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EVALUATION OF A STRATEGY TO DETECT ACTIVE HCV INFECTION USING A COMBINATION OF ELISA & TRI DOT ASSAY

Microbiology

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ABSTRACT

Background: Hepatitis C virus (HCV) is the primary etiologic agent of liver cirrhosis or hepatocellular carcinoma. HCV elevated infection rates are mostly due to the lack of an accurate and accessible screening and diagnosis, especially in low- and middle-income countries. Proper strategy will improve actual screening and treatment programs and help to reach HCV elimination.
Methods: This cross-sectional study consist of 103 subjects were included in our study who attended Dept of Microbiology, Medical College, Kolkata for anti HCV antibody detection. These include various groups who are at high risk of exposure to HCV and also low risk population between February 2018 and November 2018. A predesigned and pretested questionnaire was used to collect data and the data was summarized using descriptive statistics. Template was generated in MS excel sheet and analysis was done on SPSS 20.0 software.
Results: Out of the 103 patients, 59.2% were male and 40.8% were female. Among 64 patients who were HCV-ELISA reactive only 24 were found to be having HCV-RNA in their blood 37.5%. Specificity of ELISA was 56.52%. Specificity of ELISA and TRIDOT combined was 100%. Among 2 IDU patients positive by ELISA (both were negative in TRI-DOT). HCV-RNA could be found in one (01). Positive predictive value of ELISA in this group in 50%.
Conclusions: Combination of ELISA & Tri dot assay plays an import role to detect active HCV infection among patients.

KEYWORDS

HCV ELISA, HCV tri-dot test, hepatitis C, Combination,

INTRODUCTION:

Hepatitis C is a liver disease caused by the hepatitis C virus (HCV) that causes acute and chronic infection [1, 2]. An estimated 71 million people had chronic hepatitis C infection worldwide in 2015 [3]. Viral hepatitis caused 1.34 million deaths in 2015, a number comparable to deaths caused by tuberculosis and higher than those caused by HIV [3]. The burden of HCV infection is enormous in low and middle-income countries from the Southeast Asian region [5]. India alone is estimated to have about 6 million individuals living with chronic HCV infection, most of whom are unaware of their infection status [4, 6]. Chronic HCV infection is associated with long term complications such as liver fibrosis, cirrhosis, and hepatocellular carcinoma [7]. HCV infection is primarily spread through exposure to contaminated blood, and rates of infection are particularly high among people who inject drugs (PWID) and men who have sex with men (MSM) [8, 9]. Since HCV and HIV share similar routes of transmission, co-infection rates are high and are associated with higher morbidity and mortality as well [10–12].

Early diagnosis and curative treatment of HCV infection can reduce the risk of liver-related morbidity and mortality and serve to prevent transmission of new infections [13–16].

The detection of anti-HCV antibodies is based on the use of third generation Enzyme Immunoassay (EIA) that detects antibodies directed against various HCV epitopes. EIA tests are reproducible, inexpensive, and suitable for use in the diagnosis of HCV infection. Given the good performance of third-generation EIA tests, immunoblot tests have become obsolete in clinical practice.[17] HCV TRI-DOT test is a rapid, protein A immunofiltration assay for the qualitative detection of anti HCV antibodies in serum or plasma with a sensitivity of 100% and specificity of 91.5%.[18]

In view of increasing prevalence of HCV day by day, the present study was conducted to evaluate a strategy to detect active HCV infection using a combination of ELISA & Tri dot assay

MATERIAL AND METHODS:

In this study 64 patients who attended the in-patient and out-patient departments of Medical College, Kolkata between February, 2018 and November, 2018, and were tested positive by Anti HCV antibodies by ELISA were included after proper written consent. For control group 39 ELISA negative patients were also included for comparison.

Study Area:

Department of Microbiology, Medical College, Kolkata and ICMR Virus Unit, NICED, Kolkata.

STATISTICAL ANALYSIS:

Data was entered in Microsoft excel and analysed using SPSSv20.

INCLUSION CRITERIA :

Patients attending in-patient and out-patient departments of Medical College, Kolkata between February, 2018 and November, 2018 for Anti HCV antibody detection.

EXCLUSION CRITERIA :

Patient infected with other hepatitis viruses like HBV, HAV or HEV.

ELISA test

All samples were previously tested by a commercially available 3rd generation ELISA, HEPA-SCAN HCV ELISA, from Bhat-Biotech, using manufacturer's instruction and internal quality control sera. Before interpretation of ELISA results, the quality control sera was plotted in Levey-Jenning's chart and validated.

HCV TRI-DOT test

The samples were then tested using a 4th generation rapid antibody detection test, HCV TRI-DOT test from J. Mitra & Co, again following manufacturer's instruction and quality control sera.

STATISTICAL ANALYSIS :

The data were tabulated in Microsoft Excel 2016 and analyzed by using Statistical Package for the Social Sciences (SPSS) version 20.0 software.

RESULTS :

A total of 103 samples were tested by ELISA and Tri-Dot.

Table 1 shows that Out of the 103 patients, 59.2% were male and 40.8% were female. Majority 22.3% belong to age group of 41-50 years, followed by 19.4% 11-20 years and 51-60 years. Only 5.8% belong to age group of 21-30 years.

Among 64 patients who were HCV-ELISA reactive only 24 were found to be having HCV-RNA in their blood, a ratio of 24/64 i.e. 37.5%.

On the contrary where ELISA and TRIDOT both were reactive (n=31), HCV-RNA could be found in 21 cases. 21/31 i.e. 67.74%. However, a significant number of patients n=10 i.e. 32.26% do not have HCV-RNA in their blood. These may represent previously infected patients with no active infection**. (Table 2)**

Table 3 shows that Specificity of ELISA is 56.52%

Table 4 shows that specificity of ELISA and TRIDOT combined is 100% .

Positive predictive value and negative predictive value :

Positive predictive value of ELISA = $\frac{24}{24+30} = 44.49\%$

Negative predictive value of ELISA = $\frac{39}{39+0} = 100\%$

Positive predictive value of ELISA and TRIDOT Combination = $\frac{21}{21+0} = 100\%$

Negative predictive value of ELISA and TRIDOT Combination = $\frac{39}{39+3} = \frac{39}{42} = 92.85\%$

Positive predictive value of ELISA when TRIDOT is negative = $\frac{3}{3+30} = \frac{3}{33} = 9.09\%$

Negative predictive value of ELISA when TRIDOT is negative $\frac{39}{39+0} = 100\%$

Table 5 shows that Among the 24 thalassemia patients positive by ELISA HCV RNA could be found in 13 patients. Percentage being =

$\frac{13}{24} = 54.16\%$ and

Positive predictive value of ELISA = $\frac{13}{13+5} = 72.22\%$

The positive predictive value of ELISA when both ELISA and TRIDOT was positive

$= \frac{13}{13+0} = 100\%$

The positive predictive value of ELISA when ELISA is positive but TRIDOT is negative

$= \frac{0}{5} = 0\%$

The positive predictive value of ELISA increases significantly in high risk group like in thalassemia.

Among 2 IDU patients positive by ELISA (both were negative in TRI-DOT). HCV-RNA could be found in one (01). Positive predictive value of ELISA in this group in 50%. (Table 6)

DISCUSSION

Among 64 patients who were HCV-ELISA reactive only 24 were found to be having HCV-RNA in their blood, a ratio of 24/64 i.e. 37.5%. On the contrary where ELISA and TRIDOT both were reactive (n=31), HCV-RNA could be found in 21 cases. 21/31 i.e. 67.74%. However, a significant number of patients n=10 i.e. 32.26% do not have HCV-RNA in their blood. These may represent previously infected patients with no active infection**.**

Specificity of ELISA was found 56.52%. Specificity of ELISA and TRIDOT combined as 100%.

Positive predictive value of ELISA was 44.49% and negative predictive value of ELISA was 100%. Positive predictive value of ELISA and TRIDOT Combination was 100%. Negative predictive value of ELISA and TRIDOT Combination was 92.85%. Similar results found in Hepatitis C assays: operational characteristics, phase1 by WHO, Geneva and Kumar S S et al.[19]

Positive predictive value of ELISA when TRIDOT is negative was 9.09%. Negative predictive value of ELISA when TRIDOT is negative was 100%. Among the 24 thalassemia patients positive by ELISA HCV RNA could be found in 13 patients. Percentage was 54.16% and Positive predictive value of ELISA was 72.22%. The positive predictive value of ELISA when both ELISA and TRIDOT was positive was 100%. The positive predictive value of ELISA when ELISA is positive but TRIDOT is negative was 0%. The positive predictive value of ELISA increases significantly in high risk group like in thalassemia.

Among 2 IDU patients positive by ELISA (both were negative in TRI-DOT). HCV-RNA could be found in one (01). Positive predictive value of ELISA in this group was 50%.

CONCLUSION

Study reveals that due to low specificity of 3rd generation ELISA it cannot be recommended alone for the diagnosis of HCV infection in patients. For screening of HCV infection it can be used. For further confirmation of positive cases HCV TRI-DOT assay may be used as it is less costly. Combination of ELISA and TRI-DOT using different set of antigens are the best alternative with specificity, positive predictive value and negative predictive value each approaching 100%.

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Ethical approval: The study was approved by the institutional ethics committee

Table 1: Distribution of patients according to age (n=103)

Age (in Years)	Male		Female		Total	
	No.	%	No.	%	No.	%
≤10	4	3.9	6	5.8	10	9.7
11-20	12	11.7	8	7.8	20	19.4
21-30	4	3.9	2	1.9	6	5.8
31-40	4	3.9	8	7.8	12	11.7
41-50	16	15.5	7	6.8	23	22.3
51-60	15	14.6	5	4.9	20	19.4
>60	6	5.8	6	5.8	12	11.7
Total	**61**	**59.2**	**42**	**40.8**	**103**	**100**

Table 2 : Specificity tests of the study subject

Group	Total	HCV-RNA PCR Positive	HCV-RNA PCR Negative
ELISA TRI DOT Positive	31	21	10 (no active infection)
ELISA Positive, TRI DOT Negative	33	3	30 (number of false positive)
Total ELISA Positive	64	24	40

Table 3 : Specificity tests of ELISA

Group	Total	HCV-RNA PCR Positive	HCV-RNA PCR Negative
ELISA Positive	64	24	40 (30+10)
ELISA Negative	39	0	39

Specificity of ELISA = $\frac{39}{39+30} = 56.52\%$

Table 4 : Specificity tests of ELISA and TRIDOT combined

Group	Total	HCV-RNA PCR Positive	HCV-RNA PCR Negative
ELISA and TRIDOT Positive	31	21	10

ELISA and TRIDOT Negative	39	0	39

Specificity of ELISA and TRIDOT combined : $\frac{39}{39+0} = 100\%$

Table 5 : In high risk groups of Thalassemia of IDU

Thalassemia patients	Total	HCV-RNA PCR Positive	HCV-RNA PCR Negative
ELISA and TRIDOT Positive	19	13	6
ELISA positive and TRIDOT Negative	5	0	5
Total ELISA Positive	24	13	11

Table 6 : In high risk groups of Thalassemia of IDU

IDU patients	Total	HCV-RNA PCR Positive	HCV-RNA PCR Negataive
ELISA and TRIDOT Positive	0	0	0
ELISA positive and TRIDOT Negative	2	1	1

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