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# Beyond Routine Testing in Hepatitis B and C: Experience of A State Reference Laboratory

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**ABSTRACT**
Viral hepatitis is one of the leading causes of death globally with 1.4 million deaths per year. Hepatitis B and C viruses cause 95% of those deaths by causing chronic infection that can lead to cirrhosis and hepatocellular carcinoma. Primary diagnosis of HBV and HCV infection is done by serological tests for detecting antigens and antibodies against these viruses respectively. Qualitative and quantitative molecular tests are used to confirm primary diagnosis, quantify viral load, determine genotypes and plan treatment regimen for resistance mutants. In this study, we analyze the data of samples tested for Hepatitis B and C at a State Reference Laboratory for viral load testing. A total of 48,116 HBV and 43,596 HCV samples were screened for Hepatitis by ELISA over a period of 1.5 years; of which 2157 (4.48%) HBV and 842 (1.93%) HCV samples were further subjected to quantitative PCR testing for viral load.

## INTRODUCTION

Viral hepatitis is primary inflammation of the liver due to infiltration of hepatocytes with viral infected cells leading to parenchymal necrosis in portal and peri-portal areas[1]. Two different patterns of viral hepatitis are recognized: Acute viral hepatitis with rapid onset of infection and, usually, rapid resolution (more commonly seen with Hepatitis A and E) and chronic viral hepatitis, which is asymptomatic and often detected on routine blood tests or during screening for infection (usually seen with Hepatitis B and C) (Fig. 1)[1].

According to the World Health Organization (WHO), during 2019,296 million people worldwide are living with hepatitis B and 58 million people are living with hepatitis C[2]. In India, an estimated 40 million people are affected with hepatitis B and 6-12 million with hepatitis C (Table 1)[2].

Viral hepatitis is caused by infection with one of the five known hepatotropic viruses, which are named as hepatitis A virus (HAV), hepatitis B virus (HBV), hepatitis C virus (HCV), hepatitis D virus (HDV) and hepatitis E virus (HEV), respectively (Table 2)[3]. These viruses are quite divergent in their structure, epidemiology, routes of transmission, incubation period, clinical presentations, natural history, diagnosis and preventive and treatment options[4]. Hepatitis A and E often cause sporadic outbreaks of hepatitis whereas hepatitis B and C can either clear spontaneously or lead to chronic infection and its sequelae like cirrhosis and hepatocellular carcinoma (HCC), causing an estimated 1.4 million deaths annually[4,2].

**Hepatitis B virus:** Hepatitis B virus is a 47-nm spherical, double-stranded DNA virus, belongs to the family of hepadnaviruses that possesses several antigens of importance for diagnosis and pathogenesis[5]. There are three envelope polypeptides of particular importance: HBsAg (hepatitis B surface antigen), HBcAg (hepatitis B core antigen) and HBeAg (hepatitis B e-antigen) (Fig. 2)[5].

It is spread predominantly by percutaneous or mucosal exposure to infected blood and various body fluids, including saliva and menstrual, vaginal and

Table 1: Prevalence of Hepatitis B and C[2]

| | World | India |
|---|---|---|
| Hepatitis B | 296 million | 40 million |
| Hepatitis C | 58 million | 6-12 million |

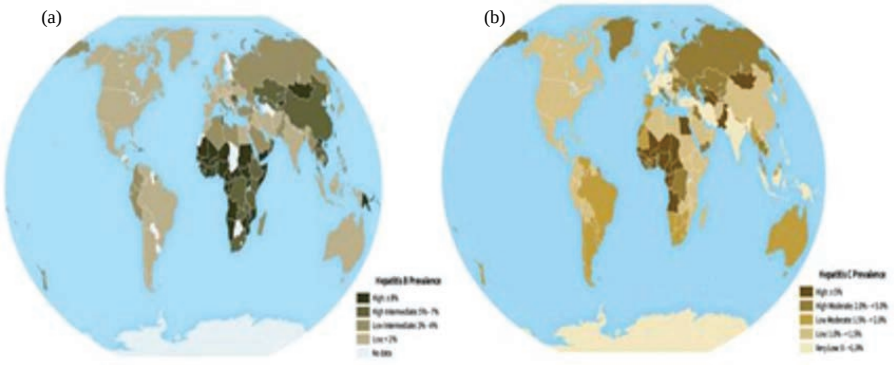


Fig. 1(a-b): Countries most affected by Hepatitis B and C[2]

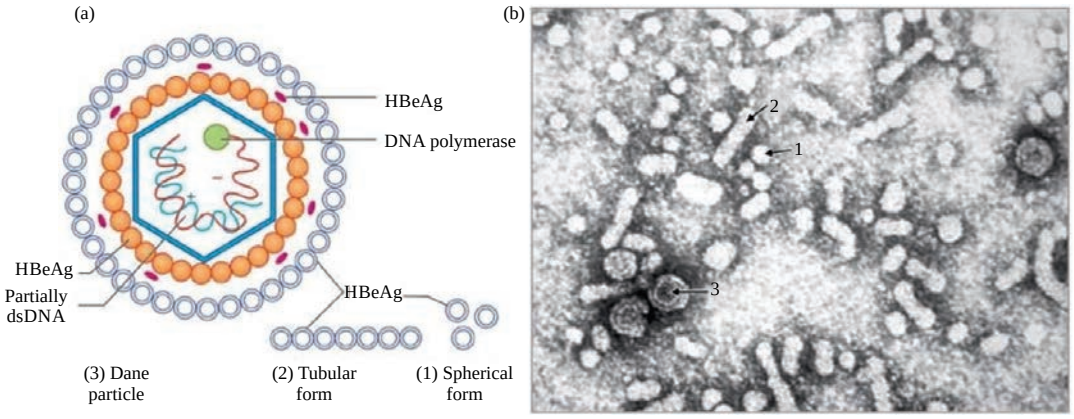


Fig. 2(a-b): A and B: (a) Schematic diagram, (b) Electron microscopic appearance of hepatitis B virus, showing 1- spherical form, 2-tubular form and 3-Dane particle

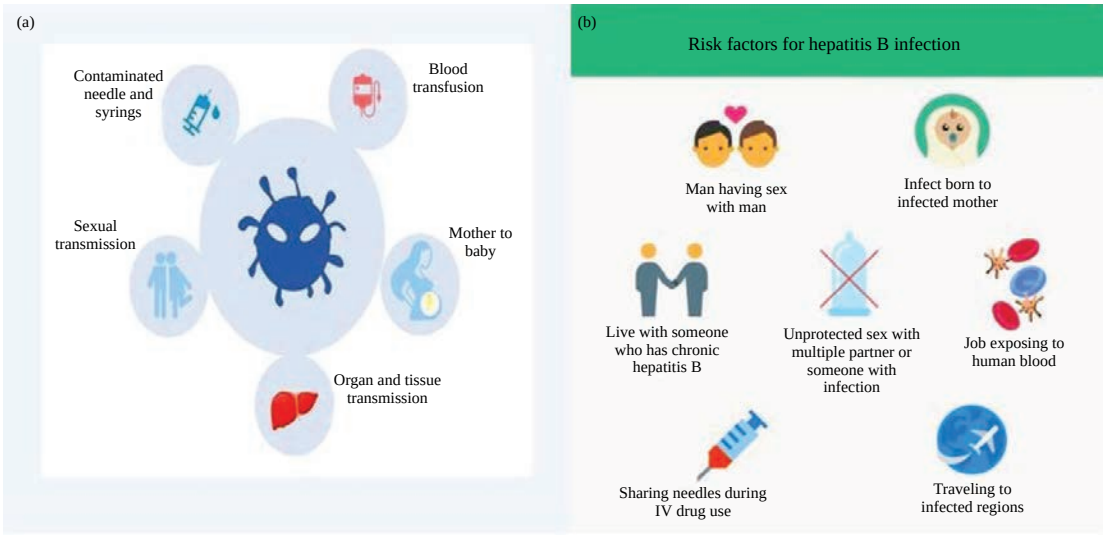


Fig. 3(a-b): A and B: (a) Modes of transmission and (b) Risk factors for Hepatitis B infection

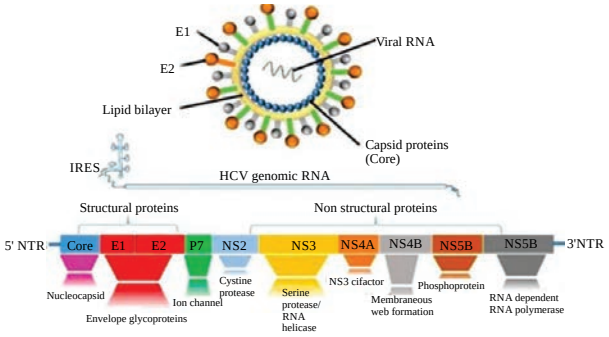


Fig. 4: Structure of hepatitis C virus

seminal fluids[6]. High risk groups prone to hepatitis B infection include unvaccinated health-care workers, patients who receive multiple transfusions, unvaccinated MSM and heterosexual persons with multiple sex partners or contact with sex workers, people using unsafe injecting equipment for drug abuse or cosmetic procedures (such as tattooing and body piercing), mobile or migrant populations (Fig. 3)[6].

**Laboratory diagnosis of HBV:** Definitive diagnosis of hepatitis B depends on the serological demonstration of the viral markers (Table 3). Detection of viral antigen, especially the surface antigen (HBsAg), plays an important role in the diagnosis of acute infection. The e-antigen (HBeAg) is detected at the same time as DNA polymerase activity, which is a marker for infectious virus; the presence of e-antigen has correlated with acute, communicable disease in many studies. Antibody to the viral core (Anti-HBc) is important diagnostically during the time when surface antigen has been cleared from the circulation and antibody to the surface antigen is not yet detectable. The presence of core antigen (HBcAg) correlates with circulating viral DNA. In some patients, a chronic infection develops in which antigenemia persists in the absence of serum antibody; this group has an increased risk for the development of chronic liver disease[5].

**Hepatitis C virus:** Hepatitis C Virus was discovered in 1989and first labeled as a “non-A, non-B hepatitis virus”[5]. Acute hepatitis C virus infection is often less severe than that of hepatitis B but the frequency of chronic hepatitis C disease is high[6]. It is an independent risk factor for hepatocellular carcinoma after the development of cirrhosis[5]. It is a linear, single-stranded enveloped RNA virus belonging to the flavivirus family[4]. The HCV genome consists of seven functional regions- the core (nucleocapsid core protein C), the envelope (two envelope glycoproteins (E1 and E2)) and the non-structural region, including six non-structural (NS) proteins (NS2, NS3, NS4A, NS4B, NS5A and NS5B) and one p7 membrane protein which functions as an ion channel (earlier considered as NS1) (Fig. 4)[1].

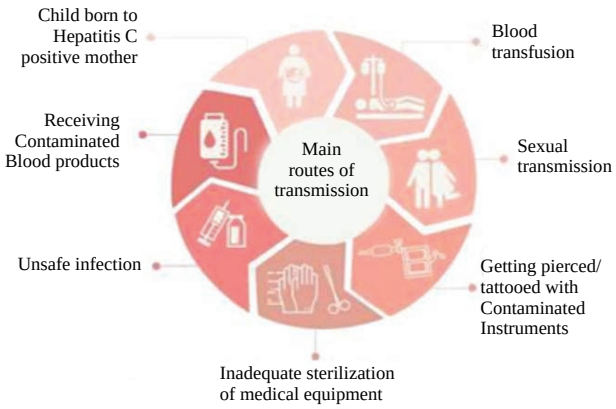


Fig. 5: Routes of transmission of hepatitis C virus


Fig. 6: CDC recommendations for hepatitis C screening[7]

Table 2: Classification of hepatitis viruses

| | |
|---|---|
| Hepatitis A virus (HAV) | Infectious hepatitis |
| Hepatitis B virus (HBV) | Serum hepatitis |
| Hepatitis C virus (HCV) | Post transfusion hepatitis. |
| Hepatitis D virus (HDV) | Defective virus, needs HBV for its replication |
| Hepatitis E virus (HEV) | Enterically transmitted non-A, non-B hepatitis |
| Hepatitis G virus (HGV) | |

Table 3: Comparison of HBV detection by HBsAg ELISA and HBVL

| HBVL ELISA | HBVL positive | HBVL negative | Total |
|---|---|---|---|
| ELISA positive | 1498 (69.44%) | 603 (27.95%) | 2101 (97.40%) |
| ELISA negative | 08 (0.37%) | 48 (2.22%) | 56 (2.59%) |
| Total | 1506 (69.81%) | 651 (30.18%) | 2157 |

There are four main routes of transmission of hepatitis C virus: Health-care-associated transmission (unsafe injection practices, invasive procedures with inadequate infection control practices, unscreened or inadequately screened blood transfusions), injectable drug use, mother-to-child transmission (MTCT) and sexual transmission[6]. Persons with HIV infection, in particular PWID and MSM, are at increased risk of HCV infection (Fig. 5)[6].

**Hepatitis C screening:** CDC recommends one-time hepatitis C screening for all individuals aged >18 years (at least once in their lifetime), all pregnant women (during each pregnancy) in areas with HCV prevalence (HCV RNA positivity) >0.1% and regularly for high-risk group, regardless of HCV prevalence (people living with HIV, IV drug users, truck drivers etc)[7]. Also, regardless of age or setting prevalence, all persons with risk factors should be tested for hepatitis C, with periodic testing while risk factors persist. Any person who requests hepatitis C testing should receive it, regardless of disclosure of risk (Fig. 6)[7].

**Laboratory diagnosis of HCV:** The diagnosis of HCV infection is made by detection of anti-HCV antibodies using immunoassays followed by detection of HCV RNA in serum or plasma[1]. The standard method of diagnosis is antibody detection by ELISA. Currently available, third generation immunoassay, which incorporates proteins from the core, NS3 and NS5 regions, detect anti-HCV antibodies during acute infection[3]. Confirmation of indeterminate anti-HCV results is by detection of HCV RNA (by PCR or transcription mediated amplification-TMA), or by determination of anti- HCV Signal-to-Cutoff Ratio (S/CO) according to CDC guidelines (Fig. 7). The most sensitive indicator of HCV infection is the presence of HCV RNA[4].

**National viral hepatitis control programme:** The WHO has introduced Global health sector strategy on viral hepatitis (2016-2021) which aims at the elimination of

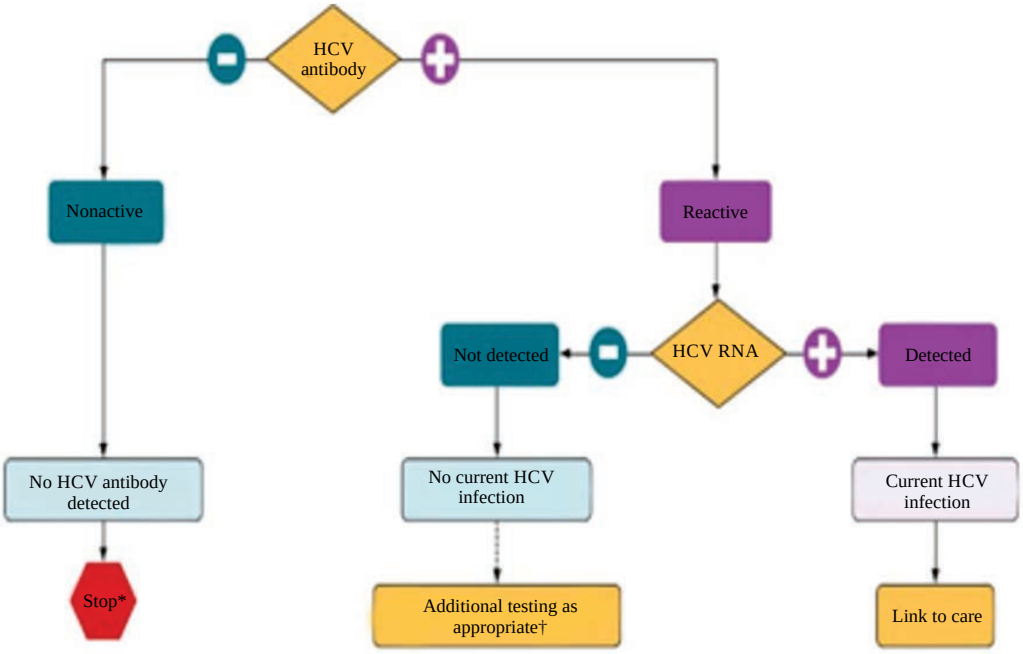


Fig. 7: Hepatitis C testing sequence[7]

viral hepatitis as a public health threat by 2030 (defined by reducing new infections by 90% and mortality by 65%). World Hepatitis Day is celebrated on 28th July every year. The Government of India is a signatory to the resolution 69.22 endorsed in the WHO Global Health Sector Strategy on Viral Hepatitis 2016- 2021 at 69th WHA[8]. 'National Viral Hepatitis Control Program' was launched in India on 28th July, 2018 with the aim to combat hepatitis and achieve countrywide elimination of Hepatitis C by 2030, achieve significant reduction in the infected population, morbidity and mortality associated with Hepatitis B and C, viz., Cirrhosis and hepatocellular carcinoma (liver cancer); and reduce the risk, morbidity and mortality due to Hepatitis A and E[8]. This is in line with our global commitment towards achieving Sustainable development goal (SDG) goal 3; target 3.3 which aims to "By 2030, end the epidemics of AIDS, tuberculosis, malaria and neglected tropical diseases and combat hepatitis, water borne diseases and other communicable diseases"[8].

Viral hepatitis is now recognized as a major public health challenge that requires an urgent response. Surveillance is recommended for acute hepatitis to detect outbreaks, monitor trends in incidence and identify risk factors for new incident infections and for chronic hepatitis to estimate the prevalence of chronic infection and monitor trends in sentinel groups. In this study, we analyze the data of samples tested for Hepatitis B and C at a State Reference Laboratory for viral load testing.

**Aims and objectives:**

- To analyze the data of samples tested for Hepatitis B and C in our institute
- To determine the significance of Viral Load testing in high-risk groups
- To detect occult hepatitis virus infection in clinically suspected patients
- To identify the demographic aspects of hepatitis patients, to investigate the factors affecting outcome

## MATERIALS AND METHODS

The study was conducted in the State Reference Laboratory in a Tertiary Care Hospital. This study includes OPD patients with suspected clinical hepatitis and those admitted for emergency and elective surgery and for conservative treatment. Retrospective analysis of samples collected over a period of One and a half year (1.5 year) was done. Samples consisted of two blood samples in plain vacutainer and EDTA vacutainer. All the samples were processed by ELISA test and HBV DNA and HCV RNA NAT testing as per the kit literature.

The details of the kits used in the study are as follows:

- Merilisa HBsAg-Meril diagnostics
- Merilisa HCV-Meril diagnostics
- COBAS® AmpliPrep/COBAS® TaqMan® HBV Test, version 2.0 (v2.0)
- COBAS® AmpliPrep/COBAS® TaqMan® HCV Quantitative Test, v2.0

Table 4: Comparison of HCV detection by HCV ELISA and HCVL

| HCVL ELISA | HCVL positive | HCVL negative | Total |
|---|---|---|---|
| ELISA positive | 241 (28.62%) | 522 (61.99%) | 763 (90.61%) |
| ELISA negative | 13 (1.54%) | 66 (7.83%) | 79 (9.38%) |
| Total 254 (30.16%) | 588 (69.83%) | 842 | |

Table 5: Hepatitis B viral load

| Patient | Age | Gender | HBVL | Diagnosis |
|---|---|---|---|---|
| 1 | 62 | F | <20 | Liver cirrhosis |
| 2 | 35 | F | 156 | Liver cirrhosis |
| 3 | 31 | F | 3813 | Liver cirrhosis |
| 4 | 52 | F | 567 | Liver cirrhosis |
| 5 | 40 | M | <20 | Liver cirrhosis |
| 6 | 29 | F | 3293 | Liver cirrhosis |
| 7 | 23 | F | 2287 | Liver cirrhosis |
| 8 | 46 | M | <20 | Liver cirrhosis |
| MEAN | 39.75 | F>M | 1272 | |

Table 6: Hepatitis c viral load

| Patients | Age | Gender | HCVL | Diagnosis |
|---|---|---|---|---|
| 1 | 39 | M | $3.56\times10^{3}$ | Liver cirrhosis |
| 2 | 50 | M | $7.92\times10^{4}$ | Liver cirrhosis |
| 3 | 31 | M | $1.71\times10^{6}$ | Liver cirrhosis |
| 4 | 47 | M | $2.96\times10^{6}$ | Liver cirrhosis |
| 5 | 54 | F | $9.69\times10^{4}$ | Liver cirrhosis |
| 6 | 54 | F | $1.75\times10^{5}$ | Liver cirrhosis |
| 7 | 28 | M | $3.48\times10^{3}$ | Liver cirrhosis |
| 8 | 77 | F | $8.06\times10^{5}$ | Liver cirrhosis |
| 9 | 37 | M | $4.25\times10^{5}$ | Liver cirrhosis |
| 10 | 27 | F | $1.06\times10^{4}$ | Liver cirrhosis |
| 11 | 74 | M | $2.32\times10^{5}$ | Liver cirrhosis |
| 12 | 53 | F | $1.72\times10^{7}$ | Liver cirrhosis |
| 13 | 23 | M | $7.87\times10^{6}$ | Liver cirrhosis |
| Mean | 45.69 | M>F | $2.43\times10^{6}$ | |

## RESULTS

A total of 48,116 samples were screened for hepatitis B virus and 43,596 for hepatitis C virus, out of which 2157 (4.48%) hepatitis B and 842 (1.93%) hepatitis C samples were further subjected to quantitative PCR testing for Viral Load.

Amongst 2157 samples subjected to HBV viral load, 2101 were HBsAg Positive samples of which 1498 (69.44%) had detectable viral load. 603 (27.95%) had no target detected. 08 (0.37%) HBsAg Negative samples had detectable viral load (Table 3).

Out of 842 samples, 763 were HCV ELISA Positive samples and amongst this viral load was detected in 241 (28.62%); target was not detected in 522 (61.99%). A total of 13 (1.54%) HCV ELISA Negative samples had detectable viral load (Table 4).

On demographic analysis, it was found that average age of patients with detectable HBV viral load was 39.75 years, females were more affected than males and the average viral load value was 1272 IU $mL^{-1}$ (Table 5).

In case of samples with detectable HCV viral load, the average age of patients was 45.69 years, males were more affected than females and the average viral load value was $2.43\times10^{6}$ IU $mL^{-1}$ (Table 6).

All patients were clinically diagnosed with liver cirrhosis.

## DISCUSSIONS

Viral load testing was done on ELISA positive and negative patients based on the clinical history suggestive of hepatitis. Negative HBV and HCV serologic markers and positive viral load can lead to improper management and complications (21 patients in our study). In such cases HBV DNA, HCV RNA and other markers help to detect occult hepatitis cases and planning of treatment and subsequent monitoring, thereby preventing adverse outcomes of Chronic Hepatitis.

The molecular detection HBV DNA is used to distinguish chronic active infection from inactive infection. The quantitative measure of HBV DNA is also used to monitor the response of the patient to therapy; with the end goal being long-term viral suppression with undetectable viral load. The quantitative detection of HCV is currently the standard of care for infected patients. The viral loads are used to monitor the response to therapy and also provide an amplicon for HCV genotyping.

## CONCLUSION

Despite the high global burden of disease due to chronic HBV and HCV infection and the advances and opportunities for treatment, most people infected with HBV and/or HCV remain unaware of their infection and therefore frequently present with advanced disease and may transmit infection to others. Testing and diagnosis of hepatitis B and C infection is the gateway for access to both prevention and treatment services and is a crucial component of an effective response to the hepatitis epidemic. Early identification of persons with chronic HBV or HCV infection enables them to receive the necessary care and treatment to prevent or delay progression of liver disease. Viral load testing should be done for detecting occult cases in patients who are negative on primary screening by serology and suspected to have hepatitis clinically/other immunocompromised patients. Hepatitis Positive patients (Serology positive/virology positive) are enrolled and provided free of cost monitoring and treatment under National Viral Hepatitis Control Program.

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