

## Original Research Article

# PREVALANCE, SEROLOGICAL & VIROLOGICAL PROFILE OF HEPATITIS B IN HEMODIALYSIS PATIENTS

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### ABSTRACT

**Background:** Hepatitis B is an important health problem in the world. It is estimated that 2 billion people have been infected in the world and more than 350 million people are chronic hepatitis B virus (HBV) carriers. Hemodialysis patients are at increased risk of this infection. **Aim of The Study:** To study the Prevalence, Serological & Virological profile of Hepatitis B infection in Hemodialysis patients.

**Materials and Methods:** A total of 150 subjects were included in the study. Serum was collected and used for detection of serological markers HBsAg, AntiHBs, HBeAg, AntiHBc & AntiHBe and also HBVDNA in serum samples. All samples were processed for detecting HBsAg, AntiHBs, HBeAg, AntiHBc & AntiHBe by using 3rd generation ELISA kits and Multiplex PCR for DNA detection.

**Results:** Out of 150 patients, 111 were males and 39 were females and the age of the patients included in the study ranges from 21 – 69 yrs and the mean age group is 45.2 years. Out of 150 patients, 5 patients got HBsAg positive, 47 were AntiHBs positive, 43 were Anti HBc positive, 22 were AntiHBe positive and none of the patients were positive with HBeAg. 0 samples were detected for HBV DNA.

**Conclusion:** Hepatitis B virus transmission is significant infection in immunocompromised population especially patients undergoing hemodialysis. Strict antiinfective measures like screening of blood, blood products and enhanced usage of Erythropoietin injections as a substitute to blood transfusions decrease the incidence of Hepatitis B in Hemodialysis patients.

**Keywords:** Hepatitis B, Hemodialysis, Serological markers, HbsAg.

## INTRODUCTION

Hepatitis B is an important health problem in the world. It is estimated that 2 billion people have been infected in the world and more than 350 million people are chronic hepatitis B virus (HBV) carriers.<sup>[1]</sup> According to WHO, Hepatitis B can be spread by needle stick injury, and exposure to infected blood and body fluids.<sup>[2]</sup> Hemodialysis patients are at risk of this infection. CKD is a broad term that describes kidney damage or a decrease in Glomerular filtration rate. It is associated with decreased quality of life, increased health care expenditures, and prematurely

death.<sup>[3,4]</sup> Hemodialysis (HD) is a procedure used for patients who are suffering with chronic renal damage for long term or permanent renal replacement therapy.<sup>[4,5]</sup> It increases the risk of transmission of blood borne viral infections such as Hepatitis B and Hepatitis C. As such CKD is an immune deficient state, Hepatitis C and Hepatitis B are important viral infections causing morbidity and mortality in maintenance hemodialysis patients.<sup>[6,7]</sup> Nosocomial transmission of HBV infection is a major cause for acquirement of infection in these patients.<sup>[8,15]</sup> Prevalence of HBV in the Indian population undergoing HD varies between 3.4 - 43%.<sup>[9]</sup> Studies shown that the prevalence of the

hepatitis B infection is higher in hemodialysis patients than general population.<sup>[9,10]</sup> Studies suggest that HBsAg positive dialysis patient seem to suffer from a high risk of developing active progressive liver disease and it runs a characteristic course with relatively less degree of Hepatic inflammation. The major complications developed are Chronic hepatitis, Cirrhosis and Hepatocellular carcinoma. A retrospective study in India, reported a higher Mortality and morbidity among HBsAg positive patients compared to HBsAg negative dialysis groups. Some studies report no major difference in mortality in HBV infected dialysis patients as compared to HBV negative HD patients.<sup>[10,11]</sup>

The present study was conducted at Gandhi Medical College/Hospital, Secunderabad and is aimed to study the Prevalence, Serological & Virological profile of Hepatitis B Virus infection (which includes detection HBsAg, Anti HBs, Anti HBc, HBeAg, Anti HBe) and Hepatitis B virus DNA in patients undergoing Hemodialysis.

### Objectives

1. To detect prevalence of Hepatitis B infection in Hemodialysis patients enrolled in hemodialysis unit of Gandhi hospital, Secunderabad.
2. To detect all Serological markers of Hepatitis B infection.
3. To detect HBV DNA in HBsAg positive & AntiHBc ab positive serum samples.

## MATERIALS AND METHODS

This study was conducted in Gandhi hospital, Secunderabad.

**Study design& period:** it is a Descriptive Observational study, done during the period of one year from May 2018 to May 2019

**Inclusion criteria & Exclusion criteria:** Patients under chronic hemodialysis of all age groups of both the sexes enrolled in Hemodialysis unit in Gandhi

The above mixture is processed for thermo cycler. PCR was done using the following primer sequences

Primers	Sequence (5'-3')	Nucleotide position
S1-F	5'CATCAGGATTCTAGGACCCCT-3'	168-199
S2-R	5'-CTTGTTGACAAGAATCCTCACA-3'	214-235
C5-F	5'-TCACCTCTGCCTAATCATC-3'	1825-1843
C6-F	5'-TTCAAGCCTCCAAGCTGTGCC-3'	2391-2371
C7-R	5'-GAGGGAGTTCTTCTTAGG-3'	2391-2371
C8-R	5'-AGGAGTGCGAATCCACTCC-3'	2277-2267

### The following temperatures are used for PCR protocol

Denaturation	94°C	5 minutes
For 40 cycles		
Denaturation	94°C	1 minute
Annealing	55°C	1 minutes
Elongation	72°C	2 minutes
Final elongation	72°C	10 minutes

### Gel electrophoresis

The amplified PCR products are subjected to Agarose gel Electrophoresis (1.5%).

### Gel documentation

The electrophoretic run is visualized in U.V illuminator and separated DNA gives an orange red fluorescence. These results are documented using a gel doc.

hospital. Patients under peritoneal dialysis are excluded.

**Sample size & collection:** A total of 150 samples were collected from patients enrolled in hemodialysis unit.

A total of 8 ml of blood was collected in two red top (plain) vacutainers under strict aseptic conditions. Centrifugation of the collected samples was done at a speed of 3000rpm for 15 minutes duration and a clear serum was obtained. The obtained Serum was separated and aliquoted in to separate aliquots and stored at minus 80 degrees centigrade. The aliquoted serum was used for detection of serological markers HbsAg, AntiHBs, Anti HBc, HBeAg, AntiHBe. A separate aliquot was kept for HBV DNA detection. Institutional ethical committee clearance was obtained prior to commencement of the study. An informed consent was obtained from all the patients enrolled in the study.

### Serodiagnosis

All samples were processed for HBsAg, HBeAg, Anti HBc Ab, Anti HBe Ab, Anti HBsAb, using third generation ELISA kits. For HBsAg (ERBA LISA SEN), HBeAg, Anti HBc Ab, Anti HBe Ab (Immu Check), Anti HBs Ab (Dia pro) kits were used. The procedure was strictly followed as per kit manufacturer's instructions. Results of all the parameters are interpreted as either Positive or negative (Qualitative ELISA).

Hepatitis B virus detection in positive (HBsAg, Anti HBc) samples.

### DNA Detection

#### DNA extraction:

All HBsAg, AntiHBc positive samples were subjected to DNA Extraction of QIAGEN kit method.

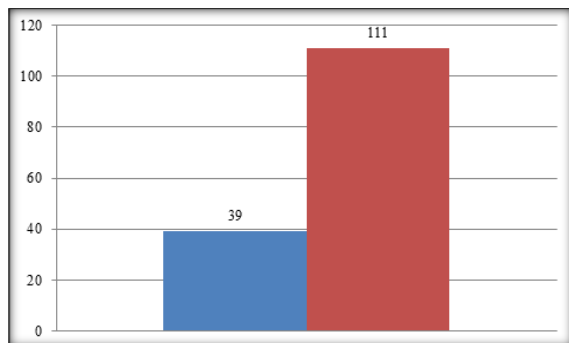
#### Multiplex PCR run for extracted DNA

PCR mastermix (containing NTP'S, DNA polymerase), nuclease free water and forward & reverse primers are added to extracted DNA.

## Data arrangement

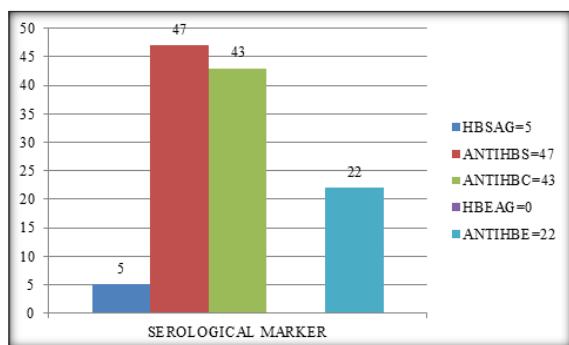
The collected data was organized under Microsoft Excel Sheet and analysis done in percentages

## RESULTS



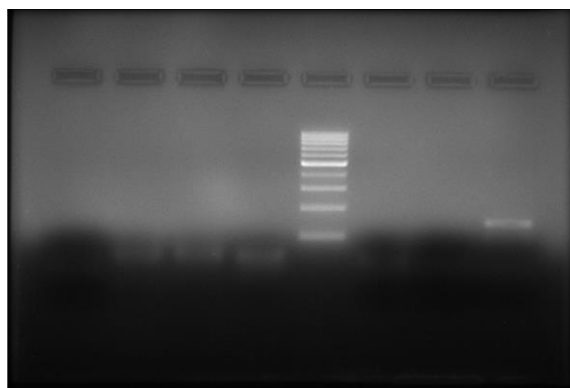
**Figure 1: Distribution of males & females affected with hepatitis-B in hemodialysis patients**

MALES=111  
FEMALES=39



**Figure 2: Distribution of hepatitis B serological markers in hemodialysis patients**

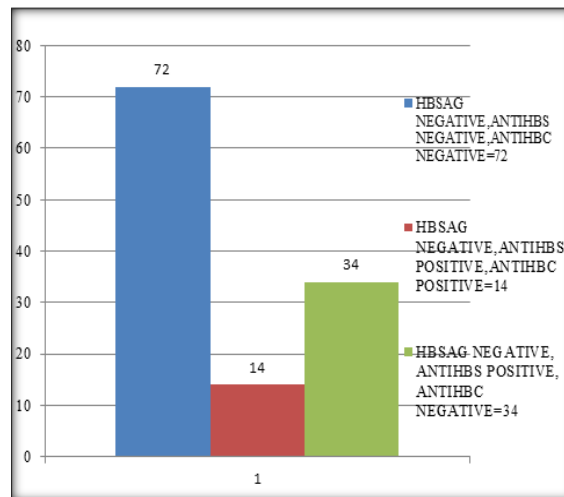
HBSAG = HEPATITIS B SURFACE ANTIGEN, HBEAG = HEPATITIS B “E” ANTIGEN, ANTIHBS = ANTIBODY FOR SURFACE ANTIGEN, ANTIHBC = ANTIBODY FOR CORE ANTIGEN, ANTIHBE=ANTIBODY FOR HEPATITIS B “E” ANTIGEN



**Figure 3: Multiplex PCR analysis of HBsAg Seropositive samples**

From left to right, Lane 1 to 4 & Lane 6 show patient samples

Lane-5: 100bp ladder, Lane-7: negative control, Lane-8: positive control.



**Figure 4: Evaluation of hepatitis b serological markers in hemodialysis patients**

A total of 150 patients under Chronic Hemodialysis enrolled in hemodialysis unit of Nephrology department are included in the study.

A Total of 111 males and 39 females are included in the present study. The male to female ratio being 2.84: 1. [Figure 1]

The mean age of the study population was 45.2 yrs. The Age of the patients included in the study varied ranges from 21 – 69 yrs. [Table 4]

Out of 150 patients 25 had a history of vaccination, 3 had a history of blood transfusion. The average duration of Dialysis of the study population being 6.8 years. [Table 1]

72 patients were negative for HbsAg antigen, AntiHBc, AntiHBs antibodies.14 patients were negative for HbsAg antigen, & positive for both antiHBs and antiHBc antibodies.34 patients were hbsAg antigen negative, antiHBc antibody negative, antiHBs antibody positive. [Figure 4]

Out of the 150 study population, 5 were Positive for HBsAg. 47 were positive for Anti HBs antibodies, 22 were positive for Anti HBe antibodies, and 43 were positive for Anti HBc antibodies. [Figure 2]

### MULTIPLEX PCR FOR DETECTION OF HBV DNA

Multiplex PCR was run for 5 seropositive cases of HBsAg and 35 seropositive cases of Anti HBc Ab. HBV DNA was not isolated among them. [Figure 3]

**Table 1: Risk factors associated with HBV in hemodialysis patients in the study group**

S.NO	RISK FACTOR	N=150
1.	GENDER	111 (M) > 39 (F)
2.	BLOOD TRANSFUSION	3/150

3.	DURATION OF DIALYSIS (AVERAGE)	6.8 YEARS
4.	HIGH RISK BEHAVIOUR (H/O needle stick injury)	2/150
5.	H/O OF VACCINATION AGAINST HEPATITIS B	25/150

**Table 2: The prevalence studies of hbsag seropositive cases in different geographical areas and percentage positivity**

S.NO	Author	Place	Percentage	Year
1.	Present study	Gandhi hospital Secunderbad	3.3%	2018
2.	Morteza <i>et al</i>	Iran	1.2%	2016
3.	Jamil <i>et al</i>	Shillong, India	2.17%	2015
4.	Perumal <i>et al</i>	Coimbatore, TN	6%	2015
5.	Filiz kizilates <i>et al</i>	Turkey	1.52	2014
6.	Pradip <i>et al</i>	Tripura	5.5	2011
7.	Kranthi kosaraju <i>et al</i>	Karnataka, India	1.52	2004-2012
8.	D. Saha and S. Agarwal <i>et al</i>	India	3.4-4.2%	2001

**Table 3: Age and sex wise distribution of patients (n=150)**

AGE	MALE	FEMALE	TOTAL
20-40YEARS	46	18	64
40-60YEARS	46	15	61
TOTAL	111	39	150

**Table 4: Prevalence of HBsAg positivity and HBsAg negativity in different age groups**

Age	HbsAg positive	HbsAg negative	Total
20 – 40	3	61	64
40 – 60	2	59	61
> 60	0	25	25
TOTAL	5	145	150

**Table 5: Seropositivity of anti HBsAb as per gender**

GENDER	ANTI HBs Ab Positive	ANTI HBs Ab Negative	TOTAL
MALE	35	76	111
FEMALE	12	27	39
TOTAL	47	103	150

ANTIHBs POSITIVE= Antibody to hepatitis B surface antigen Positive

ANTIHBs NEGATIVE=Antibody to hepatitis B surface antigen Negative

## DISCUSSION

Hepatitis B virus belongs to Hepadnaviridae family and genus Orthohepadna virus. Hemodialysis remains main stay of treatment in CKD patients. Major studies were done in estimating the risk of HCV in Hemodialysis patients. Studies on HBV infection in hemodialysis patients is limited. In our study, we observed serological profile of HBV infection in hemodialysis patients.

In the present study out of 150 HD patients 5 patients were found positive for HBsAg by ELISA and the prevalence rate was 3.3 %. The low prevalence rate correlates with the studies done by Jamil *et al*,<sup>[9]</sup> (2.17%), Morteza *et al*,<sup>[12]</sup> (1.2%) On the contrary, Perumal *et al*,<sup>[7]</sup> reported high rate of 6% of HbsAg seropositivity.

The mean age of the study population was 45.2 years which was correlating with study done by Pradip *et al*,<sup>[5]</sup> in which mean age group is 48.16 years and study done by Morteza *et al*,<sup>[12]</sup> Iran in which the mean age group was 48.94 .

The Male to female ratio of the patients included in our study was 2.84:1, which is in accordance with the study done by Morteza *et al* (3:1).<sup>[12]</sup>

Pradip *et al*,<sup>[5]</sup> Reddy *et al*,<sup>[13]</sup> Jamil *et al*,<sup>[9]</sup> in their studies suggested that blood transfusion was a major risk factor in development of HBV infection. Kranthi kosaraju *et al*,<sup>[14]</sup> in their study observed that the low prevalence of HBsAg positivity in their sample population was due to decreased blood transfusions and usage of erythropoietin as a substitute for it. The same was discussed by Renata C Ferreira *et al*,<sup>[11]</sup> in their study. Perumal *et al*,<sup>[7]</sup> Jalal *et al*,<sup>[9]</sup> on the contrary, conferred no significant effect of transfusions on HBV positivity in their study.

Kranthi kosaraju *et al*,<sup>[14]</sup> in their study observed that increased duration and frequency of dialysis was one of the important risk factors in development of Seropositivity for HBsAg. A similar opinion was suggested by Renata C Ferreira *et al*.<sup>[11]</sup> Pradip *et al*,<sup>[6]</sup> in their study conferred that the development of New HBsAg positive cases were due to lack of vaccination. Reddy *et al*,<sup>[5]</sup> Kranthi kosaraju *et al*,<sup>[14]</sup> in their study emphasized the introduction of vaccination for HBV as an important intervention in prevention of HBV infection in HD patients.

In the present study the low prevalence of HBsAg positivity might be due to vaccination of the patients prior to HD, usage of novel method like administration of Erythropoietin injections as

substitute to blood transfusion, strict adherence of infection control protocols such as usage of dedicated equipment for seropositive cases of HBV and HCV, testing for HBsAg status as a mandate prior to every dialysis procedure.

Renata C Ferreira *et al*,<sup>[1]</sup> (2002) from Central Brazil studied levels of HBsAg, AntiHBsAb, AntiHBC antibodies in 1095 dialysis patients and found that 24.8% patients showed anti HBs and Anti HBC positivity, 2.5% showed positivity for anti HBC alone, 2.4 % of patients were positive to both HBsAg and Anti HBCAb. Based on the above they determined the HBV surveillance as 29.8%.

In the present study 5 patients were HBsAg positive, 72 patients (48%) were negative for HBsAg, Anti HBs antibodies and Anti HBC antibodies suggesting that these patients are susceptible for HBV infection. 14 patients (9.3%) were negative for HBsAg, Positive for Anti HBs antibodies and Anti HBC antibodies suggesting that these patients are immune due to natural infection. 34 patients (22.6%) were negative for HBsAg, Positive for Anti HBs antibodies and negative for Anti HBC antibodies suggesting that these patients immune due to prior vaccination.<sup>[16]</sup>

Fabrizi *et al*,<sup>[17]</sup> in their study (2000 – 2004) on 585 patients found a high rate of Anti HBC Ab in dialysis patients. They stated clinical significance of this Isolated anti HBC Antibodies in dialysis patients is unclear and a certain proportion of Anti HBC Ab positive patients might be false positive depending on the test methodology used. They also say that it reflects a late immunity. Jalal *et al*,<sup>[16]</sup> (2012) in their study at Iran on 412 HD patients and 4.6% patients were positive for isolated Anti HBCAb.

Yakaryilmaz *et al*,<sup>[21]</sup> (2006) in their study at Turkey in 188 HD patients, found an isolated Anti HBC Antibodies positive in 6.4% of all patients. They inferred that a certain proportion of these may be false positives may be due to Anti HBC test methodology used. In the present study 17.3 % of patients were positive for isolated Anti HBC Antibodies.

Based on the findings of study by Fabrizi *et al*,<sup>[17]</sup> we infer that this Anti HBC antibody positivity might be IgG response indicating a previous infection or on the other hand some of the positives may be false positives based on the methodology used and an alternative more sensitive method might reveal their false positive status.

Fabrizi *et al*,<sup>[17]</sup> in their study done in 2002-2004 stated that the patients had high rate of Antihbc antibodies in dialysis patients. HBV DNA was not isolated in them stating that it was not a persistent infection. Jalal *et al* (2012,<sup>[15]</sup> Iran in their study on 412 hemodialysis patients ,4.6% were positive AntiHBC antibodies but HBVDNA was not isolated in them. In the present study, 17.3% patients were positive for antihbc antibodies and HBVDNA is not isolated. Based on the findings of study by Fabrizi *et al*,<sup>[17]</sup> we infer that high core antibody (AntiHBC) may be due to false positives based on the

methodology used & alternative method may reveal false positive status.

The study done by Filiz kizilates *et al*,<sup>[18]</sup> on 1347 HD patients & Zahedi *et al*,<sup>[19]</sup> of 228 patients HBV DNA was isolated. In our study HBV DNA was not isolated in HBsAg positive serum samples.

In the present study though HBsAg was positive in 5 patients out of 150 HD patients studied. In our study among the five HBsAg positive samples none of them were positive for HBeAg and HBVDNA indicating that there was no active viral replication in them & low viral load. The other causes may be variation in the geographical area and the population studied.

## CONCLUSION

1. Hepatitis B virus transmission is of significance in immunocompromised individuals such as those undergoing dialysis. Adherence to anti-infective strategies such as screening of blood and blood products screening for HBsAg before hemodialysis, dedicated equipment for HBsAg positives or seropositive cases.
2. Also vaccination of all HD patients with more frequency & enhanced dosage and enhanced usage of erythropoietin injections as a substitute to blood transfusions, will surely decrease the prevalence of Hepatitis B infection in patients on dialysis.
3. Patients with CKD should be screened annually to detect decline of AntiHBs titres and additional doses have to be administered accordingly.<sup>[20]</sup>

**Declaration:** I hereby declare that the work done is genuine and not copied or manipulated.

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### Conflicts of Interest:

The authors declared that there are no conflicts of interest.

### Ethical Clearance Statement:

The present study was done after taking ethical approval. (Ref no: IEC/GMC/2018/3/13 dated 27th July 2018).

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