Cost-Effectiveness of HCV Core Antigen versus PCR for Monitoring Treatment Response in DAAS-Treated Egyptian Patients

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Key words:
DAAs, SVR12, HCV core antigen

Background and study aim: PCR is currently the non-debatable proof for diagnosis of HCV infection as well as conclusion of treatment outcomes. HCV core antigen (HCVcAg) testing is a neglected, less expensive and less time-consuming test that's presumed to achieve the same aims. The aim of this study is to find the cost-effectiveness of HCV core antigen testing in the monitoring of treatment response as an alternative to the gold-standard PCR test .

Patients and Methods: 48 patients indicated for DAAs-therapy in the period from January- to July 2018. Pre- and post-treatment routine investigations including HCV-RNA levels as well as HCVcAg were done.

Results: There was a high statistically-significant difference ($p<0.001$) within the studied group as regards pre- and post-treatment results of HCV-RNA with a total SVR12 rate of 95.8% (46/48 patients). There was a high statistically-significant varaiation ($p<0.001$) as regards pre- and post-treatment levels of HCVcAg of the studied group. HCVcAg was detected in 89.5% of the included patients before treatment (43/48 patients). The 5 cases that tested negative for HCVcAg had HCV-RNA levels <2000 IU/mL. HCVcAg was undetectable in 100% of patients who achieved SVR12 (46 patients). There was a high statistically significant correlation between HCV-RNA and HCVcAg levels of the studied patients ($r= 0.677$, $p<0.001$). HCVcAg testing had an overall test accuracy of 94.79%.

Conclusion: HCVcAg is a sensitive, specific test, less expensive (cost 0.46 that of PCR per single sample) but false negative results of HCVcAg existed with low viremia (< 2000 IU/ml).

INTRODUCTION

HCV infectivity is a major public health insult. It has special prevalence rates in the Mediterranean countries and southern Europe that ranges 1-3% **[1]**.

One of the highest world-wide HCV prevalence countries is Egypt; estimated nationally at 14.7%, and more than 90% of these infections were reported to be genotype 4 **[2]** .HCV infection and its complications is a major public health challenge in Egypt. This epidemic level has been rationalized by the massive iatrogenic transmission when parenteral tartar emetics were used for treating bilharzaisis in mass-treatment campaigns **[3]**.

In 1996, the prevalence of anti-HCV antibodies in adults was more than 40%. The Demographic Health Survey (DHS) reported the same prevalence to be 14.7% and viremic prevalence of 9.7% in 2008 **[4,5]** versus 10% and 7% for seroprevalence and viremic prevalence respectively in 2015 **[6]**.

Routine screening of donated blood for anti-HCV seropositivity has markedly reduced the risk of transfusion-related HCV infection worldwide. However, it may take up to several months for anti-HCV antibodies to be detectable in blood following infection. As a result, there is still a small -but significant- residual risk of transfusion-related HCV infections resulting from donations during the early 'window phase' of infection **[10].** PCR is the most accepted proof for diagnosis of HCV infection as well as conclusion of treatment outcomes. HCV core antigen (HCVcAg) testing is however a neglected method that can be used for the same purpose **[11].**

Though less studied, HCVcAg testing has the following advantages over the gold standard PCR test: its target is a protein that's more stable than RNA, the testing procedure is simpler, less time-consuming, has fewer analytical requirements and of course less expensive **[12].**

Being a developing country with an exclusive high prevalence of HCV infection, Egypt started a series of well-organized screening and mass-treatment programs on the hope of complete HCV eradication by the year 2030. HCVcAg being an ELISA test will be a much less expensive tool to screen for HCV infection as well as treatment-response to DAAs than the gold-standard PCR test for the same aim. Inadequate and non-conclusive data or guidelines existed about the use of this tool in Egypt for evaluating its efficacy for monitoring treatment response in the DAAs era. Therefore, this work aimed to study the cost-effectiveness of HCVcAg quantification in the monitoring of treatment outcomes of DAAs in HCV-infected patients.

PATIENTS AND METHODS

This prospective study included 48 patients indicated for DDAs-therapy attending the Viral Hepatitis Treatment Unit at El-Ahrar Teaching Hospital, Zagazig, Sharqiyah governorate, Egypt in the period from January 2018 to July 2018.

Inclusion criteria: Patients with HCV infection (diagnosed by PCR), aged 18-75 years.

Exclusion criteria: Serum Albumin < 2.8 g/dl, Total serum bilirubin > 3 mg/dl, INR ≥ 1.7, Platelet count < 50000/mm, Patients who are co-infected with HIV, Patients younger than 18 or older than 75 years old, Pregnant females or those who cannot use appropriate contraceptive method and Hepatocellular Carcinoma or other extra-hepatic malignancy.

The included patients were prescribed SOF/DCV (Easy to treat) or SOF/DCV/RVN (Difficult to treat) according to the following criteria:

Easy to treat: Treatment-Naïve patients, Total bilirubin ≤ 1.2 mg/dl, Serum Albumin ≥ 3.5 g/dl, INR ≤ 1.2 and Platelet ≥ 150000/mm^3.

Difficult to treat: IFN-experienced patients, Total bilirubin ≥ 1.2 mg/dl, Serum Albumin (2.8 – 3.5) g/dl, INR (1.2 – 1.7) and Platelet count 50000-150000/mm^3.

All patients exhibited good compliance and signed an informed consent

All participants were subjected to thorough history taking with special interest on hepatic symptoms and signs, previous treatment for HCV, other chronic diseases or drug-hypersensitivity, full clinical examination. Laboratory investigations in the form of CBC and liver profile including ALT, AST, serum albumin, total bilirubin and direct bilirubin, INR, AFP, Kidney profile: S. creatinine and blood Urea using, pregnancy test (for females in childbearing period), Fasting blood glucose level, HBsAg (Hepatitis B surface antigen), HIV Abs, Quantification of HCV RNA done using COBAS® TaqMan® HCV Test, v2.0 provided by Roche, Quantification of HCV core antigen in serum: The testing was done using Quick Titer™ HCV Core Antigen ELISA Kit which is an enzyme immunotesting developed for detection and quantitation of the HCV core protein supplied by Glory Science (Glory Science Co., Ltd, 2400 Veterans Blvd. Suite 16- 101, Del Rio, TX 78840, USA). In addition, a pre-treatment abdominal ultra-sonographic study was done.

12 weeks after end of treatment: All patients were subjected to HCV-RNA quantification by PCR and HCVcAg quantification.

Statistical analysis of the data

The IBM SPSS software package version 20.0 **(**Armonk, NY: IBM Corp**)** was used for analysis. Qualitative data are characterized using numbers and percentages. Quantitative data are characterized using range (minima and maxima), means, standard deviations and medians. The significance of the resulted values were judged at the 5%.

RESULTS

The present study included 48 patients, 30 of them were males (62.5%) and the other 18 patients were females (37.5%), with a mean age of 28.4±8.5 years **Table (1)**.

Concerning clinical presentations, there was a high statistically-significant difference regarding the pre- and post-treatment prevalence of fatigue among the studied group, while there was a statistically-insignificant difference regarding pallor, palmar erythema, jaundice, lower limb edema, variceal Bleeding, hepatomegaly, splenomegaly, ascites and hepatic encephalopathy **Table (2)**

In the current study there was a statistically-significant difference within the studied group as regards pre- and post-treatment levels of ALT, while there was a statistically-insignificant difference as regards pre- and post-treatment levels of HB%, WBCs, platelet count, AST, AFP, total protein, albumin total and direct bilirubin levels, PT, INR. **Table (3)**

In the current study there was a high statistically-significant difference within the studied group as regards pre- and post-treatment levels of HCV-RNA. SVR12 was achieved in 95.8% of the included patients (46/48). **Table (4)**

There was a high statistically-significant difference as regards pre- and post-treatment quantitative results of HCVcAg of the studied patients. **Table (5)**

Also, there was a statistically-insignificant difference as regards the rate of SVR12 achievement of the studied group according to treatment regimen (SOF/DCV in 40 patients Vs SOF/DCV/RVN in 8 patients). **Table (6)**

Table (1): Demographic data of the study population

Age (years)	Mean ± SD	28.4 ± 8.5	
	Range	18 - 44	
		No	**%**
Sex	Male	30	62.5
	Female	18	37.5
Smoking	Smoker	15	31.25
	Non-smoker	33	68.75
Diabetes	Diabetic	16	33.33
	Non-Diabetic	32	66.66
Treatment Regimen	SOF/DCV X 3 months	40	83.33
	SOF/DCV/RVN X 3 months	8	16.67

Table (2): Clinical presentations of the studied group pre- and post-treatment

	Pre- treatment		**Post- treatment**		**X^2**	**P-value**
	N	**%**	**N**	**%**		
Asymptomatic	9	18.75	19	39.58	4.084	0.043
Fatigue	28	58.33	4	8.33	24.797	< 0.001
Pallor	3	6.25	5	10.42	0.136	0.711
Palmar erythema	6	12.5	6	12.5	0	1
Jaundice	1	2.08	0	0.00	1.011	0.315
LL Edema	7	14.58	3	6.25	1.005	0.316
Variceal Bleeding	3	6.25	2	4.17	0.209	0.648
Hepatomegaly	9	27.08	7	18.75	0.075	0.784
Splenomegaly	7	18.75	6	12.5	0.089	0.765
Ascites	2	4.17	0	0.00	0.511	0.475
Hepatic encephalopathy	1	2.08	1	2.08	0	1

Table (3): Lab results of the studied group before and after treatment

		Pre- treatment	Post- treatment	t-test	P
Hemoglobin(g/dL) (N: ♂ 13-18, ♀11.5-16.5 g/dL)	X ± SD	12.8 ± 1.6	13.3 ± 1.4	1.86	0.071
	Range	9.6 – 16.1	9.8 – 16.4		
TLC (X 10^3/mL**)** (N: 4-11 X 10^3/mL)	X ± SD	6.28 ± 1.4	6.89 ± 1.6	2.3	0.063
	Range	4.1 – 9.7	3.9 – 8.9		
Platelets (X 10^3/mL**)** **(N:** 150.000-450.000/mL)	X ± SD	270.9 ± 80.3	273.8 ± 79.3	0.166	0.869
	Range	134 - 432	129 - 410		
AST (N: 0-45 IU/ L)	X ± SD	43.3 ± 19.7	38.5 ± 18.7	1.14#	0.258
	Range	18 – 73	16 - 93		
ALT (N: 0-45 IU/L)	X ± SD	55.6 ± 25.5	39.1 ± 19.2	3.37#	0.002
	Range	14 – 85	17 - 97		
AFP (Alpha feto-protein) (N: 0-10 ng/ml)	X ± SD	9.563 ± 6.133	9.5 ± 5.964	1.77	0.083
	Range	2 - 32	2 - 31		
Total Protein (N: 6.3 to 7.9 g/dL)	X ± SD	7.9 ± 0.99	7.6 ± 0.69	0.102	0.936
	Range	6.6 - 8.7	5.6 - 9.2		
Serum albumin (N: 3.4 - 5.4 g/dL)	X ± SD	4.41 ± 0.69	4.4 ± 0.395	0.618	0.541
	Range	3.7 – 5.2	2.4 - 6.3		
Total bilirubin (N: 0.2-1.2 mg/dl)	X ± SD	1.13 ± 0.72	0.94 ± 0.27	1.91	0.065
	Range	0.4-3.2	0.2 - 2.6		
Direct bilirubin (N: ≤0.3 mg/dL)	X ± SD	0.136 ± 0.66	0.102 ± 0.07	2.79#	0.091
	Range	0.1 - 0.3	0.1 - 1.3		
Prothrombin time (N: 10-14 sec)	X ± SD	14.5 ± 2.6	12 ± 1.3	6.55	0.624
	Range	10 – 13	10 - 19		
INR (N: ≤ 1.1)	X ± SD	1.33 ± 0.24	1.11 ± 0.07	6.14	0.733
	Range	1 - 1.2	1 - 1.7		

= Mann-whiney test of non-parametric data

Table (4): Qualitative PCR & HCVcAg of the studied group before and after treatment

		Pre- treatment		Post- treatment		X^2	*P-value*
		N	%	N	%		
PCR	Detectable	48	100	2	4.1	88.32	*< 0.001*
	UDL*	0	0	46	95.9		
	Total	**48**	**100**	**48**	**100**		
HCV Core Ag	Positive	43	89.58	2	4.17	26.65	*< 0.001*
	Negative	5	10.42	46	95.83		
	Total	**48**	**100**	**48**	**100**		

**UDL= under detection limit (< 16 IU/mL)*

Table (5): Quantitative HCV core antigen levels of the studied group before and after treatment

	Pre- treatment	Post- treatment	Paired t-Test	P-value
Mean ± SD	96.00 ± 28.39	15.23 ± 10.25	18.644	< 0.001
Range	65.15 – 125.47	5.09 – 23.69		

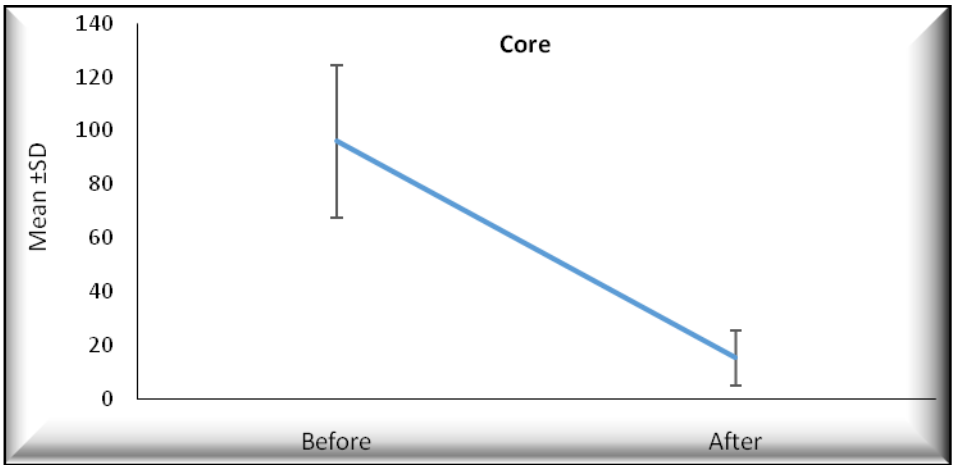

Figure (1): HCV core antigen levels of the studied group before and after treatment

Table (6): Post-treatment qualitative PCR & HCVcAg results of the studied group according to treatment regimen

		SOF/DCV *(N=40)*		SOF/DCV/RVN *(N=8)*		X^2	*P-value*
		N	**%**	**N**	**%**		
PCR	**UDL***	39	97.50	7	87.50	0.2195	0.639
	Detectable	1	2.5	1	12.5		
	Total	**40**	**100**	**8**	**100**		
HCV Core Ag	**Positive**	1	2.50	1	12.5	1.669	0.196
	Negative	39	97.50	7	87.50		
	Total	**40**	**100**	**8**	**100**		

UDL= under detection limit (< 16 IU/mL)

Table (7): Overall qualitative **HCV core antigen** and PCR results of the studied group

		PCR			Accuracy	94.79%
HCVcAg		**Positive**	***UDL***	***Total***	Sensitivity	90
	Positive	45	0	45	Specificity	100
	Negative	5	46	51	PPV	100
	Total	50	46	96	NPV	90.19

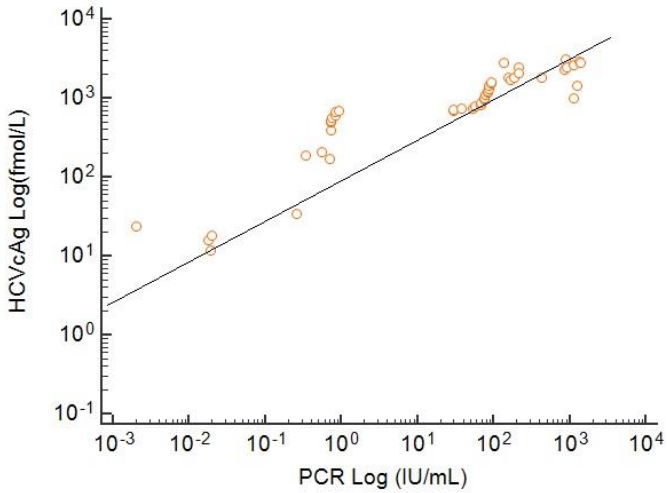

Figure (2): correlation between core Ag results and PCR results.

DISCUSSION

HCVcAg has been extensively-evaluated for establishing the diagnosis of HCV infection however, only too few studies existed about the clinical utility of the HCVAg testing to conclude treatment outcomes in DAAs-treated HCV patients **[13].** In this context, we study the sensitivity and specificity of HCVcAg quantification in the monitoring of HCV in patients treated with DAAs before- and 12 weeks after end of treatment.

The present study included 48 patients, that have undergone the pre-treatment investigations for DAAs therapy aiming at HCV eradication and classified as 'treatment-eligible' according to inclusion and exclusion criteria, 40 of them were easy to treat (83.33%) who received SOF/DCV for 12 weeks and 8 of them were difficult to treat (16.67%) who received SOF/DCV/RVN for 12 weeks. Their ages ranged 18-44 years old with mean age 28.4 years, 62.5% of them were males. **(Table 1)**

Regarding clinical presentations of the included patients, there was a high statistically-significant correlation regarding the pre- and post-treatment prevalence of fatigue among the study populations ($p < 0.001$). HCV significantly-impairs health-related quality of life which is directly-associated with lower productivity and higher disability claims**.[14]** These results are consistent with the Spanish study of **Juanbeltz et al. [15]** who reported post-treatment improvement of pain/discomfort in all patients regardless the degree of liver cirrhosis ($p = 0.036$) **[15]**.

In contrast the pre- and post-treatment prevalence of other clinical parameters namely; pallor, palmar erythema, jaundice, lower limb edema, hepato-splenomegaly, ascites and hepatic encephalopathy showed a statistically-insignificant correlation. These results are against those of **Poordad et al. [16]** and **Manns et al. [17]** who reported a significant correlation regarding the pre- and post-treatment prevalence of clinical manifestations of liver cirrhosis. This contrast may be rationalized by the small number of decompensated cases included in our study. Also, the post-treatment interval of 12 weeks may be too short (3 months) to detect decompensation or other long-term complications but this interval was mandatory for detection of SVR12.

There was a statistically-insignificant difference regarding variceal bleeding and this was in agreement with the study of **Estefania et al. [18]** where DAAs-treated patients didn't show significant improvement in the development of hepatic decompensation symptoms early after end of treatment compared to untreated compensated cirrhotic patients. **Diogo** and **Rui [19]** reported more hopeful data about the efficacy of DAAs (rate of SVR12 achievement is more than 90%) with a lower rate of development of de novo esophageal varices.

Statistical analysis of the pre- and post-treatment laboratory results of the studied group revealed a statistically-significant difference as regards levels of ALT only, this finding is in agreement with **Deterding et al. [20]** who reported normalization of ALT values of responders (different genotypes with different combinations of DAAs).

Concerning other laboratory parameters namely Hb%, WBCs, platelets, serum albumin, bilirubin, total protein, AST, AFP, PT and INR; there was a statistically-insignificant difference. This is in contrast with **Persico et al. [21]** who reported that 'SVR12 was associated with reasonable improvement of hepatic fibrosis and hepatic functional status and all of these can be detected as early as end of treatment'.

In the pre-treatment screening, HCVcAg was detectable in 89.58% of the included patients (43/48 patients) and undetectable in 10.42% of patients (5/48 patients) that had detectable HCV-RNA by PCR; the highest of them was 1887 IU/ml. Post-treatment, HCVcAg was detectable in 4.17% of the included patients (2/48 patients) (both patients had detectable viremia on PCR testing) and undetectable in 95.83% of patients (46/48 patients).

Treatment success is reported in terms of persistence of undetectable viremia 12 weeks after end of treatment (SVR12). In the present study SVR12 was achieved in 95.58% of the included patients i.e. 46/48 patients had undetectable HCV-RNA in the 12 weeks post-treatment session, while 2/48 still had detectable HCV-RNA by PCR testing (values of $827x10^3$ and $10.290X10^6$ IU/ml).

Considering HCVcAg as a screening test in the present study, 45 samples out of the 50 PCR-positive samples; tested positive for HCVcAg test, and 46 samples out of the 46 PCR-negative

samples; tested negative for HCVcAg test thus the overall sensitivity, specificity, PPV, NPV and test accuracy of HCVcAg test is 90, 100, 100, 90.19, 94.79% respectively.

In agreement with our results**, Arboledas et al. [22]** reported that the HCVcAg was detectable in 98.7% of viremic patients in the pre-treatment screening while it was undetectable in only three patients.

Also our results were in agreement with **van Tilborg et al. [23]** who recommended HCVcAg testing as a dependable, cost-saving screening tool in detection of HCV-viremic patients. 75 of 80 PCR-positive samples tested positive for HCVcAg (sensitivity 94%, 95% CI 86-98), and none of the 993 PCR-negative samples tested positive for HCVcAg (specificity 100%, 95% CI 94-100).

Also, our results come in agreement with **Garbuglia et al. [24]**; **Freiman et al. [25]; Ottiger et al. [26]** who have positioned HCVcAg as a reasonable alternate to the gold-standard PCR test, and they concluded that 3 fmol/L of HCVcAg could be equivalent to 400–3000 IU/mL HCV RNA.

There was a high statistically-significant difference regarding pre-and posttreatment levels of HCVcAg levels among the studied patients. These results suggest that the quantification of HCVcAg can help in prediction of treatment outcomes and patient adherence to treatment with such a low-cost test. In addition, there was a statistically-significant correlation between HCV-RNA and HCVcAg levels of the studied patients ($r= 0.677$, $p<0.001$) **(Table 10).** In agreement with these results, **Christine Changa et al. [27]** reported that there was strong correlation between HCV-RNA levels and HCV core Ag levels ($r=0.96$, $p < 0.001$). Again **Chevaliez et al. [13]** reported a similarly-high correlation coefficient of 0.864.

In terms of cost in this study, single HCVcAg/ PCR testing was 0.46. Taking in consideration that the HCVcAg we used in the current study are for experimental issues only (i.e. HCVcAg are not recommended for routine use in clinical labs) if HCVcAg was broadly requested in clinical labs as other ELISA tests, it would have been much less expensive.

With this number of studies that all agreed a strong correlation between HCVcAg and HCV-RNA, we can now say that HCVcAg testing can be positioned as a better cost- and time-effective tool in the screening of viremic-HCV patients than the traditional dual-step screening approach for HCV at the time. This is especially valuable in low-income and developing countries such as Egypt. However, HCV-RNA measurement by PCR is still the gold-standard in confirmation of treatment outcome and achievement of SVR12 due to false negative HCVcAg in the existence of low HCV-RNA as found in our study.

CONCLUSION

HCVcAg is a sensitive, specific tool with high accuracy and strong correlation to HCV-RNA levels that can be used to positive test, but its undetectability doesn't always reflect aviremia, due to false negative results of HCVcAg with low viremia (< 2000 IU/ml). PCR testing for HCV viremia is still the gold-standard tool for confirming or ruling out HCV-infectivity or persistence.

Abbreviations:

HCV: Hepatitis C Virus

DAAs: Direct Acting Antivirals

HCVcAg: Hepatitis C core antigen

SVR12: Sustained virologic response 12 weeks after end of treatment

Funding: No funding resources.

Conflict of interest: the authors declare that there was no conflict of interest.

Ethical consideration

This study was carried out in conformity with the Declaration of Helsinki. An informed consent was provided by all participants, and, the study protocol was notarized by the Ethics Committee of the Faculty of Medicine, Menoufia University and NCCVH.

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