding raises a question about the need for directing positive blood donors' positive for HCV IgG for national program for HCV treatment to evaluate the presence of viremia and starts the treatment if applicable to decrease the community infection pool of HCV.

Table.1 Demographic and laboratory findings of blood donors (n=100)

Age (mean± SD)years	**34.8± 10.1**
Sex **Male (No.-%)** **Female (No.-%)**	 **69 69%** **31 31%**
Residence **Rural (No.-%)** **Urban (No.-%)**	 **23 23%** **77 77%**
Albumin(gm/dl)	**4.2± 0.4**
Total bilirubin(mg/dl)	**0.7± 0.3**
ALT(IU/L)	**31.5± 13.3**
AST(IU/L)	**27.7± 12.3**
HCV IgG (No.-%)	**10 10%**
HBsAg (No.-%)	**1 1%**
Isolated HCV-RNA in PBMCs	**5 5%**

Table.2 Comparison between HCV IgG and HCV-RNA in plasma

	HCV IgG	
	Positive No. %	Negative No. %
HCV-RNA Positive Negative	 7 7% 3 3%	 0 0% 90 90%
	10 10%	90 90%
P	=0.0001	

Table.3 Demographic and laboratory data among blood donors according to presence of HCV-RNA

	HCV occult (n=5)	HCV-RNA in plasma (n=7)	Negative (n=88)	P
Age (mean± SD)years	33.2± 6.9	35.7± 13.8	34.8± 10.0	0.9
Sex Male **(No.-%)** Female **(No.-%)**	 3 60% 2 40%	 6 85.7% 1 14.3%	 60 68.2% 28 31.8%	P=0.6
Residence Rural **(No.-%)** Urban **(No.-%)**	 1 20% 4 80%	 1 14.3% 6 85.7%	 22 25% 66 75%	P=0.8
albumin(gm/dl)	4.00± 0.3	4.3± 0.4	4.3± 0.4	0.1
bilirubin(mg/dl)	1.04± 0.5	0.7± 0.3	0.7± 0.3	0.001
ALT(IU/L)	55.0± 4.9	55.0 ± 13.4	28.0± 10.0	0.0001
AST(IU/L)	47.8± 6.6	47.6 ± 14.7	24.8± 9.7	0.0001
Quantity of HCV-RNA-10^6IU/ml	9.2 ±0.7	5.4± 0.5		P=0.0001

In the present study only one blood donor had HBsAg which is similar to previous study reporting HBsAg prevalence to be 1.7% (Khattab *et al.,* 2010). The decline in hepatitis B virus prevalence in Egypt may reflect the success of the campaign of immunization against hepatitis B virus. The presence of seronegative OCI among blood donors was 5%. Previous studies about the presence of OCI in blood donors have reported similar ranges from 5% up to 7.1% in Egypt (El-Zanaty and Way, 2008; Eldaly *et al.,* 2016). Less prevalence was reported from other region such as Italy (3.3%) (De Marco *et al.,* 2009) which reflects the low prevalence of HCV in comparison to Egypt.

The presence of OCI among blood donors carries the risk of transmission of HCV especially to patients requiring multiple blood transfusions such as patients undergoing regular hemodialysis (Abdelrahim *et al.,* 2016; Shazly *et al.,* 2015). Thus, there will be a question about the safety of blood transfusion practice even after the application of blood screening for hepatitis C virus by ELISA in Egyptian blood banks. The safe blood transfusion implicates two strategies, the first is careful choose of medical eligible blood donors and the second is careful screening for blood transmitted transfusion pathogens such as HCV, HBV and HIV. Nucleic acid amplification testing (NAT) has

expanded rapidly in western blood banks to reduce the risks of blood transfusion (Safic Stanic *et al.,* 2017). However, with the emergence of OCI, there is a need to develop a new strategy for prevention of transmission of occult infections.

The remarkable findings of the present study were the association of OCI with statistically significant increase in ALT, AST and bilirubin. Previous study on OCI in non-alcoholic fatty liver diseases reported similar result (Saad *et al.,* 2011). Therefore a screening for blood donors by determination of liver enzymes and bilirubin may aid in detection of OCI. However, large number of studied blood donors are required to validate this finding.

The presence of OCI and HCV-RNA in plasma had no statistically significant association with age, sex or residence. Similar findings were reported by previous studies (Eldaly *et al.,* 2016; Saad *et al.,* 2011).

The present study highlights the prevalence of occult hepatitis C virus among blood donors. This pilot study demonstrated the presence of occult hepatitis C associated with elevated liver transaminases enzymes that can be used as a clue for such condition. There is a need for nucleic acid amplification technology in blood banks for complete assurance of blood transfusion safety. There is a need to large population studies for proper determination of occult hepatitis C in Egyptian blood donors.

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