How to Cite:
Shaji, L. K. ., Sujatha, S. R., Deepashree, R., Tejashree, A., & Satyasai, B. (2022). Comparative evaluation of chemiluminescence immunoassay and immunochromatograhic test with ELISA for detection of HCV infection. *International Journal of Health Sciences*, *6*(S9), 3044–3053. https://doi.org/10.53730/ijhs.v6nS9.13195

Comparative evaluation of chemiluminescence immunoassay and immunochromatograhic test with ELISA for detection of HCV infection

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***Abstract*---**Introduction: Hepatitis C virus still remains the major concern of post-transfusion viral infection causing chronic hepatitis, liver cirrhosis and hepatocellular carcinoma worldwide. Serological detection of anti-HCV IgG antibody is the mainstay for diagnosis of HCV. Enzyme linked immunosorbent assay (ELISA) is one of the commonest method used. Other screening test include chemiluminescent immunoassay (CLIA) and immunochromatographic test (ICT). This was a prospective laboratory based study conducted with the aim to assess anti-HCV detection method like ICT, CLIA, and ELISA with reliable sensitivity and specificity. Material and methods: A total of 92 samples that were received for routine HCV antibody detection to the microbiology laboratory were subjected to CLIA, ELISA and ICT simultaneously taking Elisa as gold standard & comparative evaluation was done. Results: Out of 92 samples, CLIA showed 34 (37%) reactive and 58 (63%) non-reactive. Whereas, ELISA and ICT showed 30 (32.6%) positive and 62 (67.3%) negative results. Conclusion: The results of CLIA & ICT was comparable with results of

ELISA. CLIA had a sensitivity and specificity of 96.6% and 91.9%, ICT had a sensitivity and specificity of 98.38% and 96.6% accordingly, when compared to ELISA.

***Keywords*---HCV- Hepatitis C virus, ELISA- Enzyme linked immunosorbent assay, CLIA- chemiluminescent immunoassay, ICT- immunochromatographic test.**

Introduction

Hepatitis C virus (HCV) is a single-stranded enveloped virus belonging to the genus Hepacivirus and family Flaviviridae. The HCV virus has a genome size of 9.6 kilobytes (Kb). [1] HCV genome encodes three structural proteins and seven non-structural proteins; core, E1, E2, and P7, NS2, NS3, NS4A, NS4B, NS5A, and NS5B. [2] Genomically HCV is classified into 7 genotypes & multiple subtypes within genotype based on Genetic variability. 1,2,3 genotypes have worldwide distribution, ubiquity of genotypes is mainly attributed to transmission rate and origin . [3] Transmission of HCV occurs via transfusion of unscreened blood, sharing of contaminated syringes for injection of drugs, sexual contact or perinatal transmission [4] It still remains the major concern of post-transfusion non A non B hepatitis. [5] It causes progressive disease resulting in chronic hepatitis, liver cirrhosis and hepatocellular carcinoma causing serious global healthcare problem. [6] Every year 3 to 4 million new infections and approximately 3,50,000 fatalities are caused by HCV.[7] Affecting 2 % to 3 % of the global population of more than 120 million to 180 million people.[8] Prevalence of HCV globally is 3%, Asian and African continents show seroprevalence of 2.3% while America, Europe, Australia is 0.93%. Predominant genotype affecting the Indian subcontinent is genotype 3,1 [9].

HCV infection diagnosis is primarily based on the detection of anti-HCV IgG antibodies as a screening test via immunosorbent assay (ELISA), immunochromatography assays etc. Samples that yielded positive results should be reevaluated by supplemental test including recombinant immunoblotting assay (RIBA), NAT and HCV RNA polymerase chain reaction (PCR). However professional technical employees, costly equipment, reagents, specialized procedural spaces, and the availability of immaculate serum or plasma samples are all required for performing these supplemental tests in the laboratory. [10][6] The enzyme-linked immunosorbent assay for antibodies to the hepatitis C virus has been the primary test for detecting HCV antibodies since its inception. Antibodies against core, NS3, NS4, and NS5 antigenic determinants are detected using a third-generation ELISA.[11.12] Currently, automated chemiluminescence immunoassays are extensively utilized because of their high accuracy and dependability.[10] In view of all this the current study was taken up to assess ICT, CLIA, and ELISA for serological detection of HCV infection with reliable sensitivity and specificity.

Materials and Methods

This is a hospital-based prospective study conducted for a period of 1 year; from March 2021 to February 2022 in the Department of Microbiology, JSS medical

college and hospital Mysore. About 92 Serum samples that were routinely received in the laboratory for Anti-HCV detection in the microbiology laboratory of all age groups and both sexes were included in the study. Lipemic samples, Haemolysed samples and insufficient samples were excluded from the study. Blood samples from the subjects fulfilling the inclusion criteria that are received were collected & samples were further subjected to CLIA (Abbot), ELISA (J. Mithra), ICT (SD BIOLINE) with appropriate controls.

CLIA – serum samples, reagents and appropriate controls were placed in the equipment in appropriate positions, since CLIA is an automated equipment it performs the test and releases the report automatically, time taken for entire procedure is around 35-40minutes and about 65 µl of serum sample is needed for detection of HCV antibodies which was performed with controls and calibrators according to manufacturers instruction. S/CO - RLU/Cutoff RLU- Relative light units are used to quantify the chemiluminescent response (RLU). S/CO ≥ 1.00 were interpreted as reactive, S/CO < 1.00 were interpreted as non reactive. Results were noted and same samples were subjected to ELISA. Where antibodies against HCV with the cascade of both structural and non-structural antigens will be detected in human serum which is a third-generation ELISA, assay was performed along with controls according to manufacturers instruction. Optical density was measured at 450nm using spectrophotometry. Cut off Value was calculated- Cut off Value = PCx(Mean absorbance of positive control) × 0.23. If the OD value is less than the cut-off value, the test specimen is negative for Anti-HCV, If the OD value is more than the cut-off value, the test specimen is positive for Anti-HCV. Results were noted and same serum samples were further subjected to lateral flow immunochromatographic test, where 10µl of serum was dispensed into the sample wells same sample pad was then treated with 3-4 drops of assay buffer. The results were interpreted 5-20 minutes after adding the assay diluent. Reactive - Appearance of the test line (T) and the control line (C), Non-reactive- Appearance of only the control line(C) without test line (T), Invalid result- Disappearance of the control line (C) after performing the test. By considering ELISA as gold standard sensitivity and specificity of CLIA and ICT was obtained.

Results

A total of 92 samples that were received for routine HCV antibody detection were included in the study and the following observations were made. Out of 92 samples tested, 54 (58.6%) were males among these 19 (35.2%) were positive for HCV antibodies and 38 (41.3%) were females among these 11 (28.9%) were positive for HCV antibodies as shown in Table 1

			Sex		Total
			male	female	
	POSITIVE	Numbers	19	11	30
		Percentage (%)	35.2%	28.9%	32.6%
	NEGATIVE	Numbers	35	27	62
		Percentage (%)	64.8%	71.1%	67.4%

HCV positivity was found more in the age group between 51-70 years of age which is around 48.3% and the least was found in <30 years of age which is around 11.1%. In a total of 92 samples, CLIA showed 34 (37%) reactive and 58 (63%) non-reactive. Whereas, ELISA and ICT showed 30 (32.6%) positive and 62 (67.3%) negative results, respectively. as shown in table 2

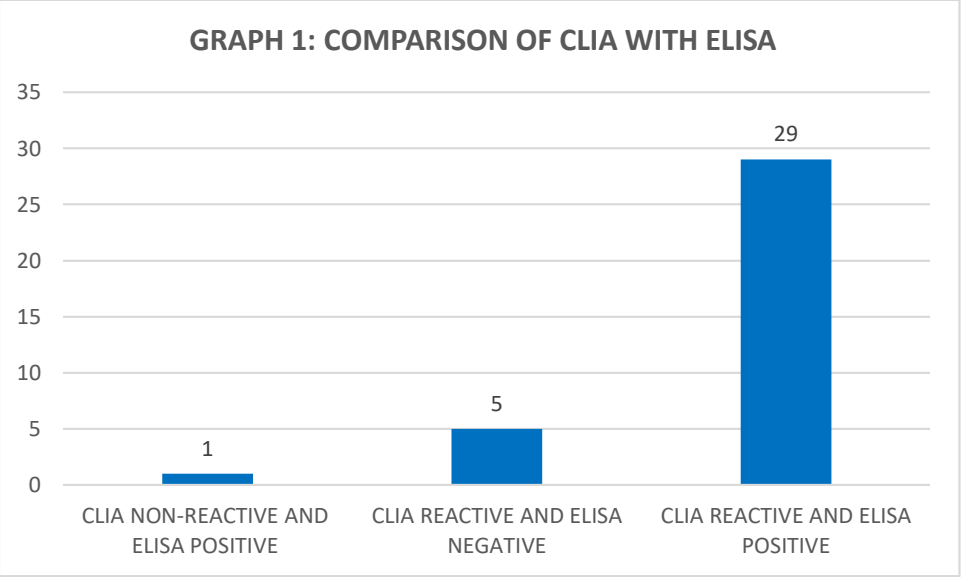
Table 2: Results obtained by CLIA, ELISA and ICT

	REACTIVE	NON-REACTIVE
CLIA	34	58
ELISA	30	62
ICT	30	62

Distribution of results of CLIA in comparison with ELISA

Out of 92 samples tested for Anti-HCV antibodies by CLIA, 34 (37%)/92 were found to be reactive, 58 (63.04%)/ 92 were found to be non-reactive. Same samples were subjected to ELISA. Out of the 58 (63.04%) samples that were non-reactive by CLIA, 57 (98.2%) samples were negative by ELISA also, and 1 (1.72%) sample which was non-reactive in CLIA was positive by ELISA. On the other hand, among the 34 (37%) CLIA reactive samples, 29 (85.2%) were found to be positive by ELISA also, 5 (14.7%) samples which were reactive by CLIA were negative by ELISA as shown in table 3 and graph 1

		ELISA RESULTS		Total	p-value
		NEGATIVE	POSITIVE		
CLIA RESULT	NON-REACTIVE	57	1	58	0.000
	REACTIVE	5	29	34	
Total		62	30	92	



Sensitivity and specificity of CLIA in comparison with ELISA

STATISTIC	VALUE
SENSITIVITY	96.6 %
SPECIFICITY	91.93 %

3) Distribution of results of ICT in comparison with ELISA

Out of the 92 samples tested for Anti-HCV antibodies by ICT, 30 (32.6%)/92 were found to be positive, 62 (67.3%)/92 were found to be negative. Elisa was performed for the same 92 ICT tested samples. Out of the 62 (67.3%) samples that were negative by ICT, 61(98.3%) tested negative by ELISA also, whereas 1(1.6%) sample that tested negative by ICT was positive by ELISA. On the other hand, among the 30(32.6%) ICT positive samples, 29 (96.6%) tested positive by ELISA also and 1(3.3%) tested positive by ICT was negative by ELISA as shown in table 4 and graph 2.

Table 4: Distribution of results of ICT in comparison with ELISA

		ELISA RESULTS			
		NEGATIVE	POSITIVE	Total	p-value
ICT RESULTS	NEGATIVE	61	1	62	0.000
	POSITIVE	1	29	30	
Total		62	30	92	

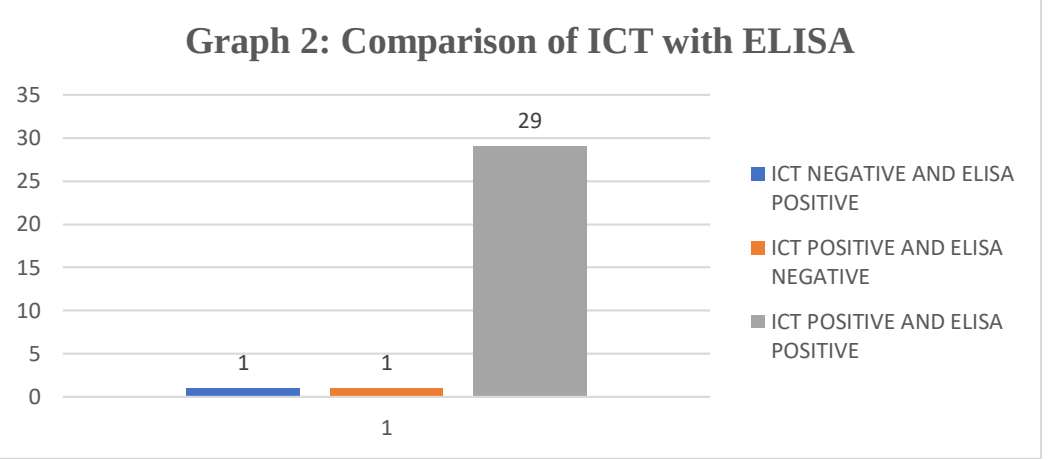


Table 5: Sensitivity and specificity of ICT in comparison with ELISA

STATISTIC	VALUE
SENSITIVITY	98.38 %
SPECIFICITY	96.66 %,

Discussion

The hepatitis C virus is one of the main concerns of transfusion-related hepatitis, and in 75% to 85% of cases, the virus survives in the host, resulting in chronic infection. [13] The ubiquity of HCV infection in India has been anticipated to be between 0.9% to 1.9%, accounting for a significant share of the global HCV incidence. [14]

In our study, demographic data reveals that out of 92 samples, 54 (58.6%) were males and 38 (41.3%) were females. Among these 54 males, 19 (35.2%) showed positive for Anti-HCV. Whereas out of 38 females 11 (28.9%) were positive for Anti-HCV. An analogous study entitled "Comparison of two rapid immunoassays for screening of HCV infection in dialysis patients" done by Hema Prakash et al Anti-HCV testing revealed that 78.7% of males were positive for Anti HCV and female seropositivity was around 21.3%, In both studies, the male population was predominant and showed high seropositivity as compared to females. [15]

In the current study, maximum number of HCV positivity results were seen in the age group of 51-70 which account for 48.3% and least of 11.1% was seen in less than 30 years of age . A comparable study entitled "Anti-hepatitis C virus seroprevalence in the working-age population in Poland, 2004 to 2014" by Aleksandra Czerw et al., showed a similar result where the maximum number of HCV-positive cases were found in the patients above 45 years of age whereas the lowest positive cases were seen between the age group of 25 to 34 years. [16]

There is a similar study entitled "Prevalence of HCV among the high-risk groups in Khyber Pakhtunkhwa" by Ijaz Ali et al., where the lowest number of seropositivity (12.20%) was found in patients under the age of 15, while the

highest (22.22%) was seen in those over the age of 50, which showed similar outcome as compared to the present study ie, high seroprevalence was observed in the middle age group while younger generation showed low seropositivity. [17] One more study conducted by Juhar SK et al., also showed that, the highest positivity was found in the age group of 40–49, with 5.5 percent, while the lowest was found in the age group of < 30 (4.4 percent). [18]

According to our study, 37% were reactive and 63% were non-reactive by CLIA. While ELISA showed 32.6% positive and 67.3% negative results. In a similar study, by Pampi Majumder et al., CLIA found that 35 % were reactive and 33 % were non-reactive out of 91 samples. ELISA, on the other hand, revealed that 33% were positive and 67.3% were negative. [6] Another study conducted by Meenu Bajpai et al., showed that out of total 1000 samples, CLIA found 6 samples to be reactive. All 6 CLIA reactive samples were subjected to ELISA. 3 of the 6 samples that were reactive by CLIA were also positive by ELISA, whereas the other 3 were negative by ELISA. [19]

Out of 92 samples tested for Anti-HCV antibodies by CLIA, 58 (63.04%)/ 92 were found to be non-reactive, and 34 (37%)/92 were found to be reactive. Same samples were subjected to ELISA. Out of the 58(63.04%) samples that were non-reactive by CLIA, 57(98.2%) samples were negative by ELISA also, and 1(1.72%) sample which was non-reactive in CLIA was positive by ELISA. On the other hand, among the 34 (37%) CLIA reactive samples, 29(85.2%) were found to be positive by ELISA also. 5 (14.7%) samples which were reactive by CLIA were negative by ELISA. In a similar study entitled “Comparison between ELISA and chemiluminescence immunoassay for the detection of Hepatitis C virus antibody” by Pampi Majumder et al., On CLIA, out of 91samples 32 (35.1%) were found to be reactive and 30 (33%) were non-reactive. ELISA test was done to re-examine the same 91 CLIA-analyzed samples. Out of these 32 which were reactive by CLIA, 30 (93.75%) samples were positive by ELISA also, while 2(6.2%) samples were determined to be negative. On the other hand, 30(33%) samples that were tested non-reactive by CLIA showed negative by ELISA also. [6]

In our study, CLIA had a sensitivity and specificity of 96.66 %, & 91.93% respectively, when using ELISA as a gold standard and their Positive predictive value and negative predictive value were found to be 98.27 % and 85.29 %, consecutively. A similar study conducted by Arshi Naz et al., entitled “Evaluation of efficacy of serological methods for detection of HCV infection in blood donors” showed a sensitivity and specificity of 100 % and 62.27% respectively. wherein his study it was concluded that the accuracy of anti HCV detection by CLIA was comparable with PCR which was used as gold standard. [20]

According to the current study, out of 92 samples, ICT showed that 32.6% were positive. A similar result was obtained in a study conducted by Ayesha Khan et al., where out of 356 samples, 22.2% showed a positive result by ICT. [21] Out of the 92 samples tested for Anti-HCV antibodies by ICT, 30 (32.6%)/92 were found to be positive and 62 (67.3%)/92 were found to be negative. Elisa was performed for the same 92 ICT tested samples. Out of the 62(67.3%) samples that were negative by ICT, 61(98.3%) tested negative by ELISA also, whereas 1(1.6%) sample that tested negative by ICT was positive by ELISA. On the other hand, among the

30(32.6%) ICT positive samples, 29 (96.6%) tested positive by ELISA also and 1(3.3%) tested positive by ICT was negative by ELISA. In a similar study entitled "comparison between ELISA and ICT techniques for the detection of anti-HCV antibody among blood donors" by Zameer M et al., by ICT, out of 130 samples, 30 samples (23.1%) were found to be HCV positive while 100 samples (76.92%) were found to be HCV negative, ELISA test was performed on all ICT-analyzed samples. Out of 30 samples that were positive by ICT, 29 (96.6%) showed positive by ELISA also and 1(3.3%) showed negative by ELISA. Whereas, in 100 samples that were tested negative by ICT, 99(99%) showed negative by ELISA also and 1(1%) was found to be positive by ELISA. [22]

Another similar study entitled "Role of Rapid Test and ELISA in the Diagnosis of HCV in Haemodialysis Patients" by Kanaga Priya et al., showed that 6 (5.6%) samples out of 106 were found to be HCV positive on ICT, whereas 100 (94.3%) samples were reported to be negative by ICT. All ICT-examined samples were subjected to the Elisa test. 6 (5.6%) samples that showed positive by ICT also tested positive for ELISA. Whereas 100 samples were tested negative by ICT, only 1 was found to be positive by ELISA. [23]

Another study similar to our study on the evaluation of ICT conducted by Mithaq Sabeeh Khudur Al-Nassary et al in a study entitled "Study of Hepatitis C Virus Detection Assays "showed that ICT had a p-value P= 0.0001 in comparison with ELISA, based on these results they have concluded that ICT is a feasible test for routine HCV screening. [24]

In our study, ICT showed a sensitivity and specificity of 98.38 percent and 96.66 percent, respectively. A similar study entitled "comparison between ELISA and ICT techniques for the detection of anti-HCV antibody among blood donors" showed sensitivity and specificity of 99 % and 96.7% accordingly. [22]

Conclusion

The sensitivity and specificity of CLIA in comparison with ELISA used as gold standard was 96.66 % & 91.93% respectively. This was comparable with the results of ELISA indicating that CLIA can be used as one of the methods for screening for HCV infection, as it has an added advantage of screening a large number of samples.

The sensitivity and specificity of ICT in comparison with ELISA was 98.38 % & 96.66 %, hence ICT can be used equally as CLIA for screening of anti -HCV since the results of ICT was comparable with ELISA, ICT is less technically demanding, low turn-around time (15-20 mins) it is useful as a point of care test. On the other hand, CLIA is expensive being automated and ELISA being labor-intensive, ICT can be used as an alternative to CLIA and ELISA in resource-limited laboratories where ICT is more cost-effective & with least turnaround time.

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