Occult Hepatitis C Virus in Blood Donors in Damietta - Egypt

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Abstract: Egypt maintains the highest prevalence of hepatitis C virus infection, affecting an estimated 15%–20% of the population, new cases reported, so we search about uncommon source of hepatitis C virus infection. Occult hepatitis C Virus infection can be detected and founded in the overall public obviously haven't any diseases, Detection of viral replication in PBMCs may increase spread and transmission of hepatitis C virus during blood transfusion, hemodialysis, and made liver disease development in occult Hepatitis C Virus infected individual. The present study aimed to detect the occult hepatitis C virus infection in healthy blood donors from Damietta-Egypt, And detection of Hepatitis C Virus core antigen. One hundred and fifty blood donors from El-Azhar University Hospital blood bank in New Damietta City were used into the present study. Detection of Hepatitis C Virus RNA in Peripheral blood mononuclear (PBMCs) and plasma by reverse transcription nested polymerase chain reaction (RT-PCR), and also detection of hepatitis C virus core antigen from plasma by ELISA. We founded that: Detection of hepatitis C virus RNA by nested PCR from PBMC among studied donors are eleven (7.9%) on the other hand Hepatitis C Virus RNA in plasma are two (1.4%) so occult C virus are 9/140 (6.4%). By comparison between results of Hepatitis C Virus core Ag and PCR evidence sensitivity 27%, specificity 98.4% in PBMC and sensitivity 100%, specificity 97.8% in Plasma.

Keywords: Hepatitis C Virus, Occult Hepatitis C Virus, PBMCs, Hepatitis C Virus Core Ag

1. Introduction

Hepatitis C Virus diagnosed by detects presence of *Hepatitis C Virus* antibodies [1] with or without detection of *hepatitis C virus* RNA in serum and plasma [2]. Occult *hepatitis C virus* is a (diverse) than *hepatitis C virus* was first described in 2004 and defined as the detection of *hepatitis C virus* RNA in the liver or PBMCs of patients whose serum tests tried negative for *hepatitis C virus* RNA by ordinary PCR examines, even presence or absence *hepatitis C virus* antibodies [3]. Occult *hepatitis C virus* can detected and founded in the overall public obviously haven't any diseases, Detection of viral replication in PBMCs may increase spread and transmission of *hepatitis C virus* during blood transfusion, hemodialysis, and made liver disease

development (e.g. liver cirrhosis and liver necrosis) in occult *hepatitis C virus* infected individual [4]. Even detection the presence of *hepatitis C virus* -RNA in liver biopsy sample is the best technique and more accurate method for the detection of occult *hepatitis C virus*, we can detect *hepatitis C virus* -RNA in PBMCs when we can't take liver biopsy [5, 6].

Hepatitis C virus core Ag consists of 191 amino acids and most founded in cytoplasm [7, 8]. During the seroconversion period and before presence of antibodies we can use *hepatitis C virus* core Ag for detection *hepatitis C virus* [9, 10], in plasma or blood transfusion in many countries [11].

2. Materials and Methods

2.1. Study Population

One hundred and fifty blood donors from El-Azhar University Hospital blood bank in New Damietta City were incorporated into this study. These cases were 15 females and 135 males. All of them were apparently free from liver diseases and negative for Hepatitis C virus IgG by ELISA.

2.2. Samples Were Negative Hepatitis C Virus Antibodies Subjected to the Following Procedure

2.2.1. Peripheral Blood Monocyte (PBMCs) and Plasma Isolation

- i Isolation of Peripheral Blood Mononuclear Cells (PBMC):

Heparinized blood was diluted by 1:1 with phosphate buffer saline (PBS) and mixed gently by inversion In Falcon tubes then centrifuged for 30 min to obtain visible pellet of PBMCs at the bottom of each tube.

- ii Plasma isolation:

Heparinized blood was centrifuged for 15 minutes to remove cells and platelets and obtain supernatant of plasma.

2.2.2. Extraction of Hepatitis C Virus RNA from PBMCs and Plasma

Using viral Gene-spin™ Kit and Tanami Virus RNA Kit in PBMCs and plasma respectively using manufacturer's catalog to extract RNA in microfuge tube then at - 20°C will store till used.

2.2.3. RT- Nested Polymerase Chain Used for Detect Hepatitis C Virus RNA

Hepatitis C virus RNA from PBMC and plasma was subjected to Nested PCR cycles using in the first round PCR Maxim RT-PCR Premix Kit and in the second round PCR Master Mix kit then by using agars gel electrophoresis examined under the UV illuminator to give band at 236bp in positive sample and negative sample give no band.

2.2.4. Hepatitis C virus Core Antigen Detection from Plasma by ELISA

Hepatitis C virus Antigen measured according to the manufacture instructions by ELISA technique to read the Absorbance and optical density of samples.

3. Results

3.1. Demographic Characteristics of Negative Hepatitis C Virus Antibodies Blood Donor Samples

This table shows that most blood donors were males in middle age group (25-35years) and from rural distribution. Seven of them made dental and surgery procedures and five had previous blood transfusion.

Table 1. Demographic characteristics of negative hepatitis C virus antibodies blood donor samples.

Socio demographic data	(No.=140) No	%
Age (years)		
18-25	50	35.7
25-35	61	43.6
35-45	29	20.7
Mean $\pm$ SD =30.1 $\pm$ 7.5		
Sex		
Female	13	9.3
Male	127	90.7
Past history		
Jaundice	0	0.0
Drug intake	0	0.0
Surgery and dental procedures	7	5.0
Previous blood transfusion	5	3.6
Residence		
Rural	81	57.9
Urban	59	42.1

3.2. Positive Hepatitis C Virus Antibodies by Elisa

This table shows that the detection of Hepatitis C Antibodies by Elisa among studied blood donors was Ten (6.7%) while 140 (93.3%) were negative

Table 2. 10 blood donors sample are positive hepatitis C virus Antibodies by Elisa so they excluded.

	(No.=150) No.	%
Hepatitis C virus Antibodies		
Positive	10	6.6
Negative	140	93.3

3.3. Detection of Hepatitis C Virus RNA from PBMC and Plasma

This table shows that the detection of Hepatitis C virus by nested PCR in PBMC among studied blood donors was eleven (7.9%) while 129 (92.1%) were negative. On the other hand detection of Hepatitis C virus by nested PCR in Plasma among considered blood donors was two (1.4%) while 138 (98.6%) were negative result

Table 3. Detection of hepatitis C virus RNA by RT- nested Polymerase chain (PCR) from PBMC and plasma.

	(No.=140) No.	%
PCR (PBMC)		
Positive	11	7.9
Negative	129	92.1
PCR (Plasma)		
Positive	2	1.4
Negative	138	98.6

3.4. Relation Between the Results PBMC & Plasma

Table 4 and figure 1 represents that eleven cases were positive from PBMC of studied blood donors, two of them were also positive in plasma so they were considered false negative by ELISA analysis, the occult cases were nine (6.4%) which (Positive in PBMC and negative by plasma).

Table 4. Relation between the results of RT- nested Polymerase chain (PCR) for PBMC & plasma.

	PCR (PBMC)		Total
	Positive cases	Negative cases	
PCR (plasma)			
(+VE) cases	2	0	2
(-VE) cases	9	129	138
Total	11	129	140

Figure 1. Relation between PCR results for PBMC & plasma.**Agars gel electrophoresis results:**

Figure 2 shows amplification results of hepatitis C virus RNA by RT-Nested PCR in PBMC. Lane 1 is 100Bp molecular weight marker, lane 2&3 is positive and negative control respectively, lanes 6, 8 are positive results and lanes 4, 5, 7 are negative.

Figure 2. Agars gel electrophoresis.**3.5. Detection of Hepatitis C Virus Core Antigen by ELISA**

This table shows that out of 140 hepatitis C virus Antibodies negative tested blood donors, only five (3.6%) were positive for core antigen detection by ELISA.

Table 5. Detection of hepatitis C virus core antigen by ELISA.

	(No.=140) No.	%
ELISA (hepatitis C virus core Ag):		
Positive	5	3.6
Negative	135	96.4

3.6. Relation Between Core Antigen and PCR of (PBMC&Plasma)

Table 6 and figure 3 shows that only three cases were detected by ELISA out of eleven cases positive for hepatitis

C virus RNA by PCR (PBMC), there was two false positive cases by ELISA. Two cases of the HEPATITIS C VIRUS RNA by PCR (plasma) positive were also positive by ELISA; this concludes that there were three false positive cases by ELISA so sensitivity 27%, specificity 98.4% in PBMC but we founded sensitivity 100% and specificity 97.8% in Plasma.

Table 6. Relation between results of hepatitis C virus core antigen and PCR of (PBMC&Plasma).

	PCR (PBMC)		PCR (Plasma)		Total
	(+VE) cases	(-VE) cases	(+VE) cases	(-VE) cases	
ELISA					
(+VE) Cases	3	2	2	3	5
(-VE) Cases	8	127	0	135	135
Total	11	129	2	138	140

Figure 3. Relation between results of hepatitis C virus core antigen by Elisa and results of hepatitis C virus RNA by RT-Nested PCR of (PBMC&Plasma).**3.7. Correlation between ELISA and PCR in Detection of Occult Hepatitis C Cases**

This table shows that only one case of OCI (11.1%) was detected by both ELISA and PCR and it was statistically significant P. value=0.003.

Table 7. Correlation between ELISA for detection of hepatitis C virus core antigen and nested PCR (PBMC) in detection of OCI cases.

	Total No.	Positive OCI		Negative OCI		Value
		No.	%	No.	%	
ELISA (+VE)	9	1	11.1	8	88.9	0.003*

3.8. General Characteristics and Results of Nested PCR

This table shows that 55% of hepatitis C virus positive cases concentrated in age group (35-45years) and it was statistically significant, P. value=0.043 and 73% of positive cases were from rural residence but was insignificant.

Table 8. General characteristics and results of nested PCR.

demographic characteristics	PCR (+VE) No.=11		PCR (-VE) No.=129		X ²	P. value
	No.	%	No.	%		
Age (years)						
18-25 (50)	2	18.0	48	37.2	5.91	0.043 *
25-35 (61)	3	27.0	58	45.0		
35-45 (29)	6	55.0	23	17.8		
Sex						
Female (13)	1	9.0	12	9.3	0.15	0.731
Male (127)	10	91.0	117	90.7		

demographic characteristics	PCR (+VE) No.=11		PCR (-VE) No.=129		X ²	P. value
	No.	%	No.	%		
Geographic distribution						
Rural (81)	8	73.0	73	56.6	0.82	0.329
Urban (59)	3	27.0	56	43.4		

*= Significant

X2= Chi- square test

3.9. Relation Between Past History and Results of Occult Hepatitis C Virus (OCI)

This table shows that previous blood transfusion, dental and surgery procedures were statistically significant as a risk factor predisposing to OCI among collected data about past history.

Table 9. Relation between past history of considered blood donors and results of Occult hepatitis C virus (OCI).

Past history	Total No 11	+VE OCI 9		-VE hepatitis C virus 131		P. Value
		No.	%	No.	%	
Jaundice	0	0	0.0	0	0.0	-----
Drug intake	0	0	0.0	0	0.0	-----
Surgery and dental procedures	7 (5.0)	4	44.4	3	2.3	0.001**
Previous blood transfusion	5 (3.6)	3	33.3	2	1.5	0.01**

----- =invalid

** = high significant

4. Discussion

High prevalence *hepatitis C virus* infection and high rate of spread infection and liver disease without known the reason [12] so, the present study was done to detect occult *hepatitis C virus* in healthy blood donors in Damietta city and its association to different factors e.g.: sex, age, residence, previous blood transfusion, dental procedures and surgery.

This was by *hepatitis C virus* RNA detecting in PBMC using RT- PCR [13] without the taken a liver biopsy [14] in absence of serum antibodies [15] and also to evaluate role of *hepatitis C virus* core Ag assay in detection of occult *hepatitis C virus* [16]. The results in this study was (7.9%), which is near to the results of other study performed by Mustafa et al. (2013) [17] reported that the detection of OCI in Egyptian volunteer blood donors (5.5%). Fewer rates were detected in Italy by De Marco et al., (2009) [18] found OCI (3.3%) in blood donors due to lower infection of *hepatitis C virus*. High rate (8.9%) detected by Kevin and his colleagues [19] in liver transplant with cryptogenic cirrhosis. Also, high rate (20%) reported by Samir et al., (2012) [20] in patients with chronic lymph-proliferative disorder in Kasr El-Eini Hospital in contrast report low rate (4%) in healthy individual.

Detection *hepatitis C virus* RNA in plasma in this study (1.4%), this reveal false negative among negative result for *hepatitis C virus* Antibodies which done already in blood

bank laboratory, because of error technique or blood donors in seroconversion period before appearance antibodies, so the true detection of OCI in our study was nine out of 140 (6.4%). This result closed to other investigation of Shazly et al. (2015) [21] who documented that OCI was 4% in healthy Egyptian individual partners of patients infected with chronic HEPATITIS C VIRUS genotype 4 infection. Also in hemodialysis patients Abdurrahman et al. (2016) [22] detect OCI (3.7%) in 81 at Mania- Egypt, Also Maria de la Luz Martinez-Rodriguez 2018[23] in Mexico City documented OCI 3.4% in blood donors Samples, Lin H 2016 documented OCI 2.2%in China [24] and Quinoa JA 2016 OCI is 2.1% Spain [25].

January 2002 European blood bank use either *hepatitis C virus* core Ag or *hepatitis C virus* RNA in blood donation test to decrease risk of spread *hepatitis C virus* infection [26], over the last decade *hepatitis C virus* core Ag used to complement *hepatitis C virus* RNA or RT nested PCR in blood test [27], In addition can used for control antiviral therapy as well as for detection of *hepatitis C virus* infection [28].

This study found that the Prevalence of *hepatitis C virus* core antigen by ELISA technique among studied blood donors for detection of *hepatitis C virus* was five (3.6%) and this result was near to result reported by and Obeid (2004) [29] who detect *hepatitis C virus* core Ag (2%) in negative blood donors sample, (negative *hepatitis C virus* Antibodies) at King Fahd University Hospital. While the result of other study performed by Gaudy et al., (2005) [30] were less than these finding (0.7%). They performed test in negative sample with high liver enzymes. This difference due to many places of the researches and variation in kits used or reagents, ELISA technique for detection *hepatitis C virus* core Ag it's more simple and easy to done in different laboratory and cost a little, and also decrease contamination and false positive result in PCR technique and can performed fast in most laboratories [31].

Hepatitis C virus core Ag results different when compared with results of RT-nested PCR from PBMCs and plasma. Only three positive samples detected of *hepatitis C virus* core Ag out of eleven positive *hepatitis C virus* RNA by PCR in PBMCs, 27% sensitivity and 98.4% specificity while the both two positive sample of *hepatitis C virus* RNA by PCR in Plasma are positive in *hepatitis C virus* core Ag by ELISA while the three other positive sample of *hepatitis C virus* core Ag are negative in plasma, 100% sensitivity and 97.8% specificity, However; this results different from reported by Daniel and his colleagues (2007) [23] who detect the sensitivity (85.3%) and specificity (95.8%) when evaluate the role of HEPATITIS C VIRUS core Ag as a sign of active HEPATITIS C VIRUS infection compared to results of PCR, Also, Gaudy et al., 2005 [30] detect sensitivity (85.3%) and specificity (99.3%) in routine examination of individual and compared with results of *hepatitis C virus* RNA detection.

Only one case (11.1%) in this study of nine occult *hepatitis C virus* samples detected by *hepatitis C virus* core Ag, however Juan and his colleagues 2006 [32] detected 4 cases

(3.4%) in 115 negative individual Also Alzahrani and Obeid 2004 [33] detected (2%) of *hepatitis C virus* core Ag in negative *hepatitis C virus* Antibodies in blood donors samples at King Fahd University Hospital.

In this study the relation between different demographic feature and *hepatitis C virus* RNA results first in age was 55% of *hepatitis C virus* positive cases founded in group their age from 35 to 45 years this result has high significance P value=0.043 and compatible with that reported with Castillo et al., (2005) [34] who founded that occult *hepatitis C virus* results in group range from 40 to 52 years and also Castillo et al., (2007) [35] reported occult *hepatitis C virus* cases centered around 46 years in patient have dual infection HBV and *hepatitis C virus* to those of patients have single occult HBV or *hepatitis C virus* infection. Also, L'opez-Alcorocho et al., (2007) [36] reported occult *hepatitis C virus* centered around 42 years and p value was 0.1.

In any case, relationship with more seasoned age wasn't set in every single linked examination. Samir et al. (2012) [37] detected occult *hepatitis C virus* in younger age (18 years) because they perform their study on patients with chronic lymph proliferative upset. But it reported insignificant by Sad et al. (2011) [38] P value was (0.646).

Second, related to the sex of the donors, it was insignificant P value was (0.716). The same result was reported by Sad et al. (2011) who reported P value (1.0) which insignificant. While, this was not in agree with other research done by Samir et al. (2012) who found that the male sex is the prevalent to OCI which may be due to more exposure. Because of, female's number in the investigation is low made it hard to estimate a strong relationship respect to this point. More numbers are expected to evaluate any possible relation between the sex and OCI.

Finally, it was insignificant in different geographic distribution (rural or urban) P value was (0.345) and this also documented by Sad et al. (2011). Different studies needed to exclude or approve any association between occult *hepatitis C virus* and geographic distribution of the individual. Many different factors affected to OCI also examined, by studying combination with previous blood transfusion, surgery, dental procedures, jaundice, drug intake, and fever. It was high significant between these factors especially history of previous blood transfusion, this result was agreed with kelvin et al. (2013) [39] who documented high significant P - value (0.02) in blood transfusion and P - value (0.001) in past history of travel to endemic places meanwhile, p - value in dental procedures is 0.601 and p - value in surgery is 0.938 were insignificant. Also, Abdel Rahim and his coworkers (2016) [40] found that blood transfusion statistically highly significant, P value was 0.002. On contrary, Samir et al. (2012) documented that blood transfusion was insignificant as possible risk factor. Many different researches are needed to detect the any risk factors for transmission occult *hepatitis C virus*.

5. Conclusions

Occult *hepatitis C virus* infection may occur in the general

population apparently diseases free. The presence of *hepatitis C virus* infection in general population seems that occult *hepatitis C virus* infection should be considered and blood transfusion may never be completely risk-free.

The *hepatitis C virus* core Ag assay does not improve the routine serological diagnosis of occult *hepatitis C virus* infection. Core antigen assay requires further evaluation prior to its use as a screening assay in blood banks. PCR is deemed to be more reliable than the *hepatitis C virus* core Ag assay.

Conflict of Interest

The authors declare no conflict of interests.

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