

## Hepatitis B and Hepatitis C - Seroprevalence and Correlation with C-Reactive Protein

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Received: 20-03-2023 / Revised: 11-04-2023 / Accepted: 05-05-2023

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Conflict of interest: Nil

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### Abstract:

**Background:** Viral hepatitis caused by Hepatitis B(HBV) and Hepatitis C virus (HCV) is a major public health problem specially in developing countries like India.

**Materials and methods:** Three hundred blood samples were collected from patients having clinical sign and symptoms of hepatitis and apparently healthy pregnant females. Detection of hepatitis B surface antigen, antibodies against hepatitis C virus and C reactive protein (CRP) was done in these samples.

**Results:** The seroprevalence of HBV and HCV in our study samples was found to be 6.3% and 2.3% respectively. CRP levels were found to be raised in both HBV and HCV positive samples.

**Conclusion:** Viral hepatitis caused by HBV and HCV continues to pose a threat as a significant health problem. Emphasis should be put on health education of the general and high-risk populations, along with health care workers to increase knowledge on preventive measures for these infections.

**Keywords:** Hepatitis B Virus, Hepatitis C Virus, Seroprevalence, C-Reactive Protein.

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### Introduction

Viral hepatitis is a public health problem worldwide and is one of the major causes of chronic liver disease and liver failure. The most commonly implicated etiological agents are the five viruses -hepatitis A virus (HAV), hepatitis B virus (HBV), hepatitis C virus (HCV), hepatitis D virus (HDV), and hepatitis E virus (HEV).[1] The World Health Organization (WHO) estimates that in 2019, 296 million persons (3.8% of the population) were living with chronic hepatitis B virus (HBV) infection in the world and 58 million persons were living with hepatitis C virus (HCV) infection in the world, accounting for

0.75% of the population.[2] The burden of hepatitis B and C infection is highest in the developing world and mostly affects resources limited countries where screening and access to care and treatment are not readily available. Although these viruses are diverse in their morphology and genomic constitution, their pathological effects on liver are similar and they cause clinically indistinguishable diseases in most cases. The most common modes of HBV and HCV infection are blood transfusion and hazardous lifestyle behaviours such as injection drug use, sharing syringes and needles and high-risk

sexual behaviours. [3] Both infections are known to have long gestation periods before the disease progresses to advanced stages resulting in chronic infection, liver cirrhosis and liver cancer, leading to high mortality especially in hepatitis C infection. Chronic HBV infection accounts for 40% of Hepato-cellular Carcinoma (HCC) and 20-30% cases of cirrhosis in India while chronic HCV infection accounts for 12-32% of HCC and 12-20% of cirrhosis. [4] C-reactive protein (CRP) is an acute phase protein synthesized by hepatocytes during acute inflammatory and infectious processes as part of the innate immune response of the host. [5]

A variety of interleukins (ILs) are closely related to HBV infection and pathogenesis. Among these interleukins, IL-6 is not only an important inflammatory cytokine, but it is also the primary regulator of CRP gene expression. In hepatocytes, IL-6 induces CRP production at the transcriptional level, which is promoted by IL-1 $\beta$ . Studies have shown that HBV upregulates the expression of the IL-1 $\beta$  and IL-6 genes consequently causing an increase in CRP levels. [6] Studies have found serum level of CRP significantly elevated in patient with HBV infection and that it correlated with disease progression. [7] CRP immune reactivity has also been quoted as a novel bio marker for poor cancer specific survival of HBV-associated hepatocellular carcinoma and also correlate with serum CRP concentration. When considering the association between HCV and CRP levels conflicting results have been found in various studies.

In one study the CRP levels were found to be in normal range in HCV infected patients while another study concludes that median values of CRP were higher among HCV-RNA positive individuals. [8,9] The present study was performed to determine the seroprevalence of hepatitis B and C in patients clinically suspected of having viral hepatitis and to determine their CRP levels for correlation.

## Material and Methods

This Prospective study was conducted in the Department of Microbiology Gajra Raja Medical College and Jaya Arogya (J.A.) Group of Hospitals for a period of 12 months in 300 patients.

Ethical approval for the study was taken from the institutional ethical committee as per certificate no. 98/IEC-GRMC/2021. Patients who were either admitted in ward or presented in the outpatient department in J.A. Group of hospital were considered to be our potential subjects of study. Patients with clinical signs and symptoms consistent with hepatitis such as anorexia, nausea, vomiting, jaundice and fever were included in the study. Forty-four apparently healthy pregnant females attending antenatal clinic were also included among the 300 study subjects. Approximately 05 ml blood sample was collected from each patient after taking their informed consent. The samples were collected with aseptic precautions in plain vials. Blood samples were allowed to clot at room temperature (20-25 degrees) and centrifuged to separate serum. The separated serum was stored in refrigerator at 2-8 degrees and each sample was subjected to following serological tests:

**a) Hepatitis B surface antigen detection by Immunochromatography (ICT) based rapid test utilising** solid phase immune chromatographic technology for the qualitative detection of HBs Ag in serum. The test was a two site immune metric assay in which a combination of monoclonal and polyclonal antibodies was used for the detection of surface antigen of HBV.

**b) Anti-hepatitis C Antibody detecting test** a rapid immune chromatographic assay intended to be used for qualitative detection of antibodies to Hepatitis C virus. The nitrocellulose membrane of HCV Rapid Test was coated with recombinant Hepatitis C virus antigen representing core NS3, NS4, and NS5 at

the test line region 'T' and control protein for build visual control line at region 'C'.

### c) Estimation of C –reactive Protein:

Qualitative and semi-quantitative analysis of CRP was done by using CRP-Latex kit which utilised latex particles coated with monospecific anti-human CRP antibodies sensitized to detect levels greater than 6 micro g/ml of CRP. The samples which were positive for CRP by qualitative method were further subjected to the semi quantitative dilution method as per protocol. The titre was calculated by taking the highest dilution which gave visible agglutination and it was multiplied with the conversion factor 6 to get the CRP values in mg/l. e.g. If the titre was 1:16, the CRP concentration was calculated as  $16 \times 6 = 96$  mg/l.

### Data analysis

Data entries were done in the Microsoft excel software and tables were prepared for pictorial representation. Chi-square test and Fisher's exact test were performed for categorical variables and statistical significance was considered at p value less than 0.05.

### Result

A total number of 300 samples from patients having clinical signs and symptoms of hepatitis including 44 apparently healthy antenatal females were included during the study period of one year from July 2021 to June 2022. Out of 300, 19 samples were positive for hepatitis B surface antigen and 7 samples were positive for anti-hepatitis C virus antibodies. Thus, the seroprevalence of HBV and HCV in our study samples was found to be 6.3% and 2.3% respectively.

**Table 1: Prevalence of Hepatitis B and hepatitis C virus**

Test	Total Patient	Total Positive	Prevalence
HBs Ag	300	19	6.3%
HCV	300	07	2.3%

Out of 170 male patients tested for Hepatitis B Virus, 11(6.4%) patients were positive. Among pregnant females the HBsAg was positive in three females among 44 females. Among non-pregnant

females the positivity of HBsAg was 5.8%. Anti HCV antibodies were positive in 07 samples out of the total 300 samples. The category wise distribution of patients is shown in the table number 2.

**Table 2: Seroprevalence of HBV and HCV in different categories**

Category	Total Patients	Hbsag Positive	Anti Hcv Positive
Males	170	11(6.4%)	04(2.3%)
Females (Non- Pregnant)	86	05(5.8%)	02(2.3%)
Pregnant Females	44	03(6.8%)	01(2.3%)
Total	300	19(6.3%)	07(2.3)

**Table 3: Distribution of samples according to age**

Age In Years	Total	Hbs-Ag Positive (%)	Hcv Positive (%)
01- 10	13	01(7.6%)	00(0%)
11-20	22	00(0%)	01(4.5%)
21-30	77	06(7.7%)	01(1.2%)
31-40	59	04(6.7%)	03(5%)
41-50	57	03(5.2%)	01(1.7%)
51-60	41	03(7.3%)	00(0%)
>60	31	02(6.4%)	01(3.2%)

The present study revealed that patients of all age groups were affected by hepatitis B and hepatitis C viruses. The age wise distribution of the samples has been shown in table no 3. The most commonly affected age group was 20 to 40 years of age. Out of the total 300 samples were tested only 1 sample was positive for both HBsAg and HCV antibodies and 1 HCV positive

sample was also found positive for HIV antibodies.

Semiquantitative estimation of CRP assay was also performed. A value greater than 0.6 mg/dl was considered positive. Out of 300 samples CRP was positive in 102 (34%) samples. The correlation between CRP and HBsAg, Anti HCV antibodies positivity has been shown in table no 4.

**Table 4: Correlation between HBV, HCV and CRP.**

	CRP Positive	CRP Negative	
<b>HBsAg Positive</b>	<b>14</b>	<b>05</b>	<b>P value &lt;0.05</b>
<b>HBsAg Negative</b>	<b>88</b>	<b>193</b>	
<b>Anti HCV Ab Positive</b>	<b>05</b>	<b>02</b>	<b>P value &lt;0.05</b>
<b>Anti HCV Ab Negative</b>	<b>97</b>	<b>196</b>	

Our study showed that 14 samples were CRP positive and 05 samples were CRP negative out of 19 Hepatitis B positive patient. 5 samples were CRP positive and 2 were negative for CRP among 7 Hepatitis C positive.

### Discussion

Considerable variation in the seroprevalence of HBV and HCV has been noted in different geographical areas of our country. According to the national programme for surveillance of viral hepatitis 2021 factsheet which followed similar methods as our study the seroprevalence of HBV and HCV in Madhya Pradesh was 0.5-1% and 0.08% respectively.[10] In our study, we found that the prevalence of Hepatitis B surface antigen and hepatitis C virus antibodies was 6.3% and 2.3% respectively. This might be due to the fact that our study was hospital based and included mostly clinically symptomatic patients as study population.

We have also calculated the prevalence on the basis of different parameters which helped us to get a better idea of the prevalence of HBV and HCV in the patients attending our hospital.

Males have been observed to be more frequently infected with HBV as compared

to females in previous studies.[11] In our study also the percentage of HBsAg positive males was approximately three times the infected females while the positivity was similar in both genders in case of HCV. The present study revealed that patients of all age group were affected by hepatitis B and hepatitis C Virus infection. The most common affected age group was 21 to 40 year and the least effected age group was 0 to 20 years which indirectly indicates the impact of successful introduction of the HBV vaccine into the National Immunization Program in India on the prevalence of HBV. The seroprevalence of both HBV and HCV was also higher in pregnant females when compared with other studies.[12,13] This can be attributed to the small sample size and convenience sampling of the subjects. The pooled HBV/HCV co-infection prevalence was found to be 1.89% according to Desikan P et al while in our study co-infection of Hepatitis B and Hepatitis C had a prevalence of 0.33%.[14] CRP is an acute phase reactant synthesized in liver by hepatocytes in response to the stimulus produced by interleukins during inflammation and infection. In our study a statistically significant positive association was observed between CRP and HBV, HCV. In a meta-analysis the pooled CRP

mean level for hepatitis C were found to be in the normal range; however, serum level of CRP was found high in hepatitis B patients. The authors suggested that the pooled mean level of CRP could be useful for the determination of the prognosis of hepatitis B and its related complications.[15]

In another study a significant negative correlation of serum CRP level in both HBV and HCV patients was found.[16] Another study concluded that HBV infection upregulated the expression of the CRP gene. The serum level of CRP was significantly elevated in patients who were infected with HBV and was correlated with disease progression. These variations can be due to the timing of CRP assay after the clinical onset of infection in addition to the fact that different studies measured CRP quantitatively with different cut-off points. A negative correlation was more commonly observed in case of HCV. It was hypothesised that in the presence of long-standing HCV infection, which is quite common since the infection remains asymptomatic for a long time, the continued replication of the virus in the liver cells may decrease their ability to produce IL-6, and consequently CRP.[17]

### Conclusion

Hepatitis B and Hepatitis C are presently regarded as a cause of great concern to public health in India and other developing country. Every year thousands of people are affected to contribute to the burden of health care. It is important to carry out such studies to better elucidate the epidemiology of HBV and identify high prevalence areas and simultaneously focus on improving public health measures to prevent disease transmission and decrease the burden of the disease. The study can serve as the baseline data about circulation of Hepatitis B and Hepatitis C virus in our region. CRP levels were found high in both HBV and HCV. Further multi-centric

studies including large number of subjects are required to confirm these findings. Preventive measures including adequate sterilization of surgical instruments and intensive screening of blood and component for transfusion and mass campaign creating awareness in the public, and health workers can reduce the incidence of these infections.

**Ethical Committee** – Approval as per certificate no. 98/IEC-GRMC/2021 Dated on 02-05-2021

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