Original Article

Seroprevalence of Hepatitis C Virus (HCV) Infection and Role of Serological and Molecular Assay in HCV Detection and Management: A Step Towards Achieving the Aim of National Viral Hepatitis Control Program (NVHCP)

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Abstract

Purpose: Hepatitis C virus (HCV) is a small, enveloped, single stranded RNA virus of Flaviviridae family. HCV causes both acute and chronic infection. Diagnosis of HCV can be done by indirect serological tests that detect antibodies against HCV (anti-HCV) and direct test that detects and quantifies components of viral RNA particles. As per CDC recommendation; HCV testing should be initiated with anti-HCV test, those anti-HCV positive/ reactive should have follow-up testing with Nucleic Acid Test. If the HCV RNA is detected, patient should be directed for the treatment with highly effective direct acting antiviral (DAA).

This study aims to determine the seroprevalence of HCV infection, to estimate presumptive HCV infection by using serological assay by anti-HCV ELISA, to estimate the current HCV infection by detecting HCV-RNA using RT-PCR molecular assay and, to highlight the role of HCV RNA for initiation of treatment.

Methods: This study is retrospective observational analysis of 36500 samples obtained for anti-HCV ELISA test from Jan to Dec 2022. The ELISA kit used was a 3rd generation sandwich assay.

All ELISA positive samples were tested for HCV RNA, by automated qRT-PCR method.

Results: Out of 36500 samples, 361 tested positive by anti-HCV ELISA with 0.9% seroprevalence.

Out of 361, only 336 were tested for HCV RNA. Of 336, 240 samples were negative as target not detected, and 96 samples showed values as viral copies in IU/mL. Most common age group affected was 18-40 years, for both serology and molecular test.

Conclusion: In current study, only 96 samples showed viral copies indicating current HCV infection and need to start treatment. Hepatitis C is curable; with the availability of highly effective DAA both in a brand and generic form can cure > 95% of infected people, so the possibility of achieving the target of elimination HCV by 2030 can be predicted.

Keywords: Serology, Molecular, DAA, NVHCP.

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Introduction

Hepatitis C virus (HCV) is a small, enveloped, positive-sense single stranded RNA virus belonging to Flaviviridae family. HCV causes both acute and chronic infection. Acute HCV infections are usually asymptomatic and most do not lead to a life-threatening disease. Around 30% (15–45%) of infected persons naturally clear the virus within 6 months of infection without any treatment. The remaining 70% (55–85%) will develop chronic HCV infection with risk of cirrhosis ranging from 15% to 30% within 20 years.[1] Diagnosis of HCV can be done by indirect serologic tests which detect antibodies against HCV (anti-HCV) using rapid diagnostic test or ELISA and direct test which detects, and quantifies components of viral RNA particles.[2] In HCV patients, anti-HCV may persist throughout life, or decrease slightly while remaining detectable, or gradually disappear after several years.[3] The serological window period is the time duration between infection and appearance of antibody, has decreased from approximately 16 weeks to 10 weeks and finally to 8 weeks with the introduction of first-, second-, and third-generation anti-HCV ELISAs, respectively.[4] Also there has been improvement in the sensitivity and specificity of anti-HCV ELISA from 70-80% sensitivity and poor specificity of first generation to greater than 99% sensitivity and specificity of third generation. There is also availability of the most recent fourth generation which detects HCV core antigen and anti HCV antibodies simultaneously.[5] Detection of HCV using the most accurate and sensitive nucleic acid test (NAT) assay likereal time RT-PCR will reduce the risk of transmission of HCV and help in the early detection even during serological window period as it can detect HCV RNA in one to three weeks after infection.[6] In recent years, the development and widespread use of techniques for detection and quantitation of HCV RNA has provided useful information on viral dynamics during natural history of the infection and antiviral treatment, and has permitted the identification of predictors of response to therapy (genotype, viral load and quasispecies).[7] As per Centers for Disease Control and Prevention (CDC) recommendation, HCV testing should be initiated with a Food and Drug Administration (FDA) approved anti-HCV test. People testing anti-HCV positive/reactive should have follow-up testing with an FDA-approved nucleic acid test (NAT) for detection of HCV RNA. If the HCV RNA is detected, the patient is considered of having current HCV infection and should be directed for the treatment. Treatment with highly effective direct acting antiviral (DAA) drugs for HCV infection is available. Over 90% of HCV infected people can be cured of their infection, regardless of HCV genotype, with 8–12 weeks of oral therapy, leading to the possibility of eliminating HCV by 2030.[8,9,10] Hepatitis C virus (HCV) infection is a major public health problem, with an estimated global prevalence of 3%. Worldwide, there are about 180 million carriers and 3-4 million new infections annually.[11] World Health Organization (WHO) has projected that 10-24 million people are living with active HCV infection in India and seroprevalence among healthy population ranged from 0.09-2.02% in India.[12] The seroprevalence of HCV infection inthe present setup is not known; hence the this retrospective study is being carried out with following aims and objective

Aims: To determine the seroprevalence of Hepatitis C virus (HCV) infection in patients attending tertiary care teaching hospital of Mumbai

Objectives:

1. To estimate presumptive (current or past) HCV infection by using serological (anti-HCV ELISA) diagnostic assays.
2. To estimate the current HCV infection by using molecular (HCV-RNA by RT-PCR) diagnostic assays.
3. To highlight the role of HCV RNA for initiation of treatment.

Material and Method

Study Type: Retrospective and Observational study.

Inclusion Criteria: All patients tested for anti-HCV ELISA and all anti-HCV ELISA positive tested for HCV RNA by RT-PCR

Exclusion Criteria: Nil

Study Duration: Jan 2022 to Dec 2022

Methodology

This study is the retrospective and observational analysis of 36500 samples obtained for anti-HCV

ELISA test over a period of one year from January 2022 to December 2022. Patients of all age groups were included in the study, which was carried out in a tertiary care teaching hospital in Mumbai. The kit used for ELISA was a sandwich format 3rd generation assay which detects antibodies against structural core and non-structural NS3, NS4 and NS5 proteins. And, all anti-HCV ELISA positive samples were tested for HCV RNA, which was detected by automated qRT-PCR method by Cobas Ampli Prep/ CobasTaqMan kit.

Anti – HCV ELISA test – Serological test

The patient's blood was collected in plain tube and serum was separated. The assay was performed on the serum samples in accordance with the manufacturer's instructions (Meril Diagnostics).

HCV Quantitative PCR- Molecular test

The anti- HCV ELISA positive samples were tested for HCV RNA by RT-PCR. Patient's samples were collected in EDTA tube and plasma was separated for the test. HCV RNA was detected by automated qRT-PCR method by Cobas Ampli Prep/ Cobas TaqMan kit (Roche Molecular Systems, Inc.). This molecular test is considered as the gold standard for detecting active HCV replication.[5]

Statistical analysis

The laboratory register were scrutinised for the results. The results of both the tests were recorded and the same were analysed. Seroprevalence was calculated as proportion of samples that tested positive for anti-HCV antibody from those that were tested. The results of RTPCR of the above antibody positive samples were analysed as both anti-HCV ELISA and PCR positive (viral copies IU/mL), and anti- HCV ELISA positive andPCR negative (Target not detected). Chi Square test was used for statistical analysis. P≤0.05 were considered statistically significant.

Ethics Approval- This study was approved by the institutional ethics committee EC/ OA-96/2023(IEC III/ OUT/ 428/2023)

Results

Out of 36500 samples received from indoor patient department (IPD) and outdoor patient department (OPD), 361 were tested positive by anti-HCV ELISA with 0.9% seroprevalence (Table-1).

Table 1: Distribution of IPD and OPD in anti-HCVELISA positive samples (n=361)

Location	ELISA Positive	ELISA Negative	Total
IPD	202(56%)	21525	21727
OPD	159(44%)	14614	14773
Total	361	36139	36500

The result is not significantat p <.05.

Gender distribution with Male (M) to Female (F) ratio was 1.2:1 (Table-2).

Table 2: Gender distribution in anti-HCVELIS Apositive samples (n=361)

Gender	IPD	OPD	Total
Male	112	88	200(55.4%)
Female	90	71	161(44.6%)
Total	202	159	361

The result is not significant at p <.05.

Of these positives, maximum samples were from Medicine department for both OPD and IPD, followed by Orthopaedics for IPD and GI Medicine for OPD (Figure -1).

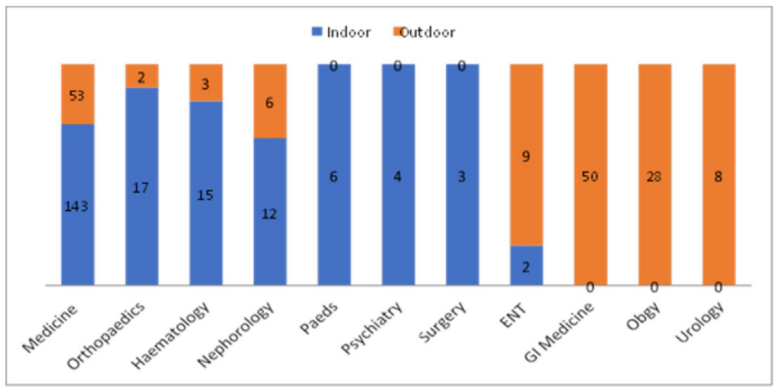

Figure 1: Department wise distribution of anti-HCVELISA positive samples

Out of 361 anti-HCV ELISA positive samples, only 336 were tested for HCV RNA by RTPCR. The default of 25 samples could be due to non-compliance, or migration or death of patients. Out of 336 HCV RNA tested, 240 samples were negative as target not detected, and 96 samples showed values as viral copies in IU/mL, of these 96, 50 were male and 46 females with M:F ratio as 1:1 (Table-3).

Table 3: Gender Distribution in RTPCR samples (n=336)

Test	Target not detected	Copiesin IU/mL	Total
Male	129	50	179(53.3%)
Female	111	46	157(46.7%)
Total	240(71.4%)	96(28.6%)	336(100%)

The result is not significant at $p < .05$

Most common age group affected was 18-40 years, for both serology and molecular test (Table-4).

Table 4: Distribution of age group in anti-HCVELISA and PCR positive samples

Age group (in years)	ELISA (n=361)	PCR (n=336)
<18	19(5.2%)	12(3.5%)
18-40	162(44.8%)	154(45.8%)
41-60	135(37.3%)	133(39.5%)
>61	45(12.4%)	37(11.0%)

Discussion

In order to estimate the seroprevalence of HCV in this region, patients visiting both IPD and OPD were screened for presence of anti-HCV antibody as per the investigations sent by the respective treating clinicians. The seroprevalence of present study is 0.9%. As reported by Goel et al. in his meta-analysis of four community-based studies from India with 11118subjects included from three states, i.e., Punjab (n=5258), West Bengal (n=2973), and Maharashtra (1054 and 1833, respectively). The anti-HCV prevalence rates varied widely being highest in Punjab (5.17%) than in West Bengal (0.87%) and Maharashtra (0.00% and 0.09%) with the weighted pooled anti-HCV prevalence of 0.85% (95%CI: 0.00–3.98%)[13], which is similar to this study. In South-East Asian countries, the seroprevalence ranged from as low as 0.23% (95%CI 0.21–0.25) in Sri Lanka to as high as 2.65% (95% CI 2.26–3.11) in Myanmar.[14] In western, northern and central Europe, HCV can be found in less than 0.5%of the population while in many countries of Eastern Europe and Central Asia the figure can be as high as 3–5%.[15] The prevalence rate as well as the significance of HCV infection varies from country to country. This may be because of cultural factors and social habits that influence its transmission. And amongst different states of country, the variation could be due to different sample size, genetic and immunity difference, socio-economical and behavioral practices. In current study, M:F ratio was 1.2:1 with 55.4% male and 44.6% female for serology test by anti- HCV ELISA, and 1:1 with 53.3% male and 46.7% female for molecular test by RT-PCR. The gender based difference was not significant. Most common age group affected was18-40 years, by both serology and molecular test. Similar results were seen in study from Haryana, with 58% male, 42% female and 21-40 years (51.86%) as the most affected age group, followed by 41-60 years (37.45%), 61/above (5.63%) and upto 20 years (5.04%).[16]The study from Ireland, in contrast, showed significantly higher seroprevalence in men (1.6%; 95%CI: 1.1–2.2%) than in women (0.42%; 95%CI: 0.25–0.71%), with age group of 30–39 years (1.9%; 95%CI: 1.2–3.1%) and 40–49 years (1.5%; 95% CI: 0.96– 2.4%) affected most.[17] As per recent reports for year 2022 of United States, high rates of new HCV infections were predominantly among young adults aged 20-29 years and aged 30-39 years[18], the present study also showed the similar age group involvement. The study from West Bengal by Chaudhary et al. reported no difference in gender but age-specific prevalence of HCV was low in children (0.31%), and increased gradually from adolescents (0.83%) to adults (1%) and older persons (1.85%).[19] Similar low prevalence of HCV infection has been reported in children (0.2%) and adolescents (0.4%) from the United States.[20] In this study pediatric age group showed prevalence of 5.2% for ELISA and3.5% for PCR.

A reactive HCV antibody result indicates any one of the situation i.e. current HCV infectionor past HCV infection that has resolved or false positive.[21] As per CDC guidelines, People testing anti-HCV positive/reactive should have follow-up testing with HCV RNA. If the HCV RNA is detected, the patient

is considered of having current HCV infection and should be directed for the treatment. In present study amongst the 336 anti-HCV positive samples which were tested for HCV RNA, only 96 samples showed viral copies indicating current HCV infection with a need to start on treatment and rest 240 samples showed target not detected with no indication for treatment. The high number of anti –HCV positive and PCR negative 240 samples, could be due to acute, chronic, resolved, or false positive condition. A false-positive HCV antibody result may occur because of cross-reactivity with other viralantigens or the presence of immunologic disorders, such as lupus or rheumatoid arthritis.[22] In the study by Berry N et al. HCV RNA and anti-HCV were present together in only7/62 (11.3%) cases and this false positive reactions of anti-HCV antibody may be due to multiple infections encountered in the tropical countries producing hypergammaglobulinemia. So, they recommended that testing for both antibody and HCVRNA would be more apt than either test alone.[7] Also the study by S Kaya et al. suggested that in routine laboratories RT-PCR appears to be a valuable assay for HCV RNA determination for diagnosis and follow up therapies and using ELISA and RT-PCR methods at the same time would help to confirm the positive results and early diagnosis.[23] Hepatitis C is curable; new antiviral medicines can cure more than 95% of infected people, reducing the risk of complications and death. Highly effective direct acting antiviral (DAA) drugs for chronic HCV infection are now available.[24] The effectiveness of antiviral treatment is assessed according to the proportion of patients achieving sustained virologic response (SVR). SVR is the essential goal of treatment and is defined as undetectable (below the lower limit of quantification) HCV RNA at 12–24 weeks after cessation of treatment. SVR rates with a DAA in combination with peg IFN plus ribavirin currently range from approx. 80–90% for treatment-naïve patients, whilst SVR rates of up to 99% have been reported with combinations of two DAAs.[25] The high price of these medications has severely limited its access, especially in low and middle income countries such as India.[26] Recently, generic DAA drugs (sofosbuvir, ledipasvir, daclatasvir, and velpatasvir) are also available and several observational reports with SVR of over 95% with these drugs have been published.[27] With the availability of effective treatment modalities, the possibility of achieving the target of elimination HCV by 2030 can be predicted. This study wants to focus the need of performing HCV RNA for all anti-HCV ELISA positive samples so we can concentrate on those 96 patients with the current HCV infection, who are in actual need of treatment with avoidance of unnecessary or unrequired exposure of medication for rest 240 patients, and thus helping in strengthening the NVHCP programme to successfully reaching its aim.

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Limitation: It is prevalence study and not incidence study.

Follow up of patients after 12 weeks of treatment is not studied so therapy outcome is not known. Further studies are required for detailed analysis of treatment and SVR (sustained virologic response).

Advantage – Large sample size. Use of third generation ELISA kit with sensitivity of 100% and specificity of >99.5%. This study is the first step towards the analysis of HCV data by both serological and molecular method for effective implementation of NVHCP programme.

Benefit of the study: This study helps in highlighting the importance of detection of HCV-RNA by RT-PCR in patients to differentiate current HCV infection that indicates active HCV replication and need for initiation of treatment from presumptive (current or past or biological false positive) HCV infection detected by ELISA test.

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Conflicting Interest: Nil

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