

**A DEMOGRAPHIC STUDY OF HEPATITIS B IN HEALTHY POPULATION OF CENTRAL INDIA**Gajendra Pal Singh Uike<sup>1</sup>, Amar Bahadur Singh<sup>2</sup>, Uday Raj Singh<sup>3</sup>, S. K. Mishra<sup>4</sup>**HOW TO CITE THIS ARTICLE:**

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**ABSTRACT: BACKGROUND:** In central India (M.P.) there is high prevalence of hepatitis; of which hepatitis B contributes as a major cause of hepatitis. In our area there were no major efforts to contain the disease. According to one estimate most of the hepatitis B occurs due to negligence transfusion of infected blood (Hepatitis B). Therefore we have tried to found percentage of population infected with this disease. **MATERIAL AND METHODS:** Study design – Prospective study. For screening donors we have used one step rapid test for the detection of HBsAg and confirmatory test was done by ELISA method. **STATISTICAL ANALYSIS:** Statistical analysis was done by using Minitab 17 Pennsylvania, USA. **RESULTS:** In our study we found 2.8% HBsAg infected blood among healthy donors; which was significantly ( $P < 0.05$ ) more in voluntary donors in comparison to replacement donors. Male between age groups 39-48 were found highly infected in comparison with other age groups. We also found prevalence was highest in Hindu community (2.9%) and more in working group (3.9%) in comparison to students and farmers. **CONCLUSION:** For effective control of transfusion of infected blood and to prevent its spread, we have to improve community knowledge towards vaccination; educational programs for blood bank personals; and government should open blood banks at Community Health Center level.

**KEYWORDS:** Prevalence of Hepatitis B, blood transfusion associated infection screening of hepatitis B.

**INTRODUCTION:** Hepatitis is term to describe a nonspecific liver inflammation. Liver is the largest and most complex internal organ of human body. It performs wide range of functions ranging from detoxification of hormonal substances to synthesis of various products. Naturally liver is vulnerable to a wide variety of insults- metabolic, inflammatory, toxic, microbial, circulatory and neoplastic. Among the patients suffering from liver diseases, viral hepatitis is a common clinical condition.<sup>1</sup>

Viral hepatitis has been a part of recorded history since the time of Hippocrates (Bockus Gastroenterology, 5<sup>th</sup> Edition) for centuries; epidemics of hepatitis have devastated military and civilian communities throughout the world.<sup>2</sup> Initially transmission of disease was attributed to non-parenteral routes, such as person to person contact or exposure to contaminated food or water. This was known as epidemic (infectious) hepatitis.<sup>3</sup>

However in 1885, in Bremen, Germany significant event occurred. Nearly 200 shipyard workers become jaundiced several months after vaccination against small pox with human lymph. Also there was no jaundice recorded in unvaccinated persons. This suggested another mode of transmission of disease with long incubation period and by parenteral route (Lurman 1885).<sup>4</sup>

Over the next century viral hepatitis was classified into two diagnostic categories- one infectious hepatitis and another serum hepatitis. Later on the names 'Hepatitis A' and 'hepatitis B' for serum hepatitis were proposed (Mac Callum, 1947).<sup>5</sup>

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No further progress in the knowledge of viral hepatitis was made until 1965, when Blumberg in Philadelphia, discovered an unusual antibody in the serum of hemophiliacs, who had received multiple blood transfusions. This antibody reacted with sera of an Australian aborigine but not with variety of sera from other people. Blumberg et al, therefore named this antigen present in sera of Australian aborigine as 'Australia antigen' This antigen is now known as the Hepatitis B surface antigen (HBsAg).<sup>6</sup> Years later in 1973, Feinstone et al discovered hepatitis A virus, the agent responsible for infectious (Epidemic) hepatitis.<sup>7</sup>

The majority of cases of hepatitis until 1975 were attributed to either HAV or HBV. But still there were some cases of hepatitis particularly post transfusion hepatitis, which could not be accounted for by either of these viruses. And to explain these cases name of "Non A, non B hepatitis (NANBH) was proposed by Feinstone in 1975.<sup>8</sup>

In 1977 another breakthrough occurred when Rizzetto identified another virus responsible for viral hepatitis and named it as Delta agent (Hepatitis D virus). This virus was an incomplete virus because it was responsible for transmission of the blood borne type of hepatitis in combination with HBV only and not individually.<sup>9</sup>

Initially the diagnosis of Non-Non-B hepatitis was made by excluding HAV and HBV infections. This led to the search of causative agent of NANBH. Various studies revealed that NANB agent is not a single virus but different viruses responsible for different types of NANBH.

These cases were then identified by Balayan<sup>10</sup> (In Russia, 1983) and Houghton.<sup>11</sup> (In USA, 1988) as to be caused by two different viruses, HEV and HCV respectively. Balayan isolated hepatitis E virus responsible for water-borne NANB hepatitis and Houghton isolated hepatitis C virus causing blood-borne NANB hepatitis. Later in 1989, Choo et al cloned the hepatitis C virus.<sup>12</sup>

The study has been undertaken to find out the prevalence of hepatitis B surface antigen & hepatitis C virus infection in the blood donors attending the blood bank of Gandhi Memorial & Sanjay Gandhi Memorial Hospital, Rewa associated with department of Pathology Shyam Shah Medical College Rewa (M.P.).

**MATERIAL AND METHOD:** This study was carried out to assess the prevalence of HBsAg infection among the blood donors, who are attending blood bank for blood donation; by detecting antibodies to HBsAg in their blood firstly by rapid kits and confirmed by ELISA method. The blood donors (voluntary/replacement) attending the blood bank of Sanjay Gandhi Memorial Hospital, Rewa were selected. This study was carried out from September 2013 to June 2014 in the department of Pathology, S.S. Medical College, Rewa (M.P.).

In our study 5281 samples were collected and screened for the presence of antibodies to HBsAg by rapid chromatographic immunoassay test strips (HEPAVIEW HBsAg QUALPRO DIAGNOSTICS, TULIP GROUP, GOA, INDIA). The reactive samples are further confirmed by Enzyme Linked Immunosorbent assay (Micro screen HBsAg ELISA KIT, Span Diagnostics Limited, SURAT, GUJARAT, INDIA).

Methodology of HBsAg One Step Rapid Kit Test (According to manufacturer's instruction) Rapid test kit for HBsAg is an in -vitro immunochromatographic, one step assay designed for qualitative determination of HBsAg in human serum or plasma. This test cassette contains a membrane strip, which is pre - coated with mouse monoclonal anti - Hbs capture antibody on test band region. The mouse monoclonal anti - HBs - colloid gold conjugate and serum sample moves along the membrane chromatographically to the test region (T) and forms a visible line as the

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antibody-antigen reaction resulting in the formation of pink/purple colour line on the test region which confirms the presence of HBsAg. The test cassette has a letter of “T” and “C” as “Test Line” and “Control Line” on the surface of the cassette.

### **Methodology of Microwell ELISA Method for HBsAg:**

**Principle:** The test is based on solid phase microplate direct ELISA (Sandwich ELISA) technique. The microwells are coated with mouse monoclonal anti-HBsAg antibodies. When patient's sample is added, if HBsAg is present in the sample, it gets bound to immobilized anti-HBsAg antibodies. After removal of non-specific antigens, peroxidase labeled anti-HBsAg antibodies are added, which bind with immobilized antibody-antigen complex. After incubating and washing. TMB (Tetramethyl benzidine) substrate is added, which gives blue coloured product due to enzymatic activity. In absence of HBsAg, the colour will not change from its original pink colour. The reaction is stopped by addition of stopping reagent, which converts the blue color to end point yellow color. In a negative test, original pink colour as seen in the above step will now become colourless. The absorbance of yellow colour is directly proportional to the concentration of HBsAg present in patient's sample. It is measured by microwell plate reader having 450nm as primary filter and 630nm as reference filter.

**PROCEDURE:** (According to manufacturer's instruction) Bring samples and all the reagents except color reagent and conjugate to room temperature before starting the procedure:

1. Dispense 100 $\mu$ l of sample Diluent to required number of wells except Blank well. (leave Blank well empty)
2. Add 100  $\mu$ l Negative control to B, C, D, and 100  $\mu$ l Positive controls to E, F. Add 100  $\mu$ l of Samples to rest of the wells, pipette up and down for mixing, seal strips securely with Adhesive strip covers.
3. Incubate for 60 minutes at 37 $^{\circ}$ C.
4. Remove and discard the Adhesive strip cover. Decant the content of wells into waste container. Fill the wells with approximately 350  $\mu$ l of Diluted washing Buffer and allow soak time of 30 seconds (programme the auto washer for soak time of 30 seconds) and then decant it in the waste container. Repeat the washing steps for 4 more times. Drain wells on a disposable absorbent pads or towels and tap firmly to remove excess of water.
5. Add 50  $\mu$ l of Conjugate Stabiliser followed by addition of 100  $\mu$ l Conjugate to each well except A (Blank well). Mix the contents of microwells by agitating the strips gently for 5-10 seconds and seal strips securely with Adhesive strip covers.
6. Incubate for 30 minutes at 37 $^{\circ}$ C.
7. Repeat step 4 for washing.
8. Add 100  $\mu$ l of colour reagent to the all the wells including Blank well. Seal strips securely with adhesive strip covers.
9. Incubate for 30 minutes at room temperature in dark.
10. Remove and discard Adhesive strip cover and add 100  $\mu$ l of stopping solution to each well. Mix the microwells by agitating the strips gently for 5-10 seconds. Blue color of Positive control/ reactive samples will turn to yellow on addition of stopping solution.
11. Read the absorbance within 30 minutes with microwell plate reader using 450nm as primary filter and 630nm as reference filter after blanking with A well(reagent well) and calculate the results.

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**ETHICAL CLEARANCE:** Ethical clearance was taken by institutional ethical committee Shyam Shah Medical College Rewa (M.P.) written consent was taken by all patients and donors, involved in the study.

**STATISTICAL ANALYSIS:** Statistical analysis was done by using Minitab 17 Pennsylvania, USA. For non-parametric data chi - square test was done. P- Value <0.05 has taken as significant for all analytical studies.

### RESULTS:

Type of Donor	No.	HBsAg Positive Cases	Prevalence %
Voluntary Donor	4431	137	3.0
Replacement Donor	850	14	1.6
<b>Total</b>	<b>5281</b>	<b>151</b>	<b>2.8</b>

Table 1 : Prevalence of HBsAg according to type of donor

**Statistical Inference for HBsAg:** Chi-square test between voluntary and replacement donor  $X^2 = 6.1$  and  $p = 0.013$  (Difference is significant since <0.05) Table 1 shows the prevalence rate of HBsAg was 2.8%. The voluntary donors accounting 3.0 % prevalence which is significantly ( $p < 0.05$ ) more than replacement donors having 1.6% of prevalence.

Gender	No.	HBsAg Positive Cases	Prevalence %
Male	4623	143	3.0
Female	658	8	1.2
<b>Total</b>	<b>5281</b>	<b>151</b>	<b>2.8</b>

Table 2 : Prevalence of HBsAg Positive cases according to gender

Table-2 shows overall prevalence rate of HBsAg infection in male and female was 2.8%. The prevalence rate of HBsAg in Male (3%) was higher than Female (1.2%).

Age Groups	HBsAg Positive Cases	Prevalence %	Male	Female
18-28	32	21.1%	31	1
29-38	43	28.4%	40	3
39-48	49	32.4%	47	2
49-58	27	17.8%	25	2
<b>Total</b>	<b>151</b>	<b>100%</b>	<b>143</b>	<b>8</b>

Table 3 : Prevalence of HBsAg according to Age Range

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Table 3 shows prevalence of various age groups in donors. The prevalence of HBsAg in age group range between 18-28 years was 21.1%, 29-38 years was 28.4%, 39-48 years was 32.4% and 49-58 years was 17.8%. The prevalence of HBsAg was higher in age group 39-48 years it was 32.4%.

Occupation	No.	HBsAg Positive Cases	Prevalence %
Working	1940	77	3.96
Student	1673	49	2.9
Farmer	912	8	0.87
Others	756	17	2.24
<b>Total</b>	<b>5281</b>	<b>151</b>	<b>2.8</b>

**Table 4 : Prevalence of HBsAg according to Occupation**

Table 4 shows the prevalence of HBsAg in donors based on their occupation. The prevalence of HBsAg in working population was 3.96%, in student it was 2.9%, in farmer it was 0.87%, and in others (laborer, house- wives etc.) it was 2.24%. Higher prevalence was found in working population.

In our study it was also found that the prevalence of HBsAg in Hindus was 2.9 % and in Muslim it was 1.6% where as in others it was 0%.

**DISCUSSION:** The present study was done on 5281 healthy blood donors attending the blood bank Sanjay Gandhi Memorial Hospital, Rewa from September 2013 to June 2014 were selected. First they screened with rapid card test; donors who show reactivity are further tested with ELISA 3<sup>rd</sup> generation. The overall prevalence of HBsAg infection was 2.8%.

In India, various studies have been done to determine prevalence of HBsAg with other blood bore diseases. On blood bank healthy blood donors studies; the overall prevalence of HBsAg ranges between 0.063 to 3.51%. Thus, prevalence observed in our study was within the range of national prevalence rate. Results obtained by various workers in India have been shown in the tables along with results of the present study.

SL. No.	Author	Year	Place	No. of cases	Prevalence rate (%)
1	Riddhi Jaiswal et al <sup>13</sup>	1997-2005	GSVM Medical college Kanpur	1,15,073	1.7
2	Karandeep singh et al <sup>14</sup>	2005-07	Kasturba Medical college, Manipal	30,428	0.74
3	Yedlapati Bhawani et al <sup>15</sup>	2004-09	KIMS HOSPITAL(A.P.)	8,097	1.41
4	Nagarekha Kulkarni <sup>16</sup>	2005-09	Vijayanagara Institute of Medical Sciences, Bellary Karnataka	19,135	0.063
5	Sayed A. Quadri et al <sup>17</sup>	2010	Al Ameen Medical college, Bijapur	4,283	1.63

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6	R. Shyamala et al <sup>18</sup>	2012	Bhaskar medical college Yenkapally Ranga Raddy District	1,980	1.06
7	Sastry jayagowri Met al <sup>19</sup>	2008- 13	Charitable hospital blood bank in Pune	13,078	1.23
8	Nilima Sawke et al <sup>20</sup>	2006- 08	People's College Of Medical Sciences & Research Centre Bhopal	5,008	2.9
9	Dharmesh Chandra et al <sup>21</sup>	2009- 13	Gajra Raja, Medical College, Gwalior	67,132	3.51
10	Nirali shah <sup>22</sup>	2006 - 13	Sheth V.S. General Hospital, Ahmedabad.	92,778	0.887
11	Present study	2013- 14	S.S. Medical College Rewa	5281	2.8

**Table 5 : Indian Studies (Hbsag Prevalence Rate in Blood Donors)**

The results of present study for HBsAg sero-prevalence was similar to findings made by Nilima sawke et al.<sup>20</sup> (2006-08) from Bhopal; She studied 5008 blood donors ( Voluntary & Replacement ) from January 2006 to December 2008 at blood bank People's College Of Medical sciences & Research Centre Bhopal by using ELISA kit and found a prevalence of 2.9% (149/5008).

The HBsAg prevalence of 2.8% seen in present study is within the range 0.063 – 3.51 according to Indian studies. Out of the various factors which need to be considered to explain this prevalence rate with other Indian studies are the epidemiology, demographic profile and social milieu of the region. No previous studies from this region are available for comparison. The nearest studies available from central India are by Nilima sawke ET al<sup>20</sup> (2006-08) from Bhopal and from Dharmesh Chandra ET al.<sup>21</sup> (2009-13) from Gwalior, which have reported prevalence rates 2.9% and 3.51% respectively.

Second factor is the effect of study design especially the size of the sample population, type of the donor (Voluntary/ Replacement) and efficacy of the assay used.

Riddhi Jaiswal et al<sup>13</sup> studied for nine year from January 1997 to December 2005 screened with ELISA kit on 1,15,073 blood donors and found overall prevalence of 1.7% and found prevalence between male and female 1.64 and 1.57% respectively; but in present study it was 3.0 and 1.2 respectively. In present study it was higher because of different epidemiological region.

In our study maximum donors presented in the age group 39-48 years (32.4%) for HBsAg prevalence.

Similar findings was found by Sayed A. Qadri et al<sup>17</sup> in 2013 at Bijapur karnatka (31.1%) but it was more in elder group that is 51-60 years. Fathi Abed A l-Gani<sup>23</sup> in 2011 in Jordan found prevalence of HBsAg 33.3% also in elder group > 50 years.

### **The cause of high prevalence in younger age in my study:**

1. Low education level - This region is not having 100% literacy so not all population properly know about these infections and its complications.
2. Un- hygienic habit – people using un-sterilized syringing, intra venous drug users because of more frequently used.
3. No vaccination by government to prevent it (Not included in national vaccination programs.)

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4. Lack of proper screening facility before blood transfusion so that professional donors not excluded by screening.

In our study we found that prevalence is more in working population (Employed population) in case of HBsAg (3.96%).

### **Causes for high prevalence in employed and student Group:**

1. Lack of proper awareness towards blood borne diseases.
2. Use of intra venous drugs by employed population and students. It's emerging fashion among these groups.
3. Lack of motivational programs to discourage use of narcotic drugs.
4. There are no strict punishments for drug suppliers.
5. Lack of use of condoms and other protective measures during sex.
6. Higher prostitution practice in Rewa region.

In our study we found that prevalence was more in hindu population 2.9% (And 1.69% in muslims; 0% in other community)

The reason for high prevalence in Hindu community is:

1. Due to high population of hindu community, this community is on greater risk of receiving infection through blood transfusion.
2. There is taboo that on donating blood they would suffer from weakness lifelong. Therefore in state of giving blood to their love-ones they prefer to take blood from addicted professional blood donors who were mostly infected by HBsAg and HCV infectious diseases.

Based on the results and the discussion the following conclusions are drawn - The overall prevalence of HBsAg (2.8%) shows that; it may be due to low literacy rate, so the educational level of population in this region has to be increase so that they aware about these infectious disease to reduce its transmission:

1. There is need to educate the people for proper hygienic habits such that- to avoid common use of syringes, avoid intra venous drugs, proper disposable of infected biohazards, wearing gloves on handling the infected samples.
2. There is need to add of vaccination for HBV in immunization schedule to reduce its prevalence in community.
3. In hospital; medical worker has to be trained, how to exclude the professional donors so that it stop the channel of transfusion of infected blood, and also to community to motivate for blood donation.
4. In case of prostitution behaviour; condoms and the other protective measures should be used to avoid blood borne diseases.
5. Younger have to be educate and discourage about harmful effects of narcotic drugs by using common syringes.
6. There is needed to make severe punishable laws towards drug suppliers to protect young population.

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7. There is need to organize more motivational programs to motivate blood donation, by which spread of infection can be checked, by avoiding blood donation by drug-abusers.

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