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ORIGINAL ARTICLE

Comparative Study between the Conventional Methods and A New Technique using Electromagnetic Waves in Diagnosis and Follow up of Treatment of Hepatitis C Virus Infections

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ABSTRACT

Background: *A simple, rapid and non- invasive electromagnetic sensor (C-FAST device) was investigated for diagnosis and follow up of HCV patients* ***Objectives****: To test the validity of the C-FAST device compared to the conventional methods used for detection of anti-HCV by ELISA and standard HCV PCR as well follow up newly diagnosed HCV patients receiving HCV treatment.* ***Methodology****: This study was carried out on 200 patients suffering from HCV infection and 100 control cases (270 were males, and 30 were females) with their ages ranged from 25-75 years old during the period from May 2014 to May 2015. The patients were diagnosed as HCV positive by clinical, laboratory and ultrasonographic methods. The included patients were selected from the Endoscopy Unit of Gastroenterology and Hepatology Unit in Kobri El Kobba Military Hospital from Outpatient Clinics. The subjects were divided into three groups: group 1 included 100 suspected HCV patients (Acute - chronic). Group 2 included 100 control subjects 50 healthy group and 50 cases positive for hepatitis B virus), group 3: included 100 HCV patients receiving HCV treatment (combination therapy with PEG-IFN and ribavirin).* ***Results****: When comparing PCR technique with, C-FAST device for diagnosis of new HCV cases and follow up of treatment, the results using receiver operator characteristic (ROC) curve; showed diagnosis of HCV by using C-FAST. Out of those diagnosed by PCR, the sensitivity findings in the different study phases were from 97.4% to 100% (98.7%) of patients with HCV had a positive C-FAST and the specificity from 95.6% to 97.6% (96.6%) of patients with HCV had a negative C-FAST. The efficacy of the C-FAST device was in the range from 96.3% to 98.2% along the study phases.* ***Conclusion****: It is a practical evidence that HCV nucleotides emit electromagnetic signals that can be used for its identification. As compared to PCR, C-FAST it is rapid, valid and non-invasive device.*

INTRODUCTION

HEPATITIS C virus (HCV) is a major worldwide public health problem due to its high prevalence and to the high risk of chronicity. It is one of the main causes of chronic liver disease worldwide[1]. HCV is a single-strand, positive sense RNA virus with a genome of approximately 10.000 nucleotides coding for 3000 amino acids [2]. The number of chronically infected persons worldwide is estimated to be about 170 million, but most cases are unaware of their infection, it ranges from 1.0% in Europe to 5.3% in Africa. At least 6 major HCV genotypes are identified, HCV genotype 4 infection is common in the Middle East and in Africa [3].

HCV infection is diagnosed serologically by detecting antibodies specific to the HCV (anti-HCV). But anti-HCV does not distinguish between an acute, chronic or resolved infection[4]. Detection and quantitation of HCV RNA by PCR nucleic acid amplification offers a measure of active viremia. Using PCR, it is possible to detect HCV viremia prior to immunological sero-conversion [5] and to detect changes in the viral load in antibody-positive chronic HCV infected patients undergoing therapy with interferon and ribavirin [6].

Now, it is generally accepted that all objects, whether living or nonliving, are continuously generating electromagnetic fields (EMFs) due to the thermal agitation of their particles that possess charges [7]. Searching in EMFs as alternative forms of cell-to-cell communication can be traced back to at least the second decade of the 20th century [7]. Interactions between EMFs and bio-systems have been thoroughly studied for over a century and a quantitative understanding of many

interaction mechanisms exists[8-9]. There is great evidence that biological processes can be induced or modulated by induction of light of characteristic frequencies [10].

The use of a nucleic acids sequence for a specific diagnostics application was developed in the early 1953 and is still growing widely. The highly specific affinity binding's reaction between virus and electromagnetic waves was used to detect the virus. This method has promoted the development of virus based sensor from the traditional method. In simple words each protein and biomolecule has its fingerprint electromagnetic characteristics that can be used for its identification[13]. Electromagnetic waves of electromagnetic radiation is a combination of electric field and magnetic fields that oscillate and propagate through space and carry energy from one place to another. This technology is very beneficial as they are odorless, noiseless, environmentally friendly, and easy to apply [15].

The C-FAST device is a biological sensor or detector in which the resonant electromagnetic energy of HCV RNA is recorded as a consensus frequency that is a molecule signature or HCV nucleic acid electromagnetic fingerprint [17]. This recorded signal is used for the detection of its identical EM match.

This study was designed to demonstrate the development and mode of action of the C-FAST device and extends to explore the proposed hypothesis of its relevance, accuracy and reliability in clinical practice.

METHODOLOGY

Subjects:

This study was conducted on 200 patients suffering from HCV infection and 100 control cases (270 were males, and 30 were females) with their ages ranged from 25-75 years old during the period from May 2014 to March 2015. The patients were diagnosed as HCV positive by clinical by laboratory and ultrasonographic methods. The included patients were selected from the Endoscopy Unit of Gastroenterology and Hepatology Unit in Kobri el Kobba Military Hospital from Out-patients Clinics.

The subjects were divided into three groups:

- Group 1:- Included 100 suspected HCV patients (Acute - chronic).
- Group 2 (control groups):- Included 100 control subjects that were divided into two subgroups:
  - Subgroup A (Healthy control group): Subjects confirmed to be negative for both hepatitis C virus and hepatitis B virus infection.
  - Subgroup B (Diseased control group): Subjects confirmed to be negative for hepatitis C virus and positive for hepatitis B virus infection.
- Group 3: - Serum samples were collected from 100 HCV patients, receiving treatment by different modalities according to the protocol of medical treatment in Armed Forces Hospitals (combination therapy with PEG-IFN and ribavirin).
  - The serum samples were collected before treatment, 12weeks from the start of treatment and 24weeks from the start of treatment for detection of HCV RNA by Real Time qRT-PCR.
  - Then patients were subjected to C- FAST for follow up treatment response. 12weeks from the start of treatment and 24weeks from the start of treatment.

***Sampling*:**

Serum samples were collected from all subjects of all groups (patients and control groups). 10 ml of venous blood were withdrawn from each patient in a sterile tubes under complete aseptic condition. Sera were separated and aliquoted into three tubes and stored at -20°C.

METHODS: Sera of all subjects were subjected to the following tests:

- HCV antibodies, by ELISA.
- HCV-RIBA for confirming diagnosis of ELISA positive HCV subjects.
- HCV-RNA quantitative real time PCR (qRT-PCR).
- C- Fast: New technology based on device electromagnetic waves for diagnosis of HCV infection.

Except subgroup B (Diseased control group) were subjected more to HBsAg detected by ELISA for detection of HBsAg in addition to HCV antibodies.

RESULTS

Detection of HCV by RT-PCR versus C-FAST Device:

For measuring how accurate is the C-FAST device in predicting HCV, the comparison between PCR and C-FAST for detection of HCV viremia was conducted throughout the whole study. HCV RNA was detected for all subjects using a real-time polymerase chain reaction (PCR) assay (COBAS TaqMan; Roche Diagnostic Systems). The C-FAST device was utilized in all subjects to detect the HCV infected patients. The results are tabulated in table 1- table 7:

Table 1: Comparison between the results among group 1 as regard patients positivity rate of qRT-PCR in relation to C- FAST negative (no deviation) or low (ranges from 5° - 40°) or moderate (ranges from 40° - 50°)or high (ranges from 50° - 90°), with strong agreement of the results between both methods:

Results of using C- FAST in diagnosis among studied group1	Group 1	Results of qRT-PCR among studied group 1				Chi-square test	
		Negative (< 15 IU/ml)	Low (15 -200000 IU/ml)	Moderate (200000 - 1000000 IU/ml)	High (more than 1000000 IU/ml)	X^2	*P*
		No/%	No/%	No/%	No/%		
Negative(no deviation)	1	1 (100%)	0 (0%)	0 (0%)	0 (0%)	61.311	0.001
Low (Ranges of deviation from 5°- 40°)	14	1 (7.14%)	4 (28.57%)	2 (14.28%)	7 (50%)		
Moderate(Ranges of deviation from 40° -50°)	52	0 (0%)	6 (11.53%)	15 (28.84%)	31 (59.61%)		
High(Ranges of deviation from 50° - 90°)	33	0 (0%)	4 (12.12%)	6 (18.18%)	23 (69.69%)		
Total	100	2 (2%)	14 (14%)	23 (23%)	61 (61%)		

Table 2: Comparison between the results among group 3 as regard patients positivity rate of qRT-PCR (negative or low or moderate or high) in relation to C- FAST[negative or low or moderate or high]with strong agreement of the results between both methods.

C-FAST	PCR before treatment								Total	Chi-square test	
	Negative		Low		Moderate		High				
	No	%	No.	%	No.	%	No.	%		X^2	P-value
Negative	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0	135.533	0.000
Low	10	55.5%	3	16.6%	2	11.1%	3	16.6%	18		
Moderate	22	34.9%	2	3.2%	14	22.2%	25	39.6%	63		
High	3	15.9%	0	0.0%	5	26.3%	11	57.9%	19		

Table (3): Comparison between group 3as regard C- FAST [negative or low or moderate or high] in relation to q RT-PCR after 12 weeks of treatment:

C-FAST	PCR after 12 weeks								Total	Chi-square test	
	Negative		Low		Moderate		High				
	No	%	No.	%	No.	%	No.	%		X^2	P-value
Negative	58	100%	0	0%	0	0%	0	0%	58	100	0.000
Low	0	0%	36	100%	0	0%	0	0%	36		
Moderate	0	0%	0	0%	5	100%	0	0%	5		
High	0	0%	0	0%	0	0%	1	100%	1		

Table (4): Comparison between group 3 as regard C- FAST, in relation to qRT-PCR after 24 weeks of treatment:

C-FAST	PCR after 24 weeks								Total	Chi-square test	
	Negative		Low		Moderate		High				
	No	%	No.	%	No.	%	No.	%		X^2	P-value
Negative	83	100%	0	0%	0	0%	0	0	100%	100	0.000
Low	0	0%	15	100%	0	0%	0	0	0%		
Moderate	0	0%	0	0%	2	2%	0	0	0%		
High	0	0%	0	0%	0	0%	0	0	0%		

Table (5): Comparison between group (3) as regard C- FAST [negative or low or moderate or high] in relation to course of medical treatment (Before treatment, after 12 weeks and after 24 weeks):

Results of using C- FAST	Before treatment	after 12 weeks	after 24 weeks	Chi-square test	
				X^2	P-value
Negative	0	58	83	245.184	0.001
Low	18	36	15		
Moderate	63	5	2		
High	19	1	0		

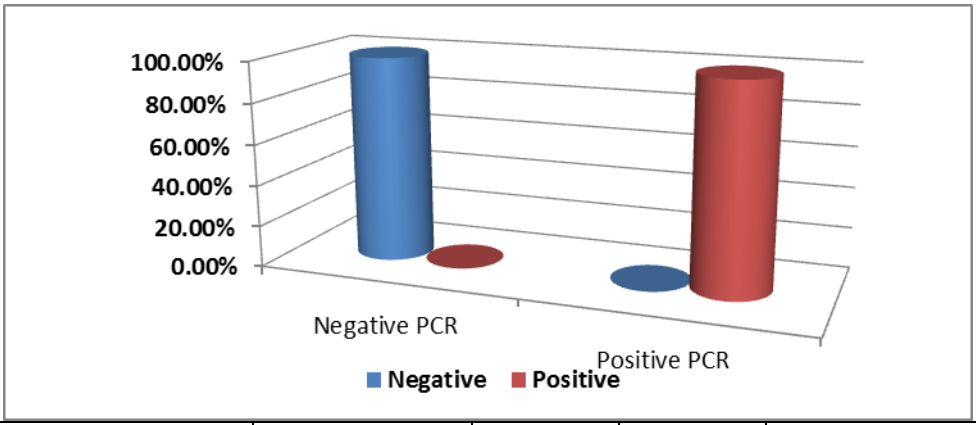
Table (6): Diagnostic accuracy of C fast at 12weeks after treatment using ROC (receiver operating characteristic curve) assessment of C- FAST: as shown in Figure (1)

C- FAST	Negative PCR		Positive PCR		Chi-square test	
	No.	%	No.	%	X^2	P-value
Negative	58	100.0%	0	0.0%	92.197	0.000
Positive	0	0.0%	49	100.0%		
Total	58	100.0%	0	0.0%		

Table (7): Diagnostic accuracy of C -FAST at 24weeks after treatment using ROC (receiver operating characteristic curve) assessment of C- FAST: as shown in Figure (2)

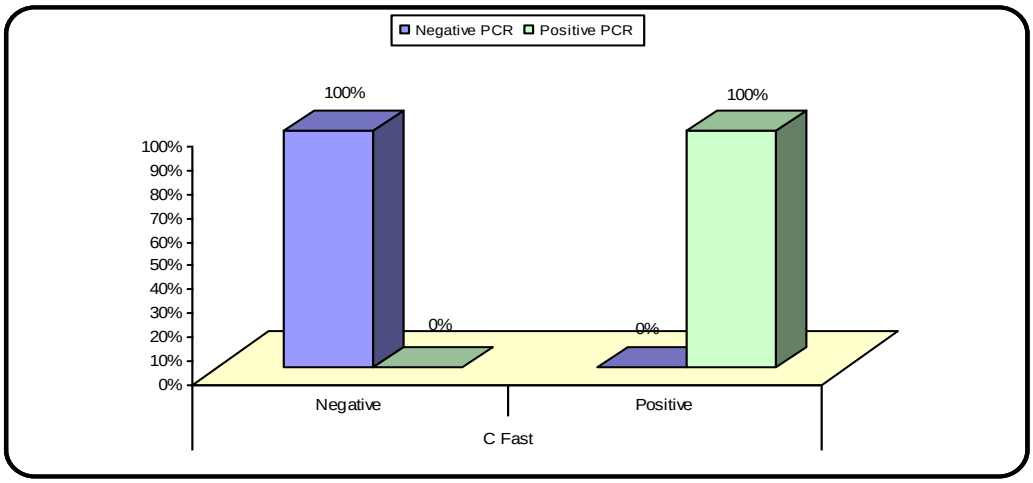
C -FAST	Negative PCR		Positive PCR		Chi-square test	
	No.	%	No.	%	X^2	P-value
Negative	83	100.00%	0	0.00%	100.000	0.000
Positive	0	0.00%	17	100.00%		

In the tables: We analysed these data using receiver operator characteristic (ROC) curve; shows diagnosis of HCV by using C-FAST out of those diagnosed by qRT-PCR. The sensitivity findings in the different study phases indicates that from 98% to 100% (99%) of patients with HCV will have a positive C-FAST and the specificity of the test indicates that 100% of patients with HCV will have a negative C-FAST. In this sense, these two statistics describe the proportion of patients in each disease category who are test positive and those who are test negative. The accuracy of the C-FAST device was in the range from 99% to 100% along the study phases. Calculations and interpretation of Sensitivity, Specificity, PPV, NPV, pre and posttest probabilities and odds, LR (+) were determined for comparison between PCR and C-FAST. (Figures 1, 2)



Sensitivity	Specificity	PPV	NPV	Accuracy
98.0%	100.0%	100.0%	98.03%	99.0%

Fig. 1: Diagnostic value of C -FAST in prediction of PCR results



Sensitivity	Specificity	PPV	NPV	Accuracy
100.0%	100.0%	100.0%	100.0%	100.0%

Fig. 2: Diagnostic value of C- FAST in prediction of PCR results.

DISCUSSION

HCV seropositive was higher among old age group (mean ± 53.09) while the HCV sero-negative was observed in younger ages (mean ± 34.44).The results indicated that the seropositivity increases with age. This agreed with a report which refered to significant difference between the studied groups[20].

In the present study the results of ELISA for diagnosis of patients in groups 1, 3 showed 100% positivity with high significant difference between the studied groups and negative in controls and this agreed with that found by Rain et al [19] who detected significant difference between the studied groups.

Using qRT-PCR technique for testing subjects with HCV infection group 1 and 3 cases under treatment proved that positive cases and negative in controls (2a, 2b) showed a significant difference between all subjects of the studied groups and this agreed with Pas et al. who stated that qRT-PCR is sensitive and specific for diagnosis of HCV infection and it is considered as a gold standard [21].

On comparison between ELISAand qRT-PCR for diagnosis of patients in groups (1), (3).In the present study two positive cases were diagnosed by qRT-PCR but not by ELISA which could be explained as false negative cases by ELISA.

In this study on examining the sera of patients group 1,3 one case was found to be negative by both C-FAST and QRT PCR and this can be explained by that the PCR load was < 15 IU/ml. another case was positive by c-fast while negative by PCR and this indicates that the c-fast can be used for detection of HCV infection early or occult HCV infections and this agreed with that reported by Gamal et al. [11] who stated that this may be due to the presence of HCV (RNA) in tissues as liver and lymph nodes with its absence in blood (no viremia). However the absence of viremia does not indicate the absence of the virus because replication occurs in the liver and extra hepatic tissues [12].

This study showed patients positive by c-fast at different deviation degrees low, moderate or high but their QRT PCR did reach 15 IU/ml (<15 IU/ml) negative and this means that the c-fast can be used for detection of HCv infection with or without viremia. So it is suggested that the c-fast can be used for early detection of HCV infection especially in acute cases (Group 1, 3 before treat) and in case of abscent viremia which agreed with Kuntzen et al. [12].

In the present study on evaluating the use of c-fast in follow up of treatment of group 3 c-fast did not show any negative case be showed (18) cases law (10) out of them are negative by QRT PCR (load < 15 IU/ml), (63) moderate positive c-fast, (22) out of them negative qRT-PCR (>15 IU/ml), and 19 high positive c-fast but 3 out of them negative <15 IU/ml by QRT PCR. That is to say that c-fast can be used to detect RNA of HCV either in case of viremia or its absence i.e. in tissues. Which agreed with that of Kuntzen et al. [12] who stated that the presence of the virus in extra. However after 12 week of the treatment table 8 (19) C-FAST demonstrated (58) cases to be negative all of them were negative by QRT PCR the cure rate of the treatment used was 58 detected by both tests and also C-FAST was low positive and load of QRT PCR was also positive low in (36) cases the same was found with the moderate and high QRT PCR positive cases were positive by c-fast. Hepatic tissues may be one of the important mechanisms for

viral break down during the treatment and relapse after the end of treatment for 24 weeks in the present study the number of cases appeared to be negative by c-fast as will as QRT PCR increased with a care rate of 83% and those still positive by both tests were decreasing 17% and showed moderate and high c-fast positive results as will as high load of QRT PCR and this means that the treatment is effective and rapid in acute, early and low load and degree of QRT PCR and c-fast respectively.

This study revealed that C-FAST is highly sensitive for detection of HCV showing sensitivity 99%, predictive value 100% and specificity of 100% and clearly demonstrates that C-FAST is a non-invasive device, no blood sample, chemicals, kits or sophisticated laboratory equipments are needed and consequently no coast per case. Moreover diagnosis of HCV using c-fast is very rapid. These advantages are expected to have a huge impact on the diagnosis and screening programs of HCV worldwide where only few millions out of 180 million. HCV patients are already diagnosed.

Taking into consideration the prospects of emerging new HCV therapies which are expected to be very potent, orally administered avoiding interferon therapy, the need for rapid and mass screening of HCV worldwide will be growing over the next few years [24].

The availability of a sample, rapid accurate and non-invasive method I or HCV diagnosis in the era of an oral effective regimen of HCV therapy could be a turning point in HCV history. Easy diagnosis and effective treatment will prolong life and prevent death from liver disease in millions of HCV patients. C-FAST is a non invasive device capable of identifying HCV infected patients instantly, making it ideal for diagnosis of HCV and mass screening program [23].

The attenuated results of this study showed that EMSD can be utilized to diagnose a specific disease using a simple device. The efficacy of C- FAST device in recording and replaying the molecule signature of HCV, the practical evidence that nucleotides in human body emit electromagnetic signals which are fully characterized and then these characteristics are used for its identification. When compared to the gold standard qRT-PCR technique, the C- FAST device has a remarkably high sensitivity and specificity. This high accuracy may solve one of the major problems in electromagnetic cellular interaction researched which is the lack of reproducibility[7], the idea of resonant absorption and resonant interactions has been proposed as an explanation for the marked sensitivity of living systems to EMFs as each biological process involves a number of interactions between proteins and their targets. These interactions are highly selective, and this selectivity is defined within the protein structure. Moreover it was shown that proteins and their targets have the same characteristic frequency in common [10]. Accordingly, the concept of recognition and interaction between a particular protein and its target at distance typically explains the very high accuracy of C- FAST device; and on the other hand the reproducibility of C-FAST in diagnosis of a very specific RNA HCV is a real life evidence if the concept of recognition and interaction between a particular nucleic acid and its targets. The concepts of bio electromagnetic communications is receiving increasing attention in the scientific community. This concept is challenging the old beliefs which are the consequences of ancient thinking, dating to Democritus, Epicurus, and Lucretius, is that all matter is composed of "imperishable" atoms, tiny indivisible particles that can be neither be created nor destroyed [18]. In accordance with the iron laws of "necessity" that were eventually replaced with Newton's Laws of Motions [13].

This study revealed that C- FAST is highly sensitive for detection of HCV showing sensitivity 99%, but this due to the low number of patients whom their qRT-PCR is negative compared to the patients whom their qRT-PCR is positive. predictive positive 100%, also this study shows specificity 100%

This study also clearly demonstrated that C- FAST is a non invasive device, no blood sample, chemicals, kits or sophisticated laboratory equipments are needed and consequently no cost per case. Moreover, diagnosis of HCV using C- FAST is very rapid. These advantages are expected to have a huge impact on the diagnosis and screening programs of HCV worldwide where only few millions of 180 million HCV patients are already diagnosed. Considering the prospects of emerging new HCV therapies which are expected to be very potent, necessialiatesthe need for rapid and mass screening of HCV worldwide will be growing over the next few years[24].

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