

A COMPARATIVE STUDY OF EFFICACY BETWEEN THIRD GENERATION ELISA AND FOURTH GENERATION TRI-DOT TEST TO DETECT SEROPREVALENCE OF HEPATITIS-C INFECTION IN PATIENTS ON HAEMODIALYSIS

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Abstract

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Introduction: 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. 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%. The study was conducted to compare the efficacy between 3rd generation ELISA and 4th generation TRI-DOT test to detect the seroprevalence of HCV and to assess the risk factors associated with HCV infection in the patients who are on haemodialysis.

Material and Methods: The study was conducted to compare the efficacy between 3rd generation ELISA and 4th generation TRI-DOT test to detect the seroprevalence of HCV and to assess the risk factors associated with HCV infection in the patients who are on haemodialysis. Sera of 150 adult CRF patients on >2 months haemodialysis tested by TRI-DOT test and then by ELISA. Demographic data of all included patients reviewed for risk factors. Statistical analysis was performed by the SPSS program for Windows, version 17.0.

Results: Out of 150 cases, 17 were found seropositive for HCV by TRI-DOT test and 16 were found seropositive for HCV by ELISA. One hundred and one patients were males and 49 were females. Maximum positive patients fell in 36-55 years age group. Duration of haemodialysis, mean blood transfusion, number of dialysis centre changed found almost double in HCV reactive patients than in non reactive. Sensitivity of HCV TRI-DOT is 100% and specificity is 99.3%, positive predictive value is 94.1% and negative predictive value is 100% in comparison of ELISA.

Conclusion: Seroprevalence of Hepatitis C in haemodialysis patients in our institute is 10.7%. Risk factors for HCV infection in haemodialysis patients included duration of haemodialysis, number of haemodialysis centre changed, number of blood transfusion unit transfused, male gender and middle age. The sensitivity of HCV TRI-DOT is equal to HCV ELISA but the specificity is slightly less.

Keywords : HCV Elisa, HCV tri-dot test, hepatitis C, haemodialysis

INTRODUCTION

Any infection that results in inflammation of liver is called hepatitis. Though Hepatitis B is the commonest type of transfusion associated hepatitis but the incidence of Hepatitis C is increasing day by day. The prevalence of hepatitis C virus (HCV) infection worldwide is 3% and the infected people are estimated at 170 millions.¹ The prevalence of HCV among dialysis patients varies worldwide, ranging from as low as 1% to as high as over 70%.^{2,3} 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.⁴ 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%.⁵ In view of increasing prevalence of HCV in haemodialysis patients day by day, this study is taken to determine the seroprevalence of hepatitis-C infection in patients on haemodialysis in our institute and to assess the risk factors which lead to HCV infection in patients on haemodialysis. The present study

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was designed to compare the effectiveness of two very important tests - 3rd generation ELISA and 4th generation TRI-DOT test.

MATERIAL AND METHODS

Patient of age ≥ 18 yrs, chronic kidney disease / chronic renal failure and patients on haemodialysis for >2 months were included in the study.

After taking the approval of ethical committee of our institute, the work was started. Proper informed consent of patients was taken in dialysis unit and they were interviewed for history and their medical records reviewed to obtain details to include or exclude in study. Demographic data of all included patients reviewed for risk factors to HCV infection and biochemical parameters noted.

Three ml of blood sample withdrawn by venipuncture of 150 patients on haemodialysis from January 2015 to December 2015. Blood sample allowed to clot, sera was separated by centrifugation and tested by J. Mitra & Co. Pvt. Ltd. HCV TRI-DOT test, then by J. Mitra & Co. Pvt. Ltd HCV ELISA kit.

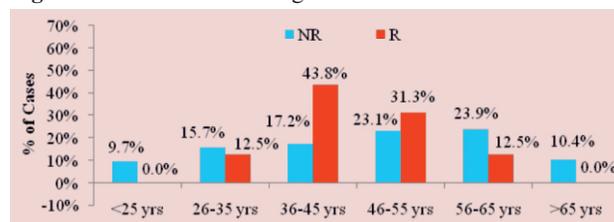
Statistical analysis was performed by the SPSS program for Windows, version 17.0 (SPSS, Chicago, Illinois). Continuous variables are presented as mean \pm SD, and categorical variables are presented as absolute numbers and percentage. Data were checked for normality before statistical analysis. Normally distributed continuous variables were compared using the unpaired t test, whereas the Mann-Whitney U test was used for those variables that were not normally distributed. Categorical variables were analysed using either chi square test or Fisher's exact test.

RESULTS

Out of total 150 cases, 101 (67.3%) were males and 49 (32.7%) were females. Thirteen were in 18-25 years age group, 23 were in 26-35 years age group, 30 were in 36-45 years age group, 36 were in 46-55 years age group, 34 were in 56-65 years age group and 14 were in >65 years age group. It has been seen that maximum numbers of the renal patients fall in age group 36-65 yrs. Out of 150 cases, 17 cases were reactive and 133 cases were nonreactive by HCV TRI-DOT test. Sixteen cases found reactive and 134 cases were nonreactive by HCV ELISA test. Out of 16 reactive cases by HCV ELISA, 7 were females and 9 males. Twelve of 16 were fell in 36-55 years age group. (Fig.-1)

Mean duration of haemodialysis is 21.52 ± 17.04 months in non reactive patients and 45.25 ± 26.21 months in reactive

Fig.-1: Correlation between Age distribution and HCV Elisa



Non Reactive (NR), Reactive (R)

patients by HCV ELISA. The difference is statistically significant ($p < 0.001$). Mean blood transfusion in reactive cases by HCV ELISA is 3.50 ± 2.73 units and in non reactive cases is 2.67 ± 2.57 units in one year. (Table-1)

The mean number of dialysis centre changed in non reactive by HCV ELISA is 1.83 ± 0.95 and in reactive is 2.44 ± 0.81 . The difference is statistically significant ($p = 0.015$). Sensitivity of HCV TRI-DOT is 100%, specificity is 99.3%, positive predictive value is 94.1%, negative predictive value is 100% and diagnostic accuracy is 99.3% in comparison of HCV ELISA. (Table-2)

Table-1: Duration of haemodialysis

	HCV Elisa						P Value
	NR			R			
	Mean \pm SD	Median	Min-Max	Mean \pm SD	Median	Min-Max	
Duration of haemodialysis (months)	21.52 \pm 17.04	18	3.0 - 96.0	45.25 \pm 26.21	36	8.0 - 96.0	<0.001

Non Reactive (NR), Reactive (R)

Table-2: Number of dialysis centre

	HCV Elisa						P Value
	NR			R			
	Mean \pm SD	Median	Min-Max	Mean \pm SD	Median	Min-Max	
Number of dialysis centre	1.83 \pm 0.95	2	01-May	2.44 \pm 0.81	2.5	01-Apr	0.015

Non Reactive (NR), Reactive (R)

DISCUSSION

Prevalence of anti-HCV antibody among hemodialysis (HD) patients is consistently higher than in general population indicating increased risk of acquiring HCV infection among HD patients. The reported incidence varies from country to country and depends upon type of assay used and execution trends for HD. HCV chronic infection causes significant morbidity and mortality among patients undergoing HD.⁶ Currently, third-generation anti-HCV ELISA is largely in use and has shown greater sensitivity and specificity in patients receiving HD.⁷ Fourth generation HCV TRI-DOT test is also in use for screening HCV infection with comparative sensitivity and specificity and less turn around time.

In present study, out of total 150 cases, 101(67.3%) were males and 49 (32.7%) were females, out of 16 reactive cases by HCV ELISA, 9 (56.3%) were males and 7 (43.8%) were females which is similar to Jasuja S et al⁷ and Khan S et al⁶ Maximum reactive cases fell in 36-45 years age group and followed by 46-55 years age group, similar is seen in study of Covic A et al.⁸ and Hegde R et al³

It revealed that as the duration of haemodialysis increases, the risk of developing HCV infection is enhanced. In this study, it has been observed that the mean duration in reactive cases is almost double of as in non reactive cases. The study of Kamyar Kalantar-Zadeh et al⁹ and Jasuja S et al⁷ stated that duration of HD was found to have significant impact on HCV positivity.

In this study difference between mean of dialysis centre changed in reactive and non reactive cases is almost twice. Jasuja S et al⁷ and Megmar AS Carneiro et al¹⁰ showed that treatment in multiple units were significantly associated with HCV positivity.

As far as the seroprevalence of Hepatitis C is concerned in present study, it is 10.7% (16 patients were found positive for anti Hepatitis C antibodies by third generation HCV ELISA). On comparative statistical analysis, one sample which was non reactive by HCV ELISA but reactive by HCV TRI-DOT, considered as false positive due to decreased specificity of HCV TRI-DOT test. Kamyar Kalantar-Zadeh et al⁹, Hegde R et al³ and Joukar F et al¹¹ had similar positive results for anti-HCV by ELISA. Megmar AS Carneiro et al¹⁰ showed out of the 428 patients, 185 (43.22%) were found to be seropositive by ELISA, which is higher than what is found in present study.

The difference between reactive and non reactive cases by HCV ELISA and HCV TRI-DOT is statistically significant. HCV ELISA was taken as gold standard between the two tests. In comparison to HCV ELISA, sensitivity of HCV TRI-DOT is 100% and specificity is 99.3%. Positive predictive value is 94.1% and negative predictive value is 100%. Diagnostic accuracy is 99.3%. Similar results found in Hepatitis C assays: operational characteristics, phase I by WHO, Geneva⁵ and Kumar S S et al¹²

One sample which was found reactive by HCV TRI-DOT test and non reactive by HCV ELISA test, is considered as false positive due to the low specificity of HCV TRI-DOT test in comparison of HCV ELISA test. As we know HCV ELISA is again not a confirmatory test for the anti HCV antibody detection in serum samples, this one sample should be confirmed by RIBA (recombinant immunoblot

assay) for anti HCV antibody or by PCR (polymerase chain reaction) for the detection of HCV RNA. In present study neither RIBA nor PCR is applied to confirm this doubtful result due to financial restriction. PCR or RIBA is recommended to confirm such type of cases which show positivity by TRI-DOT test and negative by ELISA test as they are considered as doubtful cases. Hence the more specific or confirmatory tests like nucleic acid detection assays are strongly recommended.

CONCLUSION

Risk factors for HCV infection in haemodialysis patients included duration of haemodialysis, number of haemodialysis centre changed, number of blood unit transfused, male gender and middle age. In comparison of 3rd generation HCV ELISA, 4th generation HCV TRI-DOT test is rapid, easier to perform with less technical skills and less requirement of materials. The sensitivity of HCV TRI-DOT is equal to HCV ELISA but the specificity is slightly less.

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