Comparative study for various HCV Diagnostic methods used in Basrah health institutions.

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Abstract : Hepatitis C virus (HCV) is one of the major cause of chronic liver diseases. Various screening tests of HCV was developed since the virus was first identified in 1989. Detection of HCV infection during the window phase of infection, before seroconversion, is important in blood screening. However, there is a significant delay in the time between infection and the development of antibodies. The aim of the present study was to evaluate three screening method are used for HCV detection and compare the sensitivities   of these method using PCR as gold standard .Of 160 collected blood specimens from suspected hepatitis C patients, serum separated and stored at -20º C. Then they were examined by the methods of immuno-chromatographic (ICA) rapid test, ELISA-3,Monolisa Ag-Ab test and PCR. Using ICA method, 153 cases were positive and 7 cases were found nega-tive. In ELISA-3 method,152 cases were found positive and 8 were negative. In monolisa Ag-Ab method, 159 cases were found positive and 1 case was found negative. While, using PCR method, 101 cases were real positive and 58 cases were negative. Considering real time PCR for HCV RNA as the gold standard for HCV infection determination in this patient population, ELISA-3, ICA , Monolisa Ag-Ab assay yielded a sensitivity of 99%, 99% ,100% respectively and low specificity , positive predictive value of 65.8% ,65.5% ,63.5% respectively and negative predictive value of 87.5%, 85.7% , 100% respectively . Comparing three diagnostic methods, Monolisa combined Ag-Ab test proved to be more sensitive, therefore can be applied for early de-tection of HCV infection during window period.

Keyword: Hepatitis c virus , Basraha , ELISA, poly merease chain reaction

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1 INTRODUCTION

Hepatitis C virus (HCV) represent a major health problem ,and considered the leading cause of chronic liver disease worldwide, giving rise in a significant number of infected in-dividuals to long-term complications such as cirrhosis and hepatocellular carcinoma [Spits et al 1998] . HCV is a global epidemic, and according to the World Health Organization (WHO), about 170-200 million people are infected with HCV and the virus is distributed worldwide [Brown & Gaglio. 2003] with a prevalence varying in different countries. Hepatitis C virus is one of the most common blood-borne pathogens transmitted from patients to health care worker , recipients of(blood, blood products, or organs) and injecting drug users . Patients on long-term hemodialysis also have a higher rate of HCV infection, and it is also transmitted by tattooing, body piercing, and other forms of skin penetration [Svetlana. 2013].
 The hepatitis C virus (HCV) is a member of the *hepaci –viruses*

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genus that belong to the *Flaviviridae* family of viruses [Rosen, 2011] .

HCV is a small, spherical, enveloped RNA virus that encloses its long, single-stranded, positive sense genomic RNA with an icosahedral capsid. The envelope contains two structural pro-teins, E1 and E2, and nonstructural proteins in-clude NS2, NS3, NS4A, NS4B, NS5A, and NS5B.[Jawetz, et al.2010]. The acute hepatitis C is frequently asymptomatic, so its diagnosis is still difficult and the presence of HCV-RNA in serum or liver is the first biochemical evidence of exposure to this virus[Farci , et al.1991], so it need an accurate and sensi-tive diagnosis in the early stages of HCV infection. Accurate testing for hepatitis C virus has both clinical and epidemio-logic importance. Antibody testing has been used to screen blood and organ donors. More recently, HCV RNA detection by the polymerase chain reaction (PCR) has been utilized to diagnose chronic hepatitis C and to monitor patients who are undergoing therapy with systemic interferon [Houghton et al .1991]. Laboratory assays that are available for the diagno-sis and management of HCV infection include: (a) serological tests to detect HCV antibodies (enzyme linked immuno-sorbent assay (ELISA) and recombinant immunoblot assay (RIBA), (b) molecular tests to detect and quantitate HCV-RNA and (c) genotyping [Tashkandy, and Khodari. 2007].
Current serological assays for detecting anti-HCV antibody cannot distinguish between patients with active infection and those who have cleared the virus. This is due to the fact that those who resolve the infection remain anti-HCV positive for

many years [S.Keating. 2005]. false positive and false negative are also frequent with these tests, and due to the absence of an effective culture system for HCV or assays capable of detecting viral antigens, direct detection of HCV has depended on nucleic acid amplification technology (NAT) techniques [Fabriz, and Lunghi. 2005]. Several types of HCV diagnostic methods are being used in the health institutions and laboratories. There are high rates of variations in the results and different types of interpretations can be forwarded, so utilization of a diagnostic test proved to be valid in comparison to other methods will ensure a high confidence and reliability in investigational methods and help both physicians and patients in avoiding unsecure test results. Therefore the present study aimed to evaluate various HCV diagnostic methods and find out the best test for better diagnosis.

2   Material and Methods

2.1 Study population

A total number of 160 plasma samples were included in this study were collected from suspected Patients with chronic hepatitis C. these patients attended public health laboratory and molecular Biology laboratory in Basrah during the period from September 2014 until May 2015 .These patients included 66 males and 94 females with ages ranged between (2-75) year. A questionnaire was designed in a special form by the investigators to include information concerning demographic characters .

2.2 Blood sample collection and preparation:

Blood samples were collected in EDTA tubes , the plasma separated after centrifuging at 1600 rpm for 15min. All samples were separated into two portions; one portion was for PCR and the other was for serological assay. All of them were kept at deep freezing (-°35C or -20°C) with a code number until the work time.

2.3   Laboratory tests : all blood specimens were screened for Anti-HCV by using three screening method : Immunochromatographic Anti-HCV rapid test(Plasmatec, UK); Anti- HCV antibody third generation ELISA(Foresight , REF:1231-1031, Acon laboratories. USA) ; and combo test for Antigen-Antibody detection(Monolisa HCV (Ag-Ab) Ultra, Bio Rad, REF:72562, France) according to the manufacturer's instructions. All samples for anti-HCV were tested for the presence of HCV-RNA at the molecular Biology laboratory in General

Basrah Hospital using an ABI real time PCR system 7500 (Applied bio system ,4345241 ,USA).

3   Results

Table 1 shows that a Total of 160 samples tested by ULTRA monolisa , 159 were positive and 1 was negative. These samples were tested by PCR for confirmation, 101(63.1%) were PCR positive and 58(36.9%) were PCR negative.

Table 1: Comparison between monolisa Ag-Ab ULTRA and PCR for anti-HCV positive

monolisa HCV Ag-Ab ULTRA	PCR		Total
	Positive No.	Negative No.	
Positive	**101(63.5%)**	**58(36.5%)**	**159**
Negative	0	1(100%)	1
Total	101(63.1)	59(36.9%)	160(100)

Table 2 shows that a total of 160 samples that tested by ordinary ELISA,152 were positive and 8 samples were negative . These samples when tested by PCR ,100(65.8 %) were positive and 52(34.2 %) samples were negative.

Table 2 : Comparison between ordinary ELISA and PCR for anti-HCV positive patients**.**

Ordinary ELISA	PCR		Total
	Positive n (%)	Negative n (%)	
Positive 152	100 (65.8)	52 (34.2)	152
Negative 8	1(12.5)	7(87.5)	8
Total	101(63.1%)	59(36.9%)	160

Table 3 shows that a Total of 160 positive cases tested by immunochro-matographic ICA rapid test , 153 were positive and 7 were negative. These samples were tested by PCR for confirmation,100 were PCR positive and 52 were PCR negative.

Table 3:comparative results between immunochromatographic (ICA) anti-HCV rapid test and PCR test for anti-HCV positive patients

ICA Test	PCR		Total
	Positive no (%)	Negative no (%)	
Positive	100(65.3%)	53(34.6%)	153
Negative	1(14.2%)	6(85.7%)	7
Total	101(63.1%)	59(36.9 %)	160

According to PCR results as assumed as gold standard method with sensitivity and specificity of 100%, detection rate sensitivity of EIA-3 , ICA rapid test and Monolisa HCV Ag-Ab test were 99%,99%,100% respectively and their specificities were 11.9%, 10.2%, 1.7% . Also Positive predictive value was 65.8% ,65.5% and 63.5% respectively and their negative predictive value were 87.5%, 85.5% .100% respectively. Results are summarized in table number 4.

Table 4. Summarized results of PCR and EIA-3, ICA rapid test, monolisa HCV Ag- Ab test.

Parameter	PCR	ELISA-3	ICA test	Monolisa Ag-Ab test
Sensitivity	100%	99 %	99%	100%
Specificity	100%	11.9%	10.2%	1.7%
PPV	100%	65.8%	65.5%	63.5%
NPV	100%	87.5%	85.5%	100%
Accuracy	100%	66.8%	66.2%	63.7%
False positive rate	0	34.2%	34.6%	37.1%
False negative rate	0	12.5 %	14.2%	0

4 Discussion

Hepatitis C is among the major blood transfusion transmissible diseases. In blood banking, a false negative result may lead to transfusion of infected blood to an uninfected individual , so a very sensitive method is required to detect the most hidden blood infection. A wide array of advanced immunological, molecular and histological assays are available for confirmation of diagnosis in HCV infection, assessment of prognosis and aid in clinical and therapeutic decisions making. The immunological assays include: ELISA, immunochromatography rapid test, RIBA while the molecular assays include qualitative, and quantitative PCR, whereas, invasive assessment includes examination of liver biopsy [khan et al. 2010].

Our data comparing EIA with RNA PCR suggest that the sensitivity of the third-generation EIA may be as high as 99 % for detecting active HCV infection in infected patients. However, it was apparent that a negative EIA result did not rule out HCV infection in high risk patients, since one of EIA-negative clinical specimens was positive by the RNA PCR assay. False-negative antibody results may occur in acute disease prior to seroconversion and in patients with a suppressed immune system. These result showed high frequency of false positive (34.2%) with ELISA test when compared with PCR, and this agrees with a study carried out in Brazil by Martins et al in 1994 which reported that the false positivity rate with ELISA as compared with PCR in blood donors was 23.4 % [Martins.1994] , and this results disagrees with a study conducted in Egypt 2011, by Swellam , and Mahmoud reported that sensitivity and the specificity of ELISA-3 were 78.9% and 100%, respectively [Swellam, , and Mahmoud . 2011].The false positivity rate may due to HCV past or resolved infection or patients may suffer from hepatitis C at the chronic phase of infection, existing antibody without any viruses or cross reactivity with non-specific antibodies detected by ELISA screening test [Tashkandy .2007]. The results of the present study is comparable with those of Kim et al ,in 2006 in Korea , kim and Lee , Kaya et al in Turkey in 2007, they found that the positive predictive value of anti - HCV ELISA test among anti- HCV positive patients was 63. 6 % ,57.1% ,50 % respectively [kim et al .2006, kim &Lee.2006, Kaya, et al.2007]. In contrast our result differ from an Iranian study in 2006 carried out by Ansari & Omrani in 2006 , They found that the sensitivity ,specificity ,PPV, NPV were 41.6%, 63.1%, 26.31%, 77.4% respectively, and this study found that the false positive rate was 36.8% which is comparable with our result but the false negative rate was 58.2% which is higher than our result which was 12.5 %[Khadem Ansari and Omrani .2006]. Taking into consideration the above results it can be suggested that EIA method can be considered as a suitable method for public screening, and PCR method has been a specific method with high specificity and sensitivity for diagnosis of hepatitis. Currently, there is no studies published on the combined detection of HCV antigen and antibodies in Iraq population. Therefore we assessed a combined HCV Ag/Ab assay by ELISA in comparison to PCR and anti HCV antibodies detection test for early detection of HCV infection. In present study the monolisa Ag-Ab ULTRA test, giving sensitivity of 100%, while the test shows low specificity and accuracy of 63.7% .The positive predictive value

(PPV) was(63.5%) and negative predictive value (NPP) was 100%. These result indicate that this test is sensitive and can reduce incubation (window period). especially regarding to cost, complex requirement, emergency, and logistic difficulties. Our result agrees with El- Emshaty et al , Schnuriger et al , in 2005, Laperche et al, they found that the new combined test allowed, in most cases, the detection of hepatitis C infection earlier than that by conventional serological assays. [El-Emshaty .2011, Schnuriger et al. 2005 , laperche et al.2005], and disagrees with a study carried out in Germany in 2014 by Odari et al, emphasized that despite of high sensitivity of Monolisa HCV Ag-Ab ULTRA assay , the protein detection is more of antibody- than antigen-based. and they concluded that this assay cannot be used to replace Nucleic Acid based Techniques (NAT) or core antigen-only-based assays in routine blood transfusion, and cannot be used alone for HCV diagnosis in immunocompromised patients[Odari et al.2014].

The anti- HCV rapid test evaluated in this study was found to have comparable performance characteristics to the ELISA test when compared to PCR as confirmatory test. Immunochromatographic assay (ICA) for anti-HCV Abs testing showed 99% sensitivity,10.2% specificity and 66.2% accuracy . The ability of this rapid test for detecting and predicting positive sample (tested and proved by PCR) was(65.5 %) whereas its ability in predicting PCR negative samples was (85.7 %). In this study, we found a good correlation between the rapid assay and the ELISA HCV-Ab assays. Statistically the results of two tests(ICA and ELISA) were comparable in terms of sensitivities ,specificities , PPV, and NPV. In the present study false negative results were less than false positive results. Increased rate of false positive cases may be due to unknown viral infections. In addition, in our country quality and storage of immunochromatgraphic device may be questionable. Our results agrees with Lee et al in 2011 , Smith et al, in 2011. AL-Tahish et al , they found that HCV Rapid antibody test showed clinical performance that was equivalent to current ELISA and giving sensitivity ranging between (93-98). This agrees with present study , where the two tests (ELISA& ICA rapid test) giving same sensitivity 99% , they concluded that HCV rapid test is a suitable test in the diagnosis of HCV infection and its simplicity and flexibility to use multiple specimen types, may increase testing opportunities[Lee et al .2011 , Bryce et al .2011, Al-Tahish & El-Barrawy .2013].

5 - Conclusion

This study emphasized that the monolisa HCV Ag-ab Ultra had a 100% sensitivity , simple and require no complex instrumentation , no long incubation period or considerable skills, and less cost than PCR ,therefore can be applied in blood banks for early detection of HCV infection during window period , and can applied easily in the field work, and it could be used as alternative when PCR cannot be available .

Acknowledgment

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