Role of Hepatitis C Virus Core Antigen Assay in Blood Donors Screening at Zagazig University Hospitals

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Background and study aim: Hepatitis C virus (HCV) infection is a major public health problem worldwide. Blood donations screening achieved mainly by serological identification of HCV-Antibody (Ab), has largely reduced HCV transmission. HCV Core Antigen (CAg) tests have been introduced to supplement anti-HCV tests and HCV PCR analyses. CAg may be a useful screening test for identifying window phase of HCV infected patients whom are candidate for blood donations. The study aimed to evaluate diagnostic performance of HCV core antigen in comparison to HCV-RNA quantification and anti-HCV-Ab analyses in attendances of blood bank of Zagazig University hospitals.

Patients and Methods: The study was performed on 92 participants attending the blood banks of Zagazig University Hospitals for blood donation between May 2015 to November 2017. The participants were classified into two groups; group A, which included 46 donors (32 males and 14 females) with negative HCV antibody and group B, which Included 46 patients (30 males and 16 females) with positive HCV antibody. Clinical assessment, HCV AB detection by ELISA, Prototype ELISA for HCV core antigen for presence of HCV core antigen and HCV RNA Quantitative were done for all participants.

Results: No significant differences between both studied regarding sex and age. A high significant relation between HCV AB positivity and negativity as regard HCV PCR was found in both groups. A high significant relation between HCV core antigen positivity and negativity as regard HCV PCR. There was high significant relation between HCV core antigen positivity and HCV PCR in group A patients. There was a high significant relation between HCV core antigen positivity and HCV PCR in positive HCV antibody patients and statistically a high significant relation between HCV core antigen negativity and HCV PCR in group B patients.

Conclusion: HCV core Ag can be identified by serological ELISA. This assay is cheap, easily performed, and compatible with HCV PCR. Its application may prevent the vast majority of HCV transmissions caused by the transfusion of window phase donations.

INTRODUCTION

Hepatitis C virus (HCV) is an overall disease. In 2008 about 15% of the Egyptian population, aged 15–59 years had antibodies to HCV and 10% (approximately 5 million persons) had chronic HCV infection **[1].** HCV transmission is continuous in Egypt, and the incidence rates have been estimated to be 2.4 per 1,000 person/ year **[2].**

The incubation period for newly acquired HCV infection ranges from two weeks to six months, however, viral replication can be detected as early as one week after exposure. It has been shown that total HCV core antigen levels correlate with HCV RNA levels. Core antigen kinetics run parallel to HCV RNA kinetics during chronic HCV infection. **[3].**

Diagnostic tests for hepatitis C can be divided into two general categories; serological assays that detect antibody to HCV (anti-HCV) and molecular assays that detect and/or quantify HCV RNA genomes within an infected patient. Serological assays have been subdivided into screening tests for anti-HCV, such as the enzyme immunoassay

(EIA), and supplemental tests such as recombinant immunoblot assay (RIBA). Supplemental anti-HCV tests are designed to resolve false-positive testing by EIA. Detection of HCV RNA in patient specimens by polymerase chain reaction (PCR) provides evidence of active HCV infection and is potentially useful for confirming the diagnosis and monitoring antiviral response to therapy **[4].**

Anti-HCV assays have several disadvantages, such as a high rate of false positivity, lack of sensitivity if used in the early window period of 45 to 68 days after infection, the inability to distinguish between acute (ongoing active, viremia), past (recovered), and persistent (chronic) infections, in addition to the possibility of false negativity with samples from immunocompromised patients, who may not have an adequate antibody response. Also, PCR analysis for measuring viral loads has some drawbacks including; expensive and requirement of technical skills **[5]**.

HCV core antigen (Ag) tests have been introduced to supplement anti-HCV tests or HCV PCR analysis **[6,7]**. It can be identified by routine serological ELISA in specimens from the early antibody–negative phase of HCV infection. It may be a useful test for identifying window phase blood donations from antibody negative donors infected with HCV **[8]**. These quantitative HCV Ag assays could be used for diagnosis of HCV infection as well as for monitoring of antiviral therapy **[9].** Furthermore, HCV Ag assay could also be useful in monitoring immuno-compromised patients and those undergoing regular hemodialysis **[10].**

The work aimed to evaluate the diagnostic performance of HCV core antigen in comparison to HCV-RNA quantification and anti-HCV-Ab analyses in blood donors.

PATIENTS AND METHODS

Study design and settings :
This comparative cross section study included ninety-two participants who attended the blood bank of Zagazig University Hospitals. Between May 2015 to November 2017. Written consents have been taken from all included participants.

Target population and sampling :
Individuals attending the blood bank of Zagazig University Hospitals. Participants were classified into two groups; group A, which Included 46 patients (32 males and 14 females) with negative HCV antibody and group B, which Included 46 patients (30 males and 16 females) with positive HCV antibody.

Exclusion criteria :
Patients who had HBV infection, history of immune-suppressive drugs, DM and any manifestations of chronic liver disease were excluded from the study.

Methods and study tools :
All participants were subjected to the following workup:

- Thorough history taking and full clinical examination with special stress on manifestations of chronic liver disease.
- Laboratory workup including HBsAg, HCV Ab detection by ELISA, HCV core antigen Prototype by ELISA and HCV Quantitative RNA assay.

Specimen collection and preparation:
Venous blood sample withdrawn from each participant under complete aseptic conditions using wide bore needle and slowly withdrawn from antecubital vein to avoid RBCs hemolysis. The sample was added to a sterile vacutainer dry tube, allowed to be clotted for 30 minutes, and then separated by centrifugation in PCR unit. The resulted serum was divided into 2 aliquots under strict sterile conditions and stored at -80°C to be used for HCV-Ab levels, HCV core antigen and PCR. HCV antibodies (HCV-Ab) and hepatitis B surface antigen (HBsAg) were detected by ELISA (Dia Sorin Diagnostics, Italy). This kit complements qualitative methods depended on enzyme linked immunosorbent assays (ELISA). The procedures were done according to manufacturer's instructions **[11]**. HCV core Antigen was measured by QuickTiter™ HCV Core Antigen ELISA Kit CELL (BIOLABS, INC, USA). HCV quantitative RNA detection by Real time PCR was detected using a Cobas AmpliPrep/Cobas TaqMan HCV kit (Amplicor, Roche Diagnostics, Branchburg, NJ). The lower limit of detection of this assay was 16 IU/ml **[12].**

Statistical analysis :
Data were checked, entered and analyzed using SPSS 22 for Windows. Data were expressed as mean ± SD for quantitative variable, number and percentage for qualitative one. Chi-squared (X^2) or t test and paired t test were used when

appropriate. P<0.05 was considered significant. P<0.001 was considered high significant.

RESULTS

Demographic data showed non significant difference between both groups as regard gender and age (p>0.05) (Table 1). positive HCV core Ag in group (A) was 4 cases (8.7%) and 37 cases (80.4%) in group (B) (Table 2). A high significant relation between HCV AB positivity and negativity as regard HCV PCR in both groups (p<0.001) (Table 3). A high significant relation between HCV core antigen positivity and negativity as regard HCV PCR (p<0.001) (Table 4). A high significant relation between HCV core antigen positivity and HCV PCR in group A patients (p<0.001) (Table 5). A significant relation between HCV core antigen positivity and HCV PCR in positive HCV antibody patients and also, a statistically significant relation between HCV core antigen negativity and HCV PCR in group B patients (p<0.001) (Table 6). positive HCV core Ag in 41 patients; 37 patients with positive HCV Ab and 4 patients with negative HCV ab in patients of both groups(Table7). The ability of HCV core Ag test to detect positive infected cases is 97.4% with positive predictive value of 100%, and the ability to exclude negative cases from truly negatives is 100% with negative predictive value of 70%, test accuracy is 97.8% (Table 8).

Table (1): Comparison between the studied groups as regard demographic data

Demographic data	Group (A) (N=46)		Group (B) (N=46)		Test	p-value (Sig.)
	No.	%	No.	%		
Sex						
Male	32	69.5%	30	65.2%	0.377‡	0.539 (NS)
Female	14	30.5%	16	34.8%		
Age (years)						
≤ 20 years	13	28.2%	12	26%	0.040‡	0.980 (NS)
20-30 years	15	32.6%	16	34.8%		
30-40 years	11	23.9%	10	21.7%		
≥ 40 years	7	15.3%	8	17.5%		

‡ Chi-square test. * Independent samples Student's t-test.
• Mann Whitney U test. P< 0.05 is significant. Sig.: Significance.

Table (2): HCV seromarkers in both groups

HCV seromarker	Group A (N=46)		Group B (N=46)	
	No.	%	No.	%
HCV Ab				
Negative	46	100%	0	0%
Positive	0	0%	46	100%
HCV core Ag				
Negative	42	91.3%	9	19.6%
Positive	4	8.7%	37	80.4%

‡ Chi-square test. P< 0.05 is significant. Sig.: Significance.

Table (3): HCV RNA levels among studied groups

HCV RNA levels by PCR	Group A (N=46)		Group B (N=46)		Test‡	p-value (Sig.)
	No.	%	No.	%		
<16 IU/ml	41	89.1%	7	15.2%	64.720	<0.001 (HS)
16 <10^4 IU/ml	2	4.3%	16	34.8%		
>10^4 – <10^6 IU/ml	1	2.3%	15	32.6%		
>10^6 IU/ml	2	4.3%	8	17.4%		

<16 IU/ml (below level of detection) ‡ Chi-square test.
P< 0.05 is significant. Sig.: Significance.

Table (4): Relation of HCV core antigen with HCV RNA levels by PCR in patients

HCV RNA levels by PCR	HCV core Ag				Test‡	p-value (Sig.)
	Negative (N=51)		Positive (N=41)			
	No.	%	No.	%		
<16 IU/ml	48	94.1%	0	0%	95.693	<0.001 (HS)
16 <10^4 IU/ml	2	3.94%	15	36.6%		
>10^4 – <10^6 IU/ml	1	1.96%	15	36.6%		
>10^6 IU/ml	0	0%	11	26.8%		

<16 IU/ml (below level of detection) ‡ Chi-square test.
P< 0.05 is significant. Sig.: Significance.

Table (5): Relation between HCV core Ag and HCV PCR in group (A)

HCV PCR	Negative HCV Ab (group A) (N=46)				Test	p-value (Sig.)
	Negative HCV core Ag (N=42)		Positive HCV core Ag (N=4)			
	No.	%	No.	%		
Negative	41	97.62%	0	0%	43.137‡	<0.001 (HS)
Positive	1	2.38%	4	100%		
<16 IU/ML	41	97.6%	0	0%	47.586‡	<0.001 (HS)
16-10^4 IU/ML	0	0%	2	50%		
10^4-10^6 IU/ML	1	2.4%	1	25%		
>10^6 IU/ML	0	0%	1	25%		

‡ Chi-square test. P< 0.05 is significant. Sig.: Significance.

Table (6): Relation between HCV core Ag and HCV PCR in group (B)

HCV PCR	Positive HCV Ab (group B) (N=55)				Test	p-value (Sig.)
	Negative HCV core Ag (N=9)		Positive HCV core Ag (N=37)			
	No.	%	No.	%		
Negative	7	77.78%	0	0%	37.447‡	<0.001 (HS)
Positive	2	22.22%	44	100%		
<16 IU/ML	7	77.8%	0	0%	37.913‡	<0.001 (HS)
16-10^4 IU/ML	1	11.1%	13	35.1%		
10^4-10^6 IU/ML	1	11.1%	13	35.1%		
>10^6 IU/ML	0	0%	11	29.8%		

P<0.05 is significant. Sig.: Significance.

Table (7): HCV core antigens in all HCV patients with different serological presentations

	HCV core Ag			
	Negative (N=51)		Positive (N=41)	
	No.	%	No.	%
HCV ab +ve	9	17.6%	37	90.2%
HCV ab –ve	42	82.4%	4	9.8%
<16 IU/ml	48	94.1%	0	0%
16 <10^4 IU/ml	2	3.94%	15	36.6%
>10^4 – <10^6 IU/ml	1	1.96%	15	36.6%
>10^6 IU/ml	0	0%	11	26.8%

<16 IU/ml (below level of detection)

Table (8): Validity data of HCV-core Ag in relation to PCR in diagnosis of HCV infection

Cut off value	AUC	P-value	Sensitivity	Specificity	PVP	PVN	accuracy
69.5	**1.00**	**0.000**	**97.4%**	**100%**	**100%**	**70%**	**97.8%**

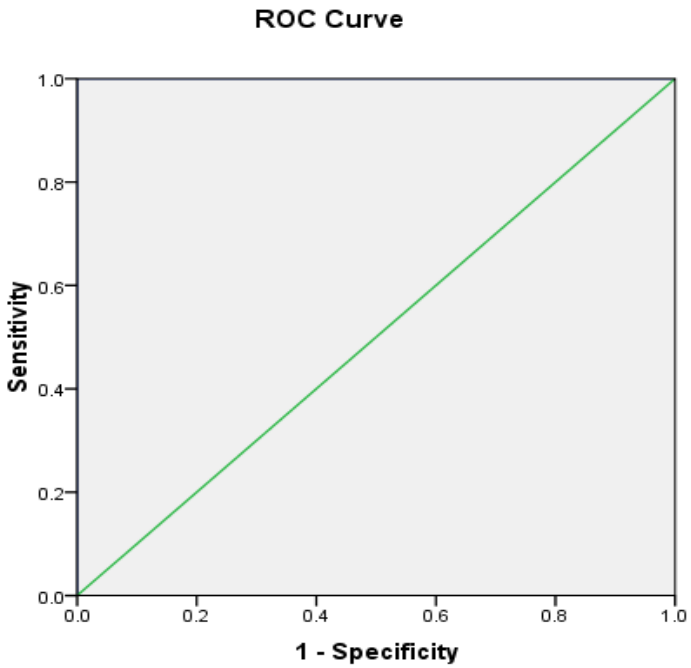

Figure (1): Receiver operating curve (ROC) for validity of HCV-core Ag in relation to PCR in diagnosis of HCV infection

DISCUSSION

WHO declared HCV infection a global health problem, with approximately 3~4% of the world's population (roughly 170-200 million people) infected with hepatitis C. In the US, approximately 3 million people are chronically infected, many of them still undiagnosed while the Egyptian prevalence rate of HCV antibody has been estimated to be 10-13% of the general population. It is well known that in HCV infection, liver fibrosis progresses as the period of infection prolongs, may reach liver cirrhosis and it may reach liver cirrhosis and if it progresses to liver cirrhosis, the risk of HCC also increases **[1]**.

HCV core antigen levels correlate well with HCV RNA levels. Core antigen kinetics runs closely parallel to HCV RNA kinetics during chronic HCV infection. The incubation period for newly acquired (acute) HCV infection ranges from two weeks to six months, however, viral replication can be detected as early as one week after exposure **[3]**.

We try to confirm the importance of HCV core antigen detection as an alternative diagnostic tool for active and chronic HCV infection that can replace HCV RNA in early detection of active HCV infection in blood donners attending blood banks especially in poor country like Egypt.

This study observed that there was no significant difference among both groups as regard age and sex. Regarding patients of group (A), 32 of them were male (69.5%) and 14 were female (30.5%). Regarding patients group (B), 30 of them were male (65.2%) and 16 of them were female (34.8%). This may be attributed to the fact that the prevalence of chronic HCV infection in Egypt is higher among men than women (12% and 8%, respectively) **[1].**

We reported a statistically significant relation between HCV core antigen positivity and HCV PCR in both study groups. This result agrees with the results obtained by Catherine Goudy et al. 2005 [13], who confirmed a 96.7% sensitivity of the HCV core Ag assay with a high significant relation between positivity and negativity of HCV core Ag and HCV PCR in positive HCV antibody patients.

Our results are partially agreed with Reddy et al. **[14]**, who reported a 60% sensitivity of HCV core Ag assay in their study that included 111 chronic renal failure patients undergoing hemodialysis. The cause of discrepancy in the results may be related to compromised immune response.

We reported 4 cases with –ve HCV ab and +ve HCV RNA by PCR. This finding is supported by Morgan et al. **[15]** who confirmed presence of HCV antigen during the early, RNA-positive phase of anti-HCV seroconversion.

This study showed a high significant relation between HCV core antigen positivity and negativity as regard HCV PCR. This goes in harmony with Medici et al. **[16]**, they reported that circulating HCV core antigen first became detectable at approximately the same time as HCV RNA. The residual risk of HCV transmission because of the antibody-negative viremia 'window phase' has been documented. They have indicated that this residual risk might be substantially eliminated by testing blood donations for HCV core antigen or HCV RNA.

Morgan et al. **[15]** evaluated the sensitivity of this prototype test in specimens from individuals undergoing seroconversion following HCV infection. They added that HCV core antigen can be identified by routine serological ELISA in specimens from the early antibody-negative phase of HCV infection. A test for HCV core antigen may be a useful test for identifying window phase of blood donations from antibody negative donors infected with HCV.

CONCLUSION

HCV core antigen ELISA assay is a simple and reliable direct method for detection of acute and chronic HCV infection. Since this assay is based on ELISA technology, it can be easily performed in most laboratories with low cost and this is a very important in devolving countries with low economic resources. HCV core Ag is compatible with HCV PCR, its wide application may prevent the vast majority of HCV transmissions caused by the transfusion of window phase donations. Moreover, HCV core antigen serve as a good method for direct HCV detection in patients during pre- seroconversion period 'window phase' when the antibody assays are negative. Also, it can be used for monitoring of antiviral therapy.

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