ORIGINAL ARTICLE

IMMUNOCHROMATOGRAPHY AND CHEMILUMINESCENCE FOR DETECTION OF ANTI HCV ANTIBODIES IN DIAGNOSIS OF HEPATITIS C INFECTION AMONG HEALTHY BLOOD DONORS

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ABSTRACT

Objective: To compare Immunochromatography with chemiluminescence for the diagnosis of Hepatitis C infection.

Material and Methods: The study was carried out at Rehman Medical Institute(RMI) laboratory, Peshawar, Pakistan, from healthy blood donors from May 2017 to July 2017. After taking history and excluding already known patients of any chronic illness and high risk people such as sex workers and I/V drug abusers, 257 samples were collected for the study. The samples were then tested on both SD bioline HCV ICT kit and Architect for detection of Anti HCV antibodies. The result were analyzed on using SPSS version 15.0.

Results: Out of 257 donors 97.7% were males and 2.3% were females. Architect machine showed that 5 (1.9%) donors were positive for HCV. Whereas HCV ICT showed reactivity of 2 (0.8%) people, it missed three cases which is positive in Architect. And the sensitivity came out to be 62.5%.

Conclusion: The sensitivity of the best available SD ICT kit in Peshawar used by most of the laboratories is very less according to our study.

Key words: HCV glycoprotein E1, Enzyme linked immunosorbent assay, Immunochromatography,Chemiluminescence, Transfusion transmitted infections.

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INTRODUCTION

Hepatitis C virus is a single stranded RNA virus which is enveloped and is from Flaviviridae family. Genome of the virus is about 9.6kb and has a single open reading frame responsible for synthesis of three structural and seven nonstructural proteins[1].This viral genome encodes a protein which has about three thousand aminoacids which is further broken down into ten single proteins. The proteins that are classified as structural proteins consist of the core protein and the enveolope glycoprotein E1 and E2. Other proteins which are nonstructural include NS2, NS3, NS4A, NS4B, NS5B and p7[2]. There are multiple subtypes into each of the seven genotypes of hepatitis C virus, it causes a variety of disorders ranging from hepatitis to hepatocellular carcinoma[3].The estimated prevalence of Hepatitis C throughout the world is thought to be 170-200 million people[4]. The people thought to be infected with Hepatitis C are about 17 million[5] 350,000 deaths annually are caused by this infection worldwide[6]. The whole genome of Hepatitis C was mapped in 2013 [7]. Europe and America has a greatest prevalence of genotype[1]. The most common genotype in India and Australia is genotype 4. Africa and Middle East have a high prevalence of genotype[3]. Hongkong, Vietnam and Australia showed a greater prevalence of genotype 6 and genotype 5 is more common in Africa[8,9] Most regions of China contain

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HCV genotype 1 and 2. Genotype 6 and genotype 3 are common in south western China[10,11]. A review in Pakistan on HCV genotyping showed that most common genotype was three alongwith high prevalence of type 1 in Punjab [12]. As already discussed 6 % of the total population in Pakistan is said to be affected by HCV infection and it carries a high rate of mortality and morbidity[13,14].

MATERIAL AND METHODS

It is a descriptive, cross sectional study, carried out at Rehman Medical Institute laboratory from May 2017 to July 2017. All the samples counting for blood donation after taking history were studied and those already having a history of HBV, HCV, HIV or any other serious illness were excluded from the study. After taking history and excluding samples based on exclusion criteria the samples were there pasted on both SD HCV ICT kit and architect. A total of 257 samples were included in the study and the sample size was calculated using the estimated prevalence of Hepatitis C infection in Pakistan. Statistical analysis was carried out using SPSS version 15.0 SD Bioline HCV: It is an immuno chromatographic single step test used for detection of HCV in serum or plasma. SD Bioline HCV is used in many laboratories as a diagnostic test whereas the literature of kit suggests that it should be used only as a screening test and positive samples should be confirmed by a superior method The principle of test is that the test device has a membrane strip coated with recombinant HCV capture antigen (core, NS3, NS4 and NS5) on test band region. The Protein A- colloid gold conjugate and serum sample moves along the membrane by chromatography to test region, and forms a visible line as the antigen-antibody protein A gold particle complex with high degree of sensitivity and specificity. Architect system HCV Qualitative II: is a chemiluminescent microparticle immunoassay (CMIA), intended to detect anti HCV in human serum /plasma. As it a qualitative test it can be used for screening of HCV infection in apparently healthy donors. It is a one-step immunoassay using CMIA immunotechnology using flexible protocols also known as chemiflex. In architect HCV Qualitative II assay, the anti-HCV coated paramagnetic micro particles and anti-HCV acridinium labelled conjugate are combined to create a reaction mixture with the sample. If HCV is present in the sample it binds to anti-HCV coated microparticles and to anti-HCV acridinium labelled conjugates. After washing ancillary washed buffer is added to the reaction mixture following another wash cycle. Pre trigger and trigger solutions are added to the reaction mixture and reactive chemiluminescent reaction is measured as relative light units (RLUs) The presence or absence of HCV can be found out by comparing the chemi luminescent signal in the reaction to the cut off signal obtained by active calibration.

RESULTS

Frequency of positive patients according to Architect is shown in Table 1. The frequency of positive patients according to ICT is shown in Table 2. Correlation of the tests are shown in Table 3. Two tailed correlation and p value of the two tests are shown in Table 4.

Sensitivity of ICT = 62.5% Sensitivity of Chemiluminescence on Architect =100%

Table 1: HCV Architect

		Frequency	Percent
Valid	Reactive	5	1.9
	Non reactive	252	98.1
	Total	257	100.0

Table 2: ICT HCV

		Frequency	Percent
Valid	Reactive	2	.8
	Non reactive	255	99.2
	Total	257	100.0

Table 3: Chi-Square Teste

	Value	df	Asymp. Sig.(2-sided)	Exact Sig.(2-sided)	Exact Sig.(1-sided)
Pearson Chi-Square	101.591(b)	1	.000	.000	.000
Continuity Correction(a)	56.391	1	.000		
Likelihood Ratio	16.678	1	.000		
Fisher's Exact Test					
Linear-by-Linear Association	101.195	1	.000		
N of Valid Cases	257				

Table 4: Correlations

		ICT HCV	HCV Architect
ICT HCV	Pearson Correlation	1	.629(**)
	Sig. (2-tailed)		.000
	N	257	257
HCV Architect	Pearson Correlation	.629(**)	1
	Sig. (2-tailed)	.000	
	N	257	257

DISCUSSION

A total of 257 subjects were included in the study out of them five subjects that is 1.9% were positive for HCV or Architect and two subjects that is 0.8% were reactive with the SD ICT kit when we compared that results of Architect with ICT p value was found to be 0.000, which is highly significant A study was carried out by Adeyami AA in Nigeria in 2013 in which he compared ICT strip test with ELISA for HCV. According to the study four subjects out of 660 that is 0.6% were positive with ELISA whereas none were positive for Anti HCV using ICT method[15]. Another study was carried out in Pakistan and it compared different ICT KITS with Gold standard fourth generation ELISA. Out of 100 cases that were ICT positive the sensitivity of Acon USA (ICT) was 93%, that of Membrane-Canada was 89% and Nobis-Germany was 86%[16]. A study carried out by CDC evlauated and compared three rapid screening test that is Chimbio, Orasure and Medmira for Anti quantitave antibody with well-established screening assays for anti HCV. The sensitivities came out to be 96.2%, 86.8% and 97.8% respectively[17,18]. A study in Brazil carried out by Pereira FM Compared Elecsys Anti HCV assay with the Architect Anti HCV and showed that Architect was more sensitive than Elecsys Anti HCV assay particularly for c 33 protein[19].

Another study carried out at UHS Lahore showed the comparison between ICT technique and EISA. This study showed that the ICT screening method had a sensitivity of 99% and specificity of 98% which was in contrast to our study[20]. A study carried out in Lahore showed HCV sensitivity by ICT to be 80.2% as compared to 100% ELISA which is comparable to our study[21]. Another study from Batool et at carried out in 2009 showed that ICT for Antic HCV antibody gave false positive results in 2.35% of subjects[22]. People working in transfusion centres are most likely to get hepatitis B and C infection due to repeated pricks.[23] According to Pakistan Medical Research Council HCV prevalence has risen to 4.8% in 2007[24]. Paid donors have more chance of transmitting hepatitis C infection and so such blood transfusion centres need standard screening procedures[25]. According to Centre of disease control (CDC) guideline Antic HCV testing should include an initial screening which will need confirmation with supplementary methods such as HCV RNA detection on Nucliec acid amplification test (NAT) for verification of diagnosis. According to CDC if the screening test is negative no further testing required but those which come out to be positive with high Signal to cut off ratio (S/CO) should be reported as positive but those with low S/CO ratio should be confirmed with NAT or Recombient Immunonblot assay(RIBA)[26].

CONCLUSION

The sensitivity of ICT in our study was very low so it is not recommended as a suitable test for screening of HCV positive patients.

Recommendations

Study was done in a tertiary care hospital where the facility of ELISA and chemiluminescence is available but due to its expenses might not be feasible to shift to these two methods in DHQ.

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AUTHOR'S CONTRIBUTION

Following authors have made substantial contributions to the manuscript as under:

Ali N:	Entering data in SPSS, write up of materials and methods, results.
Batool Z:	Conceiving idea, writing introduction and discussion.
Noor A:	Review of literature and correction of reviewers comments twice
Aurangzeb:	Running the test on ICT rapid kit and chemiluminescence on Architect machine
Khan SA:	Review of literature
Basharat S:	Collection of samples

Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.