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Comparative study between some different serological methods for detection of *Hepatitis "C" virus* antibodies in Taif Governorate

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Abstract
Objective: detection of hepatitis C virus antibodies in serum samples can be done by different methods. However, choosing between them is still a confusing matter. So, this study aimed to make a comparison between some serological methods for detection of *hepatitis "C" virus* antibodies in Taif Governorate to identify the most sensitive, specific and accurate technique to facilitate the correct diagnostic process for HCV.
Methods: a total number of 200 blood samples were collected in this study during the period from June 2017 to December 2017 in Taif Governorate for detection of anti-HCV by ELISA, CLIA and RIBA techniques.
Results: the results declared that CLIA was better than ELISA for the detection of anti-HCV with high sensitivity (98.59%), specificity (89.65%), accuracy (96%) and precision (96%). The RIBA has been used in this study as a confirmatory technique for detection of antibodies to HCV in blood samples.
Conclusion: the results of this study indicate that CLIA was more sensitive, specific and accurate than ELISA for identifying anti-HCV and confirmed by RIBA.

Keywords: HCV, ELISA, CLIA, RIBA

1. Introduction
Hepatitis C is an infection of the liver, results from hepatitis C virus (HCV) [1], and it represents a significant public health problem worldwide [2]. It has been discovered for the first time in 1989 [3]. HCV is an enveloped virus and related to the *Hepacivirus* genus of the family *Flaviviridae* [4]. It is roughly spheroid and is 55 nm in diameter [5]. Its genome is represented by a single-stranded positive-sense RNA [6].
There are two types of Hepatitis C infection, acute and chronic. Acute Hepatitis C refers to the first several months of Hepatitis C infection. Few cases of patients are able to clear or get rid of the virus without treatment in the first 6 months for reasons that are not known. On the other hand, most HCV infected people cannot clear the HCV and subsequently develop a chronic infection [7], that can cause life threatening problems and it is the main cause of progressive liver fibrosis, liver cirrhosis and hepatocellular carcinoma [8].
Hepatitis C is found worldwide, and the most affected regions and highest HCV prevalence are Africa, Central and East Asia and Latin America [9], while the lower prevalence was reported in industrialized countries in North America, Western Europe and Australia [3]. According to the WHO, Saudi Arabia has 437,292 cases of HCV infections that are officially reported among persons living in Kingdom of Saudi Arabia, with the estimated prevalence of approximately 1.8% [10]. Jeddah governorate had the majority of HCV positive cases (35.8%), then Riyadh city (20.5%) and then Eastern region (14.5%), and it is predominant in older ages (>45 years) with 59% of viral hepatitis cases [11].
There are approximately 170 million people having HCV infection [12]. 70% of patients will develop chronic hepatitis over a period of 20-30 year and about 20-30% of them will develop liver cirrhosis and 1-5% will develop hepatocellular carcinoma [13]. Around 700,000 people die annually from the chronic complications of Hepatitis C [14].
HCV infection causes a wide range of clinical manifestations, ranging from a healthy carrier state to acute and chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma [15]. More than 70% of patients with Hepatitis C are asymptomatic and they do not know they are infected [16]. Few cases may show mild or severe signs like fever, fatigue, anorexia, dark urine, grey-colored stool, nausea or vomiting, stomach pain, joint or muscle pain and jaundice [7, 17].
Laboratory assays play an important role in the early diagnosis of HCV infection [18]. According to WHO laboratory diagnosis of HCV infection include screening tests which can identify antibody-positive specimens and confirmatory tests which confirm that reactive samples contain antibodies specific to HCV [19].
About 15–45% of HCV infected people can spontaneously clear the infection without any treatment due to strong immune response. The main therapy for hepatitis C was interferon and ribavirin during the last few years. Recently, new drugs have been produced and called direct antiviral

agents (DAA), that can cure about 90% of persons with hepatitis C infection within shorter duration (usually 12 weeks) beside it is safer and more cheaper. Until now there is no vaccine for hepatitis C virus [9], the development of a prophylactic vaccine for HCV is a difficult challenge due to high genetic diversity of HCV [20]. The Prevention of HCV infection depends mainly upon reducing the risk of exposure to the HCV [9], and never sharing personal items with infected persons [7]. For healthy people, it is recommended to make screening for high risk people [9].

2 Materials and Methods

2.1 Subjects and sample collection

The study included a total of 200 blood samples from patients attending the internal Medicine and Gastroenterology Clinics of King Abdul-Aziz Specialist Hospital (known to have chronic liver disease). During the period from June 2017 to December 2017. Venous blood samples (7 mL) were obtained from all participants. Samples were allowed to clot and sera were then separated by centrifugation (3000 rpm, 15 min) and then stored in aliquots at −20 °C until used for analysis of the various parameters outlined below.

2.2 Detection of HCV antibodies by ELISA

Presence of Anti-HCV antibodies was assessed using by 3rd generation ELISA (Monolisa™ anti-HCV, Bio-Rad, France) The samples were considered positive for anti-HCV antibodies when the index values (S/CO) were ≥ 1, and negative when values were < 1.

2.3 Detection of HCV antibodies by CLIA

Chemi luminescence Immunoassay was used for detection of anti-HCV using ARCHITECT anti-HCV assay (Architect I 2000 machine, Abbott, Germany).The samples were considered reactive for anti-HCV antibodies when the index values (S/CO) were ≥ 1, and non-reactive when values were < 1.

2.4 Recombinant immunoblot assay

The results of the HCV antibodies for ELISA and CLIA tests were confirmed by the recombinant immunoblot assay (RIBA), using INNO-LIA™HCV Score supplied by (Fujirebio Europe N.V., Belgium). The INNO-LIA HCV Score is a 3rd generation line immunoassay which incorporates HCV antigens derived from the core region, the E2 hyper variable region (HVR), the NS3 helicase region, the NS4A, NS4B, and NS5A regions. Band reactivity is graded by visual calibration against IgG control bands present on each strip. The intensity of the colored bands is proportional to the amount of bound antibody and is graded as - (none), ± and 1+ to 3. A sample was considered positive when at least two HCV antigen lines have a reactivity of ± minimum or higher. Indeterminate when either a NS3 line reacts with a reactivity of ± or higher and all other antigen lines are negative, or If one HCV antigen line has a reactivity rating of 1+ or higher. And a result was considered negative either when all HCV antigen lines have a negative reactivity rating or when only one HCV antigen line has a reactivity of ±, except when the reactivity is observed for NS3.

2.5 Statistical analyses

Statistical analysis was carried out with statistical package for social science (SPSS) software version 22 for Windows and a p-value < 0.05 was considered as significantly different, and the tests used were: Sensitivity, Specificity, total accuracy, Likelihood ratio, and Spearman's correlation.

3 Results

3.1 Descriptive characteristics of study samples

All 200 study samples were assessed for the presence of anti-HCV antibodies by CLIA, ELISA, and confirmed by RIBA.

3.2 Comparison of ELISA and RIBA results

Out of the total 200 samples, 144 were anti-HCV antibodies positive and 56 were negative by ELISA. When RIBA testing was performed out of the 144 anti-HCV antibodies positive by ELISA, 133 were positive and 11 were negative. While within the 56 negative cases by ELISA, 47 were negative and 9 were positive by RIBA (Table 1).

3.3 Comparison of CLIA and RIBA results

Out of the total 200 samples, 146 were anti-HCV antibodies positive and 54 were negative by CLIA. When RIBA testing was performed out of the 146 anti-HCV antibodies positive by CLIA, 140 were positive and 6 were negative. While within the 54 negative cases by CLIA, 52 were negative and 2 were positive by RIBA (Table 2).

Table 1: Comparison of ELISA and RIBA results

		RIBA		Total
		Negative	Positive	
ELISA	Negative Count % within RIBA	47 81.0%	9 6.3%	56 28.0%
	Positive Count % within RIBA	11 19.0%	133 93.7%	144 72.0%
Total Count % within RIBA		58 100.0%	142 100.0%	200 100.0%

Table 2: Comparison of CLIA and RIBA results

		RIBA		Total
		Negative	Positive	
CLIA	Negative Count% within RIBA	52 89.7%	2 1.4%	54 27.0%
	Positive Count% within RIBA	6 10.3%	140 98.6%	146 73.0%
Total Count % within RIBA		58 100.0%	142 100.0%	200 100.0%

3.4 Comparison between sensitivity, specificity, and total accuracy of both ELISA and CLIA

The sensitivity, specificity and total accuracy of ELISA were 93.6%, 81.03% and 90% respectively, while that of CLIA were 98.59%, 89.65% and 96% respectively.

3.5 Measurement of the precision of both ELISA and CLIA

The precision of ELISA was 94% while CLIA was 96%.

3.6 Interpretation of Likelihood ratio between ELISA and CLIA

+likelihood ratio (+LR) analysis in ELISA (4.93) is small /sometimes useful test while CLIA (9.52) is moderate /often useful test correctly diagnosing the presence of a condition (disease). Analysis of -Likelihood ratio (-LR) showed that both ELISA (0.07) and CLIA (0.015) are Large/very useful test in correctly diagnosing the absence of a condition (disease).

3.7 Spearman's correlation

The analysis of spearman's correlation revealed a strong positive correlation between ELISA and CLIA (Table 3), between RIBA and ELISA (Table 4) and also between RIBA and CLIA (Table 5).

Table 3: The spearman's correlation between ELISA and CLIA

			CLIA	ELISA
Spearman's rho	CLIA	Correlation Coefficient	1.000	.699**
		Sig. (2-tailed)	.	.000
		N	200	200
	ELISA	Correlation Coefficient	.699**	1.000
		Sig. (2-tailed)	.000	.
		N	200	200

**. Correlation is significant at the 0.01 level (2-tailed).

Table 4: The spearman's correlation between RIBA and ELISA

			RIBA	ELISA
Spearman's rho	RIBA	Correlation Coefficient	1.000	.755**
		Sig. (2-tailed)	.	.000
		N	200	200
	ELISA	Correlation Coefficient	.755**	1.000
		Sig. (2-tailed)	.000	.
		N	200	200

**. Correlation is significant at the 0.01 level (2-tailed).

Table 5: The spearman's correlation between RIBA and CLIA

			RIBA	CLIA
Spearman's rho	RIBA	Correlation Coefficient	1.000	.902**
		Sig. (2-tailed)	.	.000
		N	200	200
	CLIA	Correlation Coefficient	.902**	1.000
		Sig. (2-tailed)	.000	.
		N	200	200

**. Correlation is significant at the 0.01 level (2-tailed).

4. Discussion

Hepatitis C virus infection is a major public health problem and a leading cause of chronic liver disease all over the world **[21].** So, early detection of HCV infection is the key for fast intervention to treat infected persons and avoid chronic complications.

This study employs both screening test (ELISA and CLIA) and confirmatory test (RIBA) as recommended by **[19]** for detection of HCV antibody which consider an indicative marker for HCV infection as mentioned by **[22].**

This study confirmed that ELISA was simple, economic and rapid technique in the detection of HCV antibodies and this agree with **[23]** and **[13]**, while CLIA was done for detection of HCV antibodies because it has easy steps and also more economic than molecular techniques, this is also compatible with **[24]** and **[25]** who added that CLIA can detect HCV infection between 40-50 days earlier than the third generation anti-HCV screening assays.

Out of the total 200 samples, 144 were anti-HCV antibodies positive and 56 were negative by ELISA. When RIBA testing was performed out of the 144 anti-HCV antibodies positive by ELISA, 133 were positive and 11 were negative. While within the 56 negative cases by ELISA, 47 were negative and 9 were positive by RIBA.Out of the total 200 samples, 146 were anti-HCV antibodies positive and 54 were negative by CLIA. When RIBA testing was performed out of the 146 anti-HCV antibodies positive by CLIA, 140 were positive and 6 were negative. While within the 54 negative cases by CLIA, 52 were negative and 2 were positive by RIBA. Comparing the results of ELISA and CLIA with that of RIBA in the study confirmed the same conclusion of [26] and [13] who concluded that RIBA is not only more sensitive than ELISA and CLIA in the detection of HCV antibodies, but also very important for the detection of false positive cases.

The use of RIBA in this study as a confirmatory technique for detection of antibodies to HCV in blood samples is agreeing with [27], [1], [13] and [9] who recommended the use of Line/strip immunoassays since it is not only provide greater specificity, but also can differentiate between various antigens reacting to antibodies in serum samples. Unfortunately, it was disagreeing with [28] who recommend nucleic acid tests as a confirmatory test for HCV infection.

The sensitivity, specificity, accuracy and precision of ELISA in this study were 93.6%, 81.03%, 90% and 94% respectively, while that of CLIA were 98.59%, 89.65%, 96% and 96% respectively. These results indicate that CLIA is not only highly sensitive than ELISA in detecting positive cases, but also more specific than ELISA in detecting negative cases. These results agreed with [29] who found that the sensitivity of CLIA was about 100% and the specificity was more than 96%. It is also agreeing with [30] and [31] who stated that CLIA has the advantages of high sensitivity and specificity of immunoreaction. Moreover, the accuracy and precision of CLIA in this study was also higher than that of ELISA.

5. Conclusion and Recommendation

Hepatitis C virus still one of the main causes triggering chronic hepatitis in different parts of the world. A considerable number of persons in Saudi Arabia still suffering from HCV and its complications. HCV infection is commonly asymptomatic at the beginning, so laboratory assays play an important role in the early diagnosis. The results of this study indicate that CLIA was more sensitive, specific and accurate than ELISA for identifying anti- HCV and confirmed by RIBA.

More effort should be exerted to set up strategic plans to control the spread of HCV. Further studies in this area of research must be fulfilled in multicenter all over the Kingdom of Saudi Arabia to approve this result in future on wide scale patients.

6. Abbreviations

HCV: Hepatitis C virus; RIBA: Recombinant immunoblot assay; CLIA: Chemi luminescence Immunoassay; WHO: The world health organization; DAA: direct antiviral agents; S/C: Sample\cut off; HVR: hyper variable region ; SPSS

:statistical package for social science; NS: nonstructural region; LR: Likelihood.

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Competing interests
The authors declare that they have no competing interests.

Authorship and Contributorship
All authors play a main role in study design, in the collection, analysis and interpretation of data.
All authors in read and approved the final manuscript.

Patients consent
Oral approval was taken from participants in the study. As no personal data was used for the participants.

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