

Viral Load Pattern and other Non Invasive Serological Investigations among Hepatitis B Surface Antigen Positive Patients attending Tertiary Care Centre: A Retrospective Study

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ABSTRACT

Introduction: In patients with Chronic Hepatitis B (CHB), assessing the presence of liver complications is crucial for both managing and assessing treatment response.

Aim: To study viral load levels, Non invasive Tests (NITs), and their association with disease progression in patients (CHB) attending tertiary care centre.

Materials and Methods: This retrospective study was conducted at a tertiary care centre in Maharashtra under the National Viral Hepatitis Control Program (NVHCP) from October 2021 to October 2024. Patients aged 15-70 years, including preoperative cases, blood donors, and individuals with or without liver disease, who tested positive for Hepatitis B Surface Antigen (HBsAg) were included. A total of 44,596 samples were analysed. Demographic and clinical data were retrieved from the NVHCP database. All patients were evaluated using hepatitis B viral load (quantitative Polymerase

Chain Reaction (PCR)), Aspartate Aminotransferase (AST)-to-Platelet Ratio Index (APRI), and Fibrosis-4 (FIB-4) scores to assess correlations with liver complications. Age, gender, and relevant clinical history were recorded. Statistical analysis was performed using standard software; Chi-square tests were used to compare viral load with APRI and FIB-4 scores. A p-value <0.05 was considered statistically significant.

Results: A total of 44,596 screened patients, 892 (2%) were HBsAg positive. Among these, 120 (13.4%) with viral load >20,000 IU/mL started treatment. The highest viral loads (6->7 log₁₀ IU/mL) were observed mainly in the 40-60-year age group.

Conclusion: Viral load strongly correlated with non invasive markers. APRI and FIB-4 scores are valuable prognostic tools for assessing liver disease progression and transmission risk in HBV-positive patients.

Keywords: Aspartate aminotransferase-to-platelet ratio index, Chronic Hepatitis B, Fibrosis-4 index

INTRODUCTION

Hepatitis B is a potentially life-threatening liver infection caused by the Hepatitis B Virus (HBV) [1]. HBV infection is an important global health problem [2]. India accounts for 10-15% of the entire pool of HBV carriers of the world [2,3]. HBV is spread predominantly by percutaneous or mucosal exposure to infective blood and various body fluids [3]. Modes of transmission of infection include sexual, perinatal mother to child transmission, infected needles, transfusion of infected blood and blood, products [3,4]. Not all people with HBV infection have symptoms. Symptoms can range from asymptomatic or mild disease to, rarely, fulminant hepatitis. Some of the common symptoms of hepatitis B infection are abdominal pain, nausea, or vomiting, dark urine or clay-coloured stools, fever, jaundice, joint pain and loss of appetite.

Hepatitis B can cause a chronic infection and puts people at high-risk of death from cirrhosis and liver cancer. Viral hepatitis B is a vaccine preventable disease. Prevention of perinatal /vertical transmission is also possible through hepatitis B vaccination at birth [4]. Routine assessment of HBsAg-positive persons is needed to guide hepatitis B management. This includes assessment of: Complete Blood Counts (CBC) including platelet count, Liver Function Test (LFT), Ultrasound (USG) of abdomen, renal function test and NITs such as Aspartate Aminotransferase (AST)-to- APRI score and the FIB-4 [4]. Serum HBV DNA levels/viral load quantified by real-time PCR correlate with disease progression and are used for decision to initiate treatment [5,6]. It also helps the patients

by delaying the disease transmission, progression. It also helps to monitor treatment response thereby decrease the morbidity and mortality associated with it. Hence it ultimately improves the quality of life [7-9]. This study addresses a critical gap in the existing literature by concurrently examining prevalence, viral load stratification, and non invasive fibrosis assessment (APRI, FIB-4) among patients with CHB, among patients with CHB, unlike prior reports that evaluated these parameters in isolation. The analysis integrates these parameters with treatment initiation criteria as per NVHCP guidelines. This approach not only provides novel insights into age-specific viral load patterns and fibrosis risk but also underscores the clinical utility of cost-effective, non invasive tools in resource-constrained settings, thereby strengthening the evidence base for early detection and optimised management of Hepatitis B.

The aim of this study was to evaluate the prevalence, viral load distribution, and liver fibrosis status in patients with CHB, and to assess treatment eligibility in accordance with NVHCP guidelines. Primary objective was to determine the proportion of CHB patients requiring treatment based on viral load thresholds and NVHCP criteria. Secondary objectives were to analyse the distribution of viral load across different age groups among patients with CHB. To assess the degree of liver fibrosis using non invasive markers (APRI and FIB-4) in relation to viral load. To explore the potential role of simple fibrosis markers in guiding clinical decision-making, particularly in resource-limited settings.

MATERIALS AND METHODS

This was a retrospective study conducted at the Department of Microbiology, Dr. Shankarrao Chavan Government Medical College, Nanded a nodal centre for the NVHCP, Maharashtra, India. All investigations and management of patients were carried out according to standard NVHCP guidelines. The study included data from a three-year period, from October 2021 to October 2024. HBsAg screening tests (HbsAg rapid test) were performed for all suspected patients and HBsAg positive patients were referred for baseline investigations and HBV DNA viral load testing as per NVHCP guidelines [4,10]. The study (October 2021 to October 2024) was time-bound because it only includes data from that three-year period. The study was approved by the Institutional Ethics Committee (IEC) of Dr. Shankarrao Chavan Government Medical College, Nanded (Approval No.: Outward No. 001/25). Written informed consent was obtained from all participants prior to enrolment.

Inclusion criteria: Age 15-70 years, preoperative patients, blood donors, and patients with or without liver disease were included in the study.

Exclusion criteria: Patients co-infected with HIV or HCV, patients with a liver transplant, and pregnant women were excluded from the study.

Study Procedure

Sample collection: Five millilitres (5 mL) of venous blood was collected from each participant. Three millilitres (3 mL) were used for baseline investigations, including CBC and LFTs. The remaining 2 mL was stored and transported to Hind Labs, Kharghar, Mumbai, for HBV DNA viral load testing.

APRI and FIB-4 Scores [3]:

$APRI = \{AST / AST \text{ (Upper Limit of Normal)}\} \times 100 / \text{platelet count} (10^9/L)$ [3]

APRI > 0.5: suggestive of significant fibrosis

FIB-4 = (age (y) x AST (IU/L)/

(platelet count ($10^9/L$ x \sqrt{ALT} (IU/L))) [3]

FIB-4 < 1.45: low probability of significant fibrosis

FIB-4 ≥ 1.45 : intermediate to high probability of significant fibrosis

Sample storage and transport: Short-term: Refrigerator (2-8°C) for up to 24 hours; samples transported the next day to the designated viral load testing facility.

Long-term: Deep freezer (-20°C to -80°C), avoiding repeated freeze-thaw cycles.

Transport: Cold boxes (2-8°C) with a thermometer and appropriate triple-layer packing.

HBV DNA Quantification: HBV DNA viral load testing was performed at the reference centre in Mumbai using available PCR kits (AltoStar HBV Viral Load RT-PCR Kit 1.5) [11]. Kits with high sensitivity and specificity were preferred. Most samples were tested using the AltoStar HBV Viral Load RT-PCR Kit 1.5 [10] (Limit of detection: 10.2 IU/mL; Linear range: $20-1 \times 10^7$ IU/mL). RT-PCR with TaqMan fluorescent chemistry amplified the target sequence of the HBV and detected its DNA genome. An internal control was included to identify possible PCR inhibition and confirm reagent integrity.

Interpretation of Viral Load [10]:

<20 IU/mL: HBV DNA detected but below lower limit of quantification;

$20-1 \times 10^7$ IU/mL: HBV DNA detected and quantified;

1×10^7 IU/mL: HBV DNA detected but above upper limit of quantification.

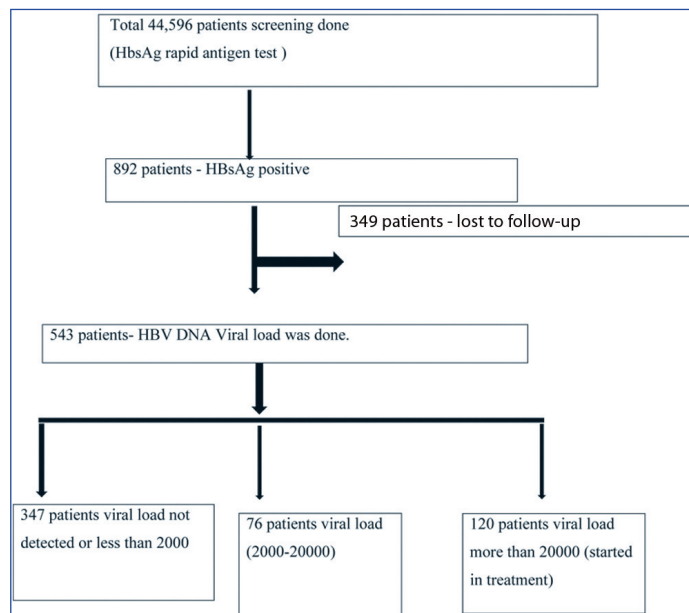
STATISTICAL ANALYSIS

Data were recorded in a master chart. Categorical variables (age, sex, AST, ALT, viral load, APRI, FIB-4) were analysed using the Chi-square test. Associations between patients' NITs and chronic

hepatitis were examined. Dependent variables included age, sex, number of comorbidities, baseline investigations, and follow-up viral load results. Statistical significance was set at $p < 0.05$. All analyses were performed using Statistical Package for the Social Sciences (SPSS) version 21.0 Statistics.

RESULTS

A total of 44,596 patients were screened using the HBsAg rapid test (MAKESURE) HBsAg Rapid Test Card, of these, 120 patients were enrolled in the study [Table/Fig-1]. The majority of affected individuals belonged to the 20-40 years age group {47 (39.2%)}, followed by the 41-60 years age group {40 (33.3%)}. A clear male preponderance was observed, with a male-to-female ratio of 3:1; overall, males were 91 (75.8%) and females were 29 (24.2%). The different viral load levels and those with high ALT levels (>40 IU/L), AST levels (>50IU/L) are mentioned in [Table/Fig-2].



[Table/Fig-1]: Flow chart illustrating patient screening and selection.

Variables	Frequency (%)
Age (years)	
20-40	47 (39.2)
41-60	40 (33.3)
>60	33 (27.5)
Gender	
Male	91 (75.8)
Female	29 (24.2)
Viral load (LOG₁₀)	
<3	25 (20.8)
3-4	05 (4.2)
4-5	16 (13.3)
5-6	14 (11.7)
6-7	28 (23.3)
>7	32 (26.7)
ALT levels (>40 IU/L)	59 (49.2)
AST levels (>50IU/L)	55 (45.8)

[Table/Fig-2]: Demographic and biochemical parameters of the study population (n=120).

The details of HBV viral load with respect to the different age groups were mentioned in [Table/Fig-3]. Viral load distribution among patients showed 28 (23.3%) in the log₁₀ group and 32 (26.6%) in the >7 log₁₀ group. The Chi-square test showed a statistically significant association between increasing age and higher viral load, indicating that older patients are more likely to experience progressive HBV

Age group (years)	<3 log ₁₀	3-4 log ₁₀	4-5 log ₁₀	5-6 log ₁₀	6-7 log ₁₀	>7 log ₁₀	Total n (%)
20-40	6 (12.8)	2 (4.3)	6 (12.8)	5 (10.6)	10 (21.3)	18 (38.3)	47 (39.2)
41-60	12 (30.0)	1 (2.5)	2 (5.0)	3 (7.5)	14 (35.0)	8 (20.0)	40 (33.3)
>60	7 (21.2)	2 (6.1)	8 (24.2)	6 (18.2)	4 (12.1)	6 (18.2)	33 (27.5)
Total	25 (20.8)	5 (4.2)	16 (13.3)	14 (11.7)	28 (23.3)	32 (26.7)	120 (100)

[Table/Fig-3]: Age group wise co relation of viral load levels.

Chi-square test 18.65, df=10, p-value= 0.0449.
statistically significant (p-value <0.05)

disease and related complications. The significant p-value (p-value <0.05) suggests that there is strong association between the two. This finding can be taken into consideration when there is limited availability of viral load testing and limited resources.

The association between viral load and FIB-4 score was calculated and mentioned in [Table/Fig-4]. Based on the findings and Chi-square test results, FIB-4 can be considered as prognostic marker stating the risk of progressive disease and transmission among the HBV positive patients. The FIB-4 score can also be considered alternative to viral load value for the management and follow-up purpose.

Similarly, the association between APRI score and viral load had been demonstrated and mentioned in [Table/Fig-5]. This suggests that APRI score can also be considered as alternative to the viral load test in resource limited area for the treatment and follow-up purpose.

Name of test parameter/ viral load (log ₁₀)	3-6	>6	Total
FIB-4 score ≤1.45	19 (38%)	31(62%)	50
FIB-4 score >1.45	41(59%)	29 (41%)	70

[Table/Fig-4]: Association of FIB-4 score with viral load level (n=120).

Chi-square test 4.937, df=1, p-value= 0.0203.
statistically significant. (p-value <0.05)

APRI score	3-6 log ₁₀ n (%)	>6 log ₁₀ n (%)	Total n (%)
≤0.5	29 (43.3)	38 (56.7)	67 (55.8)
>0.5	34 (64.2)	19 (35.8)	53 (44.2)
Total	63 (52.5)	57 (47.5)	120 (100)

[Table/Fig-5]: Association of APRI score with viral load levels (n=120).

Chi-square test 5.167, df=1, p-value= 0.023.
statistically significant. (p-value <0.05)

The APRI and FIB-4 scores showed a direct correlation with HBV DNA levels. In patients with viral load <3 log₁₀, APRI, FIB-4 score suggested HBV associated liver damage/inflammation.

This association was particularly evident in patients with a viral load of 6-7 log₁₀ IU/mL, where both scores were significantly elevated. These findings suggest that with increasing viral replication, there is a parallel rise in non invasive fibrosis markers, indicating a greater risk of hepatic inflammation and progression towards advanced liver disease. Hence, APRI and FIB-4 may serve as reliable surrogate markers to assess liver damage severity in chronic HBV infection, especially in patients with moderate-to-high viral loads.

Among 120 patients, thirty-two (32/120) (26.7%) had more than 7 (Log₁₀) Viral load and 4 (12.5%) (8-fold increased risk) developed Hepatocellular Carcinoma (HCC).

Current study showed the prevalence of hepatitis B patients as two in this region.

Prevalence rate:

$$\text{(Number of existing cases / Total population)} \times 100$$

$$892/44596 \times 100 = 2$$

In present study, the compliance rate of 45% was achieved. Out of 120 patients 54 are compliant and 66 are non compliant patients. Appropriate patient counselling from the medical team at the beginning and during the course of treatment. Patients feel much more confident when a medication works well and causes an early

virological reduction. This opens the door for consistent medication use and appropriate follow-up.

DISCUSSION

The most commonly affected age group was the young and adult population [5,6,8]. The majority of patients were males under 60 years of age, consistent with the reported male preponderance among HBV-infected individuals with detectable viral load [8,12,13]. The proportion of males and females among HBV-infected patients with detectable viral load was calculated as the number of males/females divided by the total number of patients, and differences between groups were assessed using the chi-square test. The involvement of young and adult population with male preponderance suggests that these two demographic factors make the patient at increased risk of HBV positivity. Males may be more prone to HBV infection due to the effect of sex hormones such as androgen and oestrogen, which are produced in males and females, respectively. By attaching to particular receptors, such as Oestrogenic Receptors (ERs) or Androgen Receptors (ARs), these hormones carry out their biological role by initiating signal transduction pathways. Because it produces both ARs and ERs, the liver is thought to be a sexually dimorphic organ that responds to sex hormones. It has been demonstrated that HBV X protein increases AR transcriptional activity in a manner that is dependent on androgen concentration, thereby exacerbating the sex difference in HBV-infected individuals [14]. Approximately, 50% of total patients had high liver enzymes when tested suggesting the active liver inflammation. AST and ALT levels significantly increased with higher FIB-4 values [15].

In the study population, 78% (423/543) of patients had viral load less than 20,000 IU/mL and required only follow-up, whereas 22% (120/543) had higher viral load necessitating active management. Higher serum HBV DNA levels were observed in patients with liver fibrosis compared to those without, indicating a direct correlation between elevated viral load and fibrosis-related complications. Similar finding was mentioned in one of the studies and states that the high viral load is always correlated well with the increased complication of HBV infection [8].

Among 120 patients, thirty-two (32/120) (26.7%) had more than 7 (Log₁₀) Viral load and 4 (12.5%) (8-fold increased risk) developed HCC. High-level HBV DNA (>10 000 copies/mL) was strongly associated with both HCC and cirrhosis (17- and 39-fold increased risk) [8,12].

The HBV viral load (4-6 Log₁₀) may indicate the presence of significant hepatic inflammation and fibrosis, and a subsequent increase in the hepatocyte proliferation rate, which is a major risk factor for HCC development [12]. The high HBV DNA level (6 Log₁₀) is associated with hepatocarcinogenesis through both direct (HBV DNA integration) and indirect (inflammation and fibrosis) pathways [12].

A single undetected HBV DNA test result in conjunction with a positive anti-HBV antibody status does not confirm resolved HBV infection. Hence, repeat follow-up testing is needed at frequent intervals, (regular follow-up of HBV positive patient is needed at three months, six months, 12 months and two years when the patient is on treatment) and while those patients with negative or undetected HBV viral load needs follow-up with viral load testing at

every six-month interval [4]. Such regular follow-up with HBV viral load testing (with average cost 3000-4000rs), may not be possible in every healthcare facility for HBV positive patients each time [16,17]. Hence, there was need of cost-effective test which can be easily available especially in periphery or resource limited area.

NVHCP guidelines suggests that baseline investigations such as CBCs including platelet count (CBC), LFT, USG of abdomen, HBV DNA quantitative and renal function test should be done for every six months. The patients can be managed according to the results of such findings [2,4,5].

A statistically significant association was found between FIB-4 score and APRI score with viral load levels. Similarly, HBV viral load is significantly correlated with APRI and FIB-4 scores in a study [12].

Though liver biopsy remains the gold standard [3-5,18], However, liver biopsy carries the risk of representing only a very small portion of the entire liver. In addition to technical problems, liver biopsy requires an experienced pathologist and a well-equipped hospital, and the invasive nature of the procedure carries a risk of complications, such as haemorrhage, pneumothorax, and peritonitis, which may be rare but fatal. Blood and serum markers for fibrosis, including APRI and FIB-4, or transient elastography (Fibro Scan) are performed to rule out advanced fibrosis [5]. APRI and FIB-4 are recommended as the preferred NIT to assess for the presence of cirrhosis (APRI score >2 : FIB-4 >3.25 in adults). Transient elastography (e.g., Fibro Scan) may be the preferred NITs in settings where they are available, and cost is not a major constraint [4].

When the correlation between the calculated FIB-4 and APRI scores of the patients was evaluated, there was a statistically significant linear relationship between the FIB-4 and APRI scores (p -value= 0.0203 and 0.023) [2,5,6]. In the study, APRI score of 53 patients out of 120 have shown >0.5 . According to NVHCP guidelines, irrespective of ALT or HBV DNA viral load if APRI score is ≥ 2 then it indicates cirrhosis and treatment is recommended.

Other studies have shown that the APRI score alone is not sufficiently sensitive to exclude fibrosis, and its use in combination with other scores will provide higher diagnostic accuracy [5]. High APRI also correlated with severity of cirrhosis and its complications. Thus, APRI may be used as a simple, bedside, non invasive, and inexpensive tool for evaluating portal hypertension and complications of cirrhosis [19,20].

Out of 120 patients, 70 (58.3%) patients showed FIB-4 score >1.45 . Sensitivity and specificity were 83% when the FIB-4 score was ≤ 1.45 , and the sensitivity and specificity of FIB-4 score ≥ 3.25 , showing significant fibro- sis, were 47% and 80%, respectively [5].

Among 70 patients, 29 (41%) showed >3.25 . According to NVHCP, guidelines, irrespective of ALT or HBV DNA viral load if FIB-4 score is ≥ 3.25 then it indicates liver fibrosis and treatment is recommended. This is quite high number of cases in the study, which had FIB-4 score > 3.25 . there was also approximately 60% patients had FIB-4 score >1.45 indicating their increased risk for fibrosis and need of immediate management but further study is required for the calculation of its relative risk and odds ratio. Other studies suggest that most of the patients could have a major problem due to advanced liver fibrosis not previously identified [15].

Therefore, FIB-4 ≥ 3.25 represented the first warning to consider the presence of liver fibrosis [15]. CHB or CHC patients were found to bear a significantly higher annual HCC incidence if they had a basis FIB-4 ≥ 3.25 . The accuracy of FIB-4 was comparable to the liver stiffness measurement in predicting mortality and was significantly better than APRI in CHC patients [21]. When the goal is to identify those with advanced fibrosis, the use of the FIB-4 scores should take in to account [22].

Since majority of infected people remain asymptomatic, and low viral load (as found in 78% (423/543) hepatitis B positive patients), often

present with advanced disease, early diagnosis is critical to timely initiation and scale up of treatment for viral hepatitis B. Inadequate public and healthcare provider awareness; the asymptomatic nature of infection during the early stages, lifelong treatment and access to quality diagnostics are some of the challenges to scaling up management of viral hepatitis B [7].

Single and first HBV viral load result at the start of treatment suggested the treatment response and prognosis of patient [3,4]. HBV viral load investigation results were found as excellent parameter in determination of severity and seriousness of the chronic HBV disease [8,12]. Assessment of significant fibrosis is an important step for decision-making of antiviral treatment in chronically HBV-infected patient. The diagnostic value of non invasive blood parameters (APRI, FIB-4, and HBV viral load) was compared for assessing liver fibrosis in patients with CHB infection and found that these blood parameters as excellent for ruling-out significant fibrosis, presence of active liver inflammation. Hence, the APRI, FIB-4 as NITs can be considered as follow-up tests in context of limited resources.

In the study, 892 out of 44,596 patients were HBsAg positive showing the prevalence as 2. This is accordance with the literature which states that India is in intermediate endemicity i.e., 2-8% of population [7]. Globally, the prevalence of HBV infection varies greatly; in the Asia-Pacific area, which accounts for three-quarters of all chronic HBV-positive individuals, the prevalence is intermediate to high [3]. In the study, the poor compliance to the treatment was due to the asymptomatic nature of disease (423/543) 78% which make the patient to wait till they become symptomatic. The time to become symptomatic may vary. This makes it difficult to contain the progression of disease. Hence, this was the main reason that needs to be needs to consideration as priority. The patients were actively counselled and educated about the nature of the disease and its management in the nodal centre. This was further enhanced with the help of the peer positive patients who is also an employee.

Limitation(s)

Retrospective nature not compared with the liver biopsy which is gold standard test and not compared NIT with either fibro scan or liver biopsy in all 543 patients where HBV viral load was measurable but less than 20000.

CONCLUSION(S)

The study demonstrated a prevalence of hepatitis B of 2% in the region. APRI and FIB-4, as NITs, can be considered for follow-up in symptomatic patients with limited resources, particularly when HBV viral load ranges from 3-6 \log_{10} IU/mL and viral load testing is unavailable. Patient compliance to treatment in the region was observed to be poor, highlighting the need for reinforcement. Comparison of APRI and FIB-4 scores with HBV DNA viral load levels showed good performance in identifying patients with liver fibrosis. The use of NITs in place of HBV viral load testing in selected symptomatic patients is cost-effective when applied appropriately, especially in resource-constrained settings.

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