

SEROPREVALENCE OF HBSAG AMONG BLOOD DONARS IN AND AROUND BHOPAL

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ABSTRACT

Hepatitis is a global health problem. About 350-400 million peoples are chronically infected with Hepatitis B in the world. It is important not only because it is associated with acute and chronic hepatitis, liver cirrhosis and primary hepato-cellular carcinoma but also because it carries the challenge of children who often develop chronicity and then represent the most important reservoir of infection in the community. Hepatitis B is known cause of infective hepatitis with high degree of morbidity and mortality. It is also the leading cause of death on the World[30].

The object of this study was to determine the seroprevalence of HBsAg infection and possible risk factor among Blood Donars of different age group in hospital based population in Bhopal. Serum sample were collected over a period of 7 months (from June2007-dec.2007) from Blood Bank of Peoples hospital, which is a tertiary care hospital located on Bypass road Bhanpur, Bhopal. The present study focuses on the fact that the Blood Donars who are 956 in number are were found to be 2.5% positive for HBsAg. This work is done to prevent the hepatitis by vaccination & other preventive measures with the advantage of low cost. There is no specific treatment for the hepatitis B, so the prevention is essential.

Keyword: Morbidity, Seroprevalence, HBsAg, IPD, OPD, Endemicity, HBV

INTRODUCTION

The term viral hepatitis refers to infections of the liver caused by five well characterized hepatotropic viruses which are designated as hepatitis A,B,C,D and E³. The viral hepatitis caused by hepatitis B is termed as "Serum hepatitis"¹. It belongs to a family of enveloped DNA virus, the Hepadnaviridae. Related virus in this group cause chronic hepatitis.[1] All hepatitis viruses are RNA except for hepatitis B, which is a DNA virus[11].

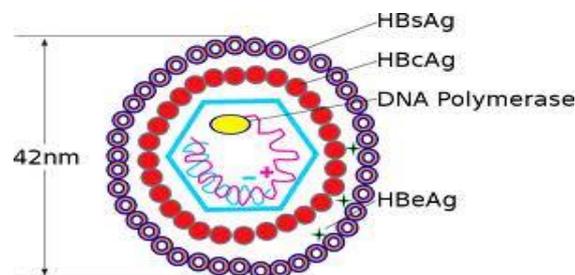
The discovery of HBV was serendipitous first isolated in 1963[10]. In 1965 Blumberg *et al* in Philadelphia found an antibody in two multiple transfused hemophiliac patient, which react with an antigen in a single serum in their panel which came from an Australian Aborigine[17]. Hepatitis B virus (HBV) is most common cause of serious liver infection in the world. It is estimated that more than 2 billion people have been infected by Hepatitis B virus & 350 billion people have been chronic infection (Dorsten *et al* 2004). More than 520,000 die each year from HBV related acute & chronic liver diseases[14].

The viruses infect only humans and some other non-human primates. Viral replication takes place predominantly in hepatocytes and to a lesser extent in the kidney, pancreas, bone marrow and spleen[29]. Hepatitis B is commonly transmitted by percutaneous exposure to contaminated blood and as little as 0.001 ml can transmit infection making them a big culprit[34]. Presence of HBsAg indicates symptomatic and asymptomatic carrier states which may progress to serious consequences like cirrhosis and a hepatocellular carcinoma[23].

Hepatitis B virus has a long incubation period (45-160 days)[16]. The acute illness is usually mild from the acute infection and the development of immunity[13]. In newly infected persons, HBsAg is present in serum 30-60 days after exposure to HBV and persists for variable period. Transient HBsAg positivity (lasting \leq 18 days) can be detected in some patients during vaccination [20].

Acute HBV infection is subclinical. The incubation period after infection last 1-4 months.

Chronic infection is define as hepatitis B surface antigen positive for atleast 6 months[22]. It has been estimated that 12% of patients with chronic HBV infection develop hepatocellular carcinoma [4]. The absolute lifetime risk of death from cirrhosis of hepatocellular carcinoma is 15-25%[8].



Risk of transmission of hepatitis B virus (HBV) through percutaneous exposure is 9-30%[25]. It is commonly transmitted by Sharing drug needles, by engaging in high risk sexual behavior (especially anal sex), from mother to her baby, during child birth and in health care setting[7].

The carrier rate of HBsAg varies worldwide from 0.1-0.2% in Britain, The USA and Scandinava, to more than 3% in Greece and Southern Italy and even upto 10-15% in Africa and far East. Carriage of HBsAg is even higher in some isolated communities: 45% in Alaska Eskimos and 85% in Austrian Aborigines[27]. More than 2000 million peoples alive today have been infected with the hepatitis B virus. Based on different HBsAg carrier rates, countries of the region can be divided into 3 epidemiological patterns. Type 1 occurs in Nepal & Srilanka characterized by a low HBsAg carrier rate of 0.9- 10 %. second pattern found in Bhutan, India, Indonesia & Maldives where carrier rate is high in general population (5-10%). Type 3 is observed in Bangladesh, DPR Korea, Myanmar & Thailand, where the carrier rate is very high & ranges from 9-12%[25].

The over population in under developed region have high endemicity (carrier rate 8%), low endemicity in the developed countries (carrier rate is less than 2%), and intermediate endemicity in other areas (carrier rate 2-7%)[9]. Out of 350 million carriers about 45 million carriers are in India which has the second largest carrier pool next only to China[7]. In India rate of HBV infection is around 4.7%[33]. In India carrier of HBsAg in hospital staff has been found to be higher 10.87% and in general population 5%[8]. In a study by Yousuf FG *et al* in Bhopal, out of 300 surgical patients, 18.66% were positive for HBV and 6.33% For HCV[5].

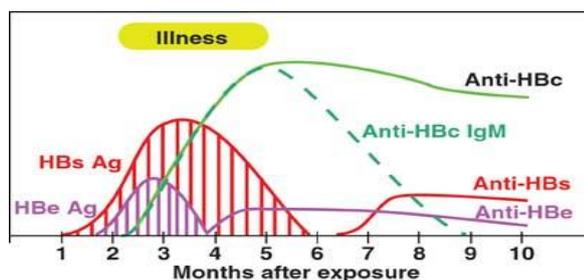
In the year 1990 the test for HBsAg by RPHA (Reversed Passive Hemagglutination) was conducted in M.P. (Tribal population) under the age of 6 to 18 years, out of which 15.7% of test were reactive for HbsAg⁷.

Infectivity of HBV is 8 times greater than HIV[35]. Surgeon, theatre staff nurses and other health care workers are at great risk of acquiring these infections[27]. Groups at increase risk for HBV Infection are Person with a history of sexually transmitted disease, Household contacts of HBV infected persons, Health care workers, Hemodialysis patients, Intravenous drug users, Infants born to HBV infected mothers, Immigrants and children of immigrants from hyper-endemic areas, Men who have sex with men, Person who have more than one sexual partner in a 6 month, Sexual partner of HBV infected persons [8].

HBV DNA is the most sensitive index of viral replication. It is detected by PCR. Using PCR, HBV DNA can be found in serum and liver, after the loss of HBsAg particularly in those receiving antiviral treatment[31]. DNA HBV in serum detected by PCR is a good marker of level of viraemia, can be correlated with serum transaminase levels and parallels the presence of HBsAg in serum[6]. Following are the antigens with viral DNA polymerase form useful diagnostic markers for HBV:-

1. HBV DNA, 2. HBsAg, 3. Anti HBs, 4. HBeAg, 5. Anti HBe, 6. IgM anti HBe, 7. Anti HBe Total

Hepatitis Antigen, Antibodies in patient recovering from illness.



Effective vaccine for HBV have been available in United State since 1982. Early strategies targeted high risk groups, but were not successful in decreasing the incidence rates[26]. The only safe and effective measures for prevention are Universal active immunization. The most simple vaccine is derived from heat inactivated plasma containing HBsAg and is based on the original observation of Krugman [21]. In 1992, WHO recommended that hepatitis B vaccine be integrated into National immunization programme of all countries with a rate of chronic HBV infection of 80% or higher by 1995 and into the programme of all countries by 1997. To date two drugs have been approved for treatment of chronic hepatitis B. Injectable interferon (IFN) alpha and two oral antiviral agent 1) Lamivudine (Epivir)- approved by FDA in 1998[11]. 2) Adefovir dipivoxil (Hepsera) – approved by FDA in September 2002[2].

Several other drugs are in the progress of efficiency testing in clinical trial[11].

WHO recognized vaccine in India is [30]:-

- Type- R-DNA yeast derives vaccine recombinant.
- Dose- 1ml in adult and 0.5 in pediatric at birth.
- Schedule- 0- at birth

1 - After 1 month

6 - after 6 months of first dose.

Certain standard precautions are needed to be followed in all health care settings such as Hand washing before and after all patients or specimen contact, Wearing gloves for potential contact with blood and body fluid, Disposal of syringes immediately after use in nearby imperable container. No recapping or manipulation of needle should be done in any way. Processing all laboratory specimens as potentially infections[15].

MATERIAL AND METHOD

The present study was conducted with 956 blood donors; under various age groups were registered from June 2007 to December

2007 in People's College of Medical Sciences & Research Centre, Bhanpur, Bhopal.

Information was collected in a Performa that included donar demographic data name, age, sex, address, previous history of Jaundice any time in life and vaccination. Those giving any history suggestive of Jaundice were deferred from blood donation. It includes also the history of any of the following risk factors such as multiple sex partner, tattooing, Intravenous drugs used, drug addiction, any major or minor surgery including transfusion of blood \ blood components during last 6 months.

Blood samples (2.5-3 ml preferably fasting) of all the voluntary blood donors were collected in clean plain vials, by venepuncture, using aseptic precautions and were brought to the laboratory as soon as possible for testing. In case of delay, serum sample can be separated from blood and stored for 1-2 days in refrigerator.

All blood samples were allowed to clot and clean serum samples were separated in a clean test tubes. Kit used for testing was HEPALISA-Microwell ELISA Test (J. Mitra & Co. pvt. Ltd, New Delhi).

Fit the strip holder with the required number of HEPALISA strip. Arrange the assay control wells so that well A-1 is the reagent blank. From well A-1 arrange all controls in a horizontal or vertical configuration.

1. Leave A-1 well as blank.
2. Add 50µl Negative control in each well No. B-1 & C-1 respectively.
3. Add 50µl positive control in D-1, E-1 & F-1 wells.
4. Add 50µl of sample in each well, starting from G-1.
5. Add 50µl of working Enzyme conjugate to each well except A1. Gently shake the plate for 2-3 seconds to mix the sample & conjugate.
6. Cover the plate & incubate in an incubator at 37°C + 1°C for 60 minutes.
7. Dilute the wash buffer concentrate with distilled water to 1:25 dilution.
8. At the end of incubation period, take out the plate from incubator & wash with working wash buffer.
9. Wash each well five times in total & allow to dry.
10. In the dry well after washing add 100 µl of working substrate solution in all wells including A1.
11. Cover the plate with an aluminium foil & incubate at room temperature (20 –25°C) for 30 minutes in dark.
12. Stop the reaction by adding 50 µl of stop solution to each well, mix gently.
13. Read the absorbance of the wells at 450nm in a ELISA Reader after blanking A-1 well.

Cut-off value: -Can be determined by using the following Formula.

$$\text{Cut-off value} = \text{NCx} + 0.1$$

Where NCx=mean absorbance (0.0) of negative control.

The absorbance of the unknown sample is compared with the calculated cut-off value.

- Test specimen with O.D. value less than cut-off value are non reactive and may be considered as negative for HBsAg.
- Test specimen with O.D. value greater than or equal to cut-off value are reactive for HBsAg.
- Test specimen with O.D. value within 10% below the cut-off value should be considered suspected for the presence of HBsAg.

- Specimen with absorbance value equal to or greater than the cut-off value is considered initially reactive.
- The O.D. for crystal clear negative samples can be in minus. However, the minus O.D. does not in any way affect the result interpretation. It rather gives better specificity.

RESULT

956 blood donors were tested for the presence of HBsAg in blood sample by ELISA.

Results obtained were as follows:-

Table I: Showing reactivity percentage of blood donors from June 2007-December 2007.

S. No.	Months (2007)	Total no. of Donors	Total no. of HBsAg Reactive	Percentage
1	June	157	4	2.5
2	July	152	9	5.9
3	Aug.	126	4	3.1
4	Sept	178	1	0.5
5	Oct.	107	1	0.9
6	Nov.	120	1	0.8
7	Dec.	116	4	3.4
TOTAL		956	24	2.5

Table I shows that out of 956 blood donors 24 no. are reactive for HBsAg i.e. reactivity is 2.5% all the blood donors are male only.

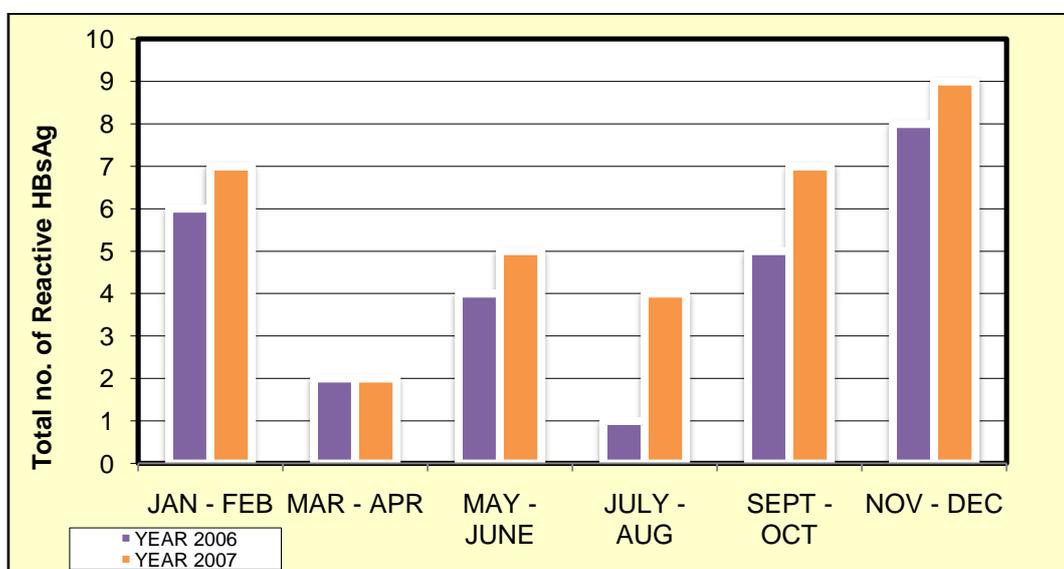
Table II: Showing age-wise distribution & total no. of reactive HBsAg in blood donors.

S. No.	Age	Total no. of reactive (HBsAg) Donors
1	18-22	3
2	23-27	5
3	28-32	5
4	33-37	4
5	38-42	1
6	43-47	1
7	48-52	0

Table no. II shows that age ranging from 23-32 years the reactivity of HBsAg is high.

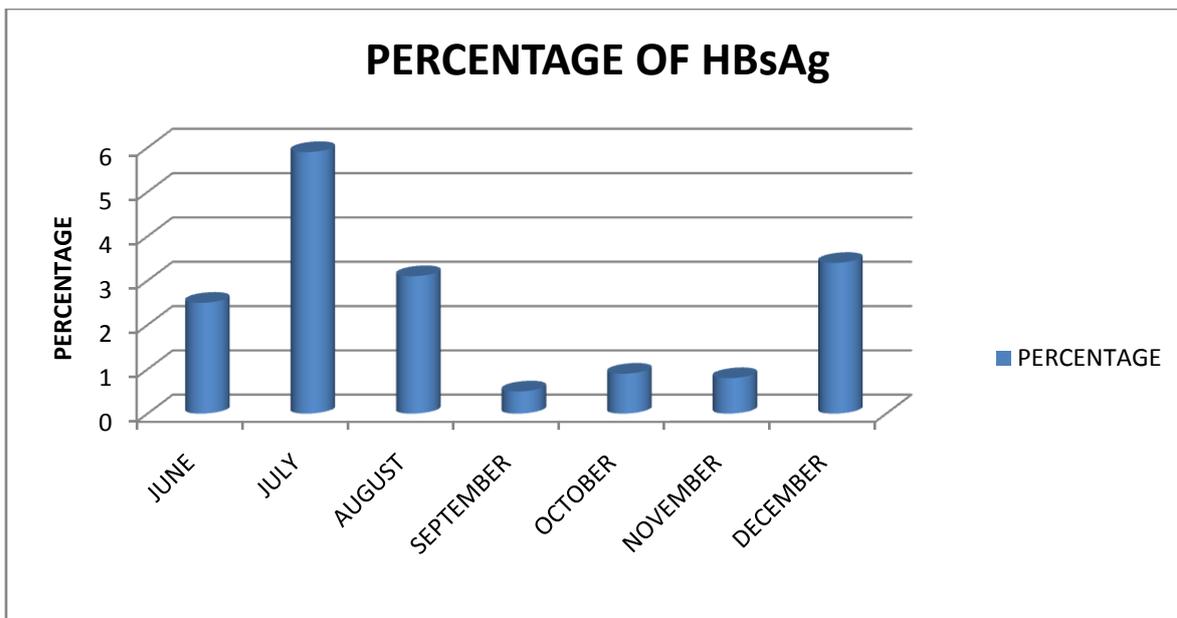
Table III: Showing six monthly HBsAg reactive cases among donors from July05 - Dec07.

S. No.	Month/ Year	Total Donors	No. of Reactive HBsAg	Percentage %
1	Jul - Dec.2005	316	08	2.5
2	Jan-June 2006	428	12	3.2
3	July-Dec.2006	676	16	2.3
4	Jan- June2007	766	14	1.8
5	July-Dec.2007	799	20	2.5
TOTAL		2985	72	2.4%



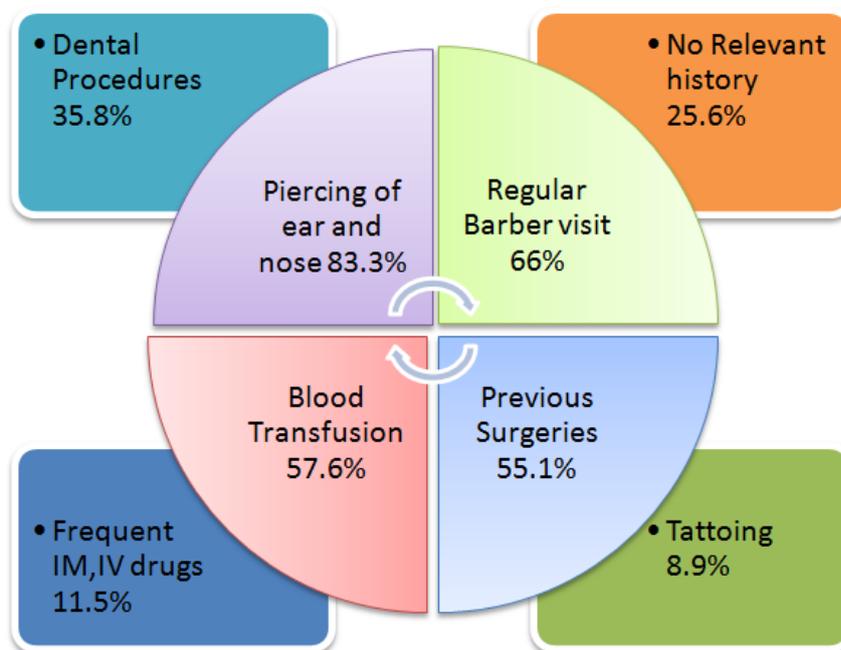
Graph I: Graph showing comparison of HBsAg reactivity during the year 2006-2007

Graph I shows that reactivity of HBsAg is gradually increased during the year 2007 as compare to 2006.



Graph II: Graph showing reactivity percentage of blood donors from June 2007-December 2007.

Risk factors of all patients are shown in the figure given below:-



DISCUSSION

In the present study the HBsAg carrier rate was 2.5% among blood donors by ELISA. This study was undertaken to compare sensitivity & specificity between ELISA & RAPID. Among them Rapid test appears to be the method of choice because of its speed & simple procedure, but ELISA is still more sensitive test to screen blood donors to prevent transmission of hepatitis to patients & the hospital personnel. Filter paper technique is suitable for detecting HBsAg carriers because the healthy individuals, specifically children, do not agree to provide blood samples by venepuncture. Collection of blood samples on filter paper may provide a convenient & suitable method for future community based studies in India.

Our result are agreeable with those of Jagvir Singh, Ragesh, S.K.Patnaik *et al* [32]who found that HBsAg carrier rate in Rajahmundry is 3.3% and in Banglore is 4.2%.

Ray *et al* found that 2.6% of Blood donars carried HBsAg in their blood by CIEP and 11.8% of donars by RPHA.

Kothari observed that out of total 200 Blood donars 3% were positive for HBsAg[18].

Rehman K *et al* and pizado *et al* has reported HBsAg was positive in 5-9% health care personnel[28].

Dienstag and Isselbacher in 1998 in Nigeria found that higher HBsAg seroprevalence was observe among 20-30 years of age category of Blood donars[11]. Our research also show the same result were 23-32 years has highly reactive HBsAg. This could be associated with sexual activity and intravenous drug used.

In our study there is gradual increasing in the prevalence of HBsAg as compare to 2005-2006 (P<0.05 significantly different) because there is lack of routine serological screening in many Hospitals

which may probably is one of the factor responsible for increasing the disease transmission and there is lack of proper precautions while managing infected patients & lack of vaccination among surgeons and healthcare workers lead to transmission of infection from patients to them and vice versa. In India there are only 306 licensed blood banks and the evidence of past transfusion hepatitis in multiple transfused patients is as high as 18-30%[25].

Yosaf & Taj MN⁵. CJ Uneke, O Ogbu, PU Jnyama etal, studies show higher results.

Erdens, Palanduz s, et al studies show lower values[15].

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