International Journal of Medical and Allied Health Sciences

ISSN: 2583-1879
Available at http://www.ijmahs.org/

Original Research Article

Diagnosis of HCV Infection Using Different Rapid Methods, ELISA and Anti HCV Test

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Article Info: Received on: 26/06/2021, Revised on: 10/07/2021, Accepted on: 11/07/2021, Available online: 16/07/2021

Abstract

Background: *Hepatitis C virus (HCV) mainly cause liver associated problem worldwide. Most of the affected people develop liver cirrhosis which develop as a major health issue.*

Aim: *This study aimed towards HCV infection in rural population in India.*

Materials and Methods: *This study was conducted at Rama Medical College and Research Centre over a period of 6 months from 1 January to 30 June, 2021. A total of 721 patients of age between (1 – 85) years were screened and checked for presence of anti-HCV antibody using ELISA and Immunochromatography test.*

Result: *A total of 53 (7.35%) from 721 samples were positive 26 (49.05%) male and 27 (50.9%) females using Immunochromatography test, and then these 53 positive samples were further tested by ELISA as confirmatory test. Out of 53 samples 48 samples were found true positive while 5 samples were found false positive.*

Conclusion: *HCV Infection in this study was found 6.65% in rural area in Hapur District of Utter Pradesh. Disease mostly observed in elder person (50-65) then younger ones. About 49.05 % males and 50.9 % female were found positive.*

Keywords: *HCV, Immunochromatography test, ELISA*

1. Introduction

There are many infectious diseases known which also include HCV infection. HCV contain single stranded RNA as genetic material [2]. Hepatitis C virus was firstly recognized in 1989, is the leading cause of blood borne infection that dramatically developed into serious complications i.e., cirrhosis and hepatocellular carcinoma [3]. Initial infection shows mild or no symptoms sometimes fever, stomachache and yellow skin observed. Initially virus associate with liver (70-80%).

Chronically disease cause liver infection and lastly develops cirrhosis. Sometimes cirrhosis may lead to liver failure and cancer of hepatic cells. The prevalence of HCV infection in high-risk group of patients is found to be more when compared to general population, which include patient undergoing intravenous drug use or blood transfusion, haemodialysis, perinatal transmission. Hepatitis develops in 170 million people including cirrhosis and cancer of hepatic cells which leads to 3, 50,000 deaths per year [4]. Diagnosis of HCV infection can be done initially by screening high risk groups for antibodies to HCV. Nucleotide amplification test (NAT) is used for diagnosis of virion based on RTPCR. The present normal management for chronic HCV is mixture of alpha interferon and ribavirin for a duration of 12 months. Prevention includes avoiding contact with infected person. Avoid intake of alcohol.

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http://doi.org/10.54618/IJMAHS.2021117

2. Materials and methods

Total 721 samples were collected from both male and female patients of age 1-85 years (Table-1). This study was conducted in RAMA Super specialty Hospital over a period of 6 months from January to June, 2021. Blood samples were collected by using disposable 3ml syringe.

Centrifugation of collected samples was done at 3000 rpm for 10 min to obtain serum. Then collected serum is transferred in sterile tube by using micropipette and placed in refrigerator. Samples were taken to virology lab and processed. Each of specimen was subjected to rapid anti HCV antibody that was purchased from Medsource Ozone Biomedicals Pvt. Ltd. These tests were further confirmed by using indirect ELISA technique, test kit was purchased from J. Mitra & Co. Pvt. Ltd.

2.1 Rapid Anti HCV test procedure

1. The test kit was kept at room temperature.
2. Added one drop (25 µl) of serum or plasma into the sample window.
3. Added one drop of assay buffer provided in the dropper bottle into the same sample window.
4. Results were observed in 15-20 minutes.

2.2 ELISA test procedure

1. The microtiter plates were arranged according to the number of the samples.
2. One negative control A-1, three positive control B-1, C-1, D-1 microtitre were labeled.
3. Added 100 µl Negative control in A-1 well and 100 µl Positive control in B-1, C-1, D-1 well.
4. Added 100 µl sample diluent in each well starting from E-1 well.
5. Added 10 µl sample in wells from E-1 well.
6. Plate was sealed with a cover and incubated for 30 minutes at 37˚C and washed 6 times with working washing buffer.
7. 100 µl working Enzyme was added in all the wells and incubate for 30 minutes at 37˚C.
8. After washing with working wash buffer 100 µl of working substrate was added in all the wells.
9. Incubated at room temperature for 30 minutes.
10. 100 µl stop solution was added in all the wells.
11. The O.D. absorbance at 450 nm in microplate reader was taken immediately after adding the stop solution.

Table 1: Patients distribution according to their age group and gender

Age in years	Male	Female	Total
Below 20	22	40	62
20-35	87	120	216
35-50	59	124	183
50-65	72	105	177
65-80	39	34	77
Above 80	04	06	10
Total	283	438	721

3. Results and Discussion

A total of 721 samples of HCV were collected, out of these 283 (39.25%) were male and 448 (60.74%) were female. The Serum of all samples was used for testing HCV with Rapid Test (Fig-1), 53 (7.35%) of the showed positive result by this test and rest were negative (Table-2). Different age group showed variable degree of positivity and highest no of positive cases were found between age group 50-65 years, while above 80 years no positive case were detected (Table-3). Rapid test positive was further confirmed through ELISA (Fig-2) and demonstrated 48 (90.56%) true positive rest 5 (9.43%) were false positive (Table-4 and 5). Chronicity of HCV differs across different geographical areas and population [5].

Collected samples were firstly diagnosed using Immunochromatography test. Samples diagnose with anti-HCV antibody were again tested using ELISA, then confirming positive sample and eliminating false positive. In our study a prevalence of HCV was reported 6.65% in patients attending the Rama Medical College and Hospital. In other study from National Referral Hospital in Rwanda a high prevalence of 16% was reported. This study shows more chronicity in females than in males, does not correlate with the study of Rao et.al. [6].

Table 2: Result of samples by Immunochromatography

Result	Male	Female	No. of Patients	%
Positive	26	27	53	7.35%
Negative	257	411	668	92.64%
Total	283	438	721	100%

Table 3: Rapid Immunochromatography test results for Anti-HCV Antibody in different age groups.

Age groups (yr)	Positive	Negative	Total
Below 20	07	55	62
20-35	12	204	216
35-50	08	175	183
50-65	20	157	177
65-80	06	67	73
Above 80	00	10	10
Total	53	668	721

Figure 1: showing negative result by ICT

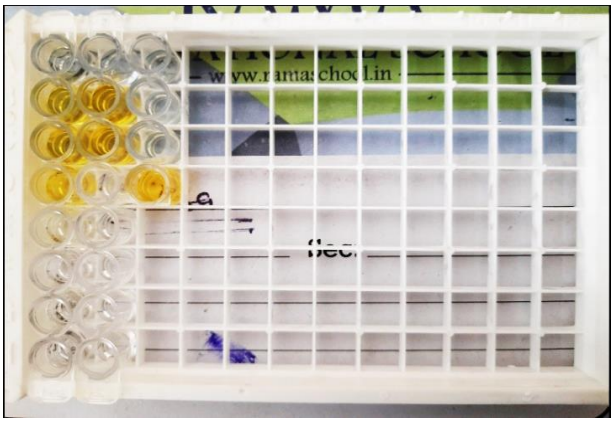

Figure 2: Negative and positive result by ELISA.

Elder ones are more susceptible to disease as reported in Uganda [7] and this may due to continuous spread of virus [8]. More diagnosis is performed in (50-65) year age group because they show more chances infection. Treatment depends on the type of Hepatitis virus; the patient has treated initially with anti- HCV drugs and possibility of cirrhosis. Direct acting anti-HCV drug reduces the number of infected patients [9]. Globally significant numbers of viral infected persons, transmission, infection and mortality is of great concern. Health Care Workers (HCWs) and higher risk in vulnerable/ susceptible groups have been major cause of concern [10].

Table 4: Positive sample confirmation by ELISA

Age groups	Total	Positive	Percentage
Below 20	07	07	14.58%
20-35	12	11	22.9%
35-50	08	08	16.66%
50-65	20	18	37.5%
Above 60	06	04	8.33%

Table 5: True positive and true negative tests results

Positive	True positive 48 (92.64%)	False positive 5 (9.43%)
Negative	False negative 00	True negative 668(92.64%)

4. Conclusion

Our study shows the infection rate of 6.65%. Diseases mostly observed in elder person (50-65yr) then younger ones. The Positivity rate of samples was found to be 6.65%. This study shows more chronicity in female then male. Chronic cases can be cured in more than 90% of people with medication. The present management for chronic infection is the mixture of alpha interferon & Ribavirin for a duration of 12 months before the development of cirrhosis.

Funding

The study was not funded by any organization.

Competing interest

The authors declare that there are no conflicts of interest.

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