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Original Research Article

Seropositivity of hepatitis B viral infection among liver disorder patients, voluntary blood donors and hospital personnel

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ABSTRACT

Background: HBV is highly infectious when compared with HIV even minute traces of infected blood as little as 0.0001 ml can transmit infection. Several of the routine therapeutic, diagnostic, prophylactic invasive procedures are capable of spreading HBV infection. Objectives of the study were to study the prevalence of hepatitis B surface antigen in voluntary blood donors, health care workers (medical and paramedical personnel) and patients with liver disorders
Methods: Present study was hospital based cross sectional study carried out among 75 subjects during a period of one year. These 75 subjects consisted of 25 patients with liver disorder, 25 health care workers and 25 voluntary blood donors. All of these were selected randomly. After obtaining informed consent, detailed history and examination was carried out. The blood samples were collected and sent for detection of HBsAg by ELISA. Data was entered and analyzed using proportions.
Results: Maximum subjects 40 (53.3%) belonged to the age group of 21-30 years overall as well as among the voluntary blood donors i.e. 60%. Among voluntary blood donors, 96% were males. 60% belonged to urban areas. Among rural subjects, maximum (53.3%) were having liver disorders. Only 28% were illiterate. Liver disorder was more common among illiterates i.e. it was 76.2%. Overall only 24% were having high risk behavior and all of them were heterosexuals. 76% were not having any high risk behavior. Liver disorder was more common (66.7%) among heterosexual high risk behavior subjects compared to only 22.8% with no high risk behavior. Seropositivity was found highest among patients with liver disorder (64%) followed by among health care workers (12%). No one was seropositive among voluntary blood donors. Overall the seropositivity was 25.4%.
Conclusions: Patients with liver disorders especially chronic hepatitis were mostly seropositive. Among health care workers, being a doctor was an important risk factor for acquiring hepatitis B infection. Hence all health care workers should be immunized for hepatitis B.

Keywords: Hepatitis B, Health care workers, HIV, Seroprevalence

INTRODUCTION

Hepatitis B viral infection occurs throughout the world. Natural infection occurs only in humans and there are no animal reservoirs for hepatitis B virus (HBV). The virus is maintained in large pool of carriers whose blood contains circulating virus for long period. There are over 450 million carriers worldwide and among them 45

million are in India, the second largest carrier pool next to China in the world. HBV is a blood borne virus and infection is transmitted by Parenteral, sexual, perinatal and other miscellaneous modes of transmission. The virus is also present in all body fluids and excretions due to its infection such as tears, breast milk, vaginal secretions, semen, urine, bile, and feces etc which are also capable of transmitting the infection to healthy individuals.[1]

Transmission via infected blood is one of the commonest modes of transmitting the HBV infection, needs strict screening for safe blood transfusion. Sexual transmission of HBV occurs in, commercial sex workers, homosexuals and those who idulge in high risk behaviour i.e. heterosexuals.[2] Certain occupational groups carry higher risk of HBV infection including medical and para-medical personnel in the hospital, in the areas like blood bank, dialysis unit etc.[3]

The presence of hepatitis B surface antigen HBsAg in relation with hepatitis and its prolonged course leads to high incidence of complications like cirrhosis of liver, chroninc active hepatitis and hepato cellular carcinoma. The prolonged course and prevalence of HBV among the community results in the horizontal spread and thereby leads to high morbidity and mortality.[4] HBV is highly infectious when compared with HIV even minute traces of infected blood as little as 0.0001 ml can transmit infection. Several of the routine therapeutic, diagnostic, prophylactic invasive procedures are capable of spreading HBV infection.[5]

So it is in this connection this study was carried out to know the prevalence of hepatitis B surface antigen in voluntary blood donors, health care workers (medical and paramedical personnel) and patients with liver disorders.

METHODS

Present study was hospital based cross sectional study carried out among 75 subjects during a period of one year. These 75 subjects consisted of 25 patients with liver disorder, 25 health care workers and 25 voluntary blood donors. All of these were selected randomly.

After obtaining Institutional Ethics Committee permission and informed consent, detailed history and examination was carried out. The blood samples were collected and sent for detection of HBsAg by ELISA.

Inclusion criteria

- Subjects willing to participate in the study
- Health care workers and voluntary blood donors free from any diseases
- Patients with liver disorder confirmed by physician

Exclusion criteria

- Subjects not giving consent
- Any subjects suffering from severe debilitating disease

Sample collection

Blood samples were collected with informed consent for serological diagnosis of HBV infection by following aseptic procedures i.e. disinfecting the site collection of blood sample by veni puncture method. 3-5 ml of blood sample was collected with 5 ml syringe and transferred to sterile test tubes. The collected blood samples were transferred to department of Microbiology. The collected blood samples were allowed to clot for 30 minutes and were centrifuged at 3000 rpm for 15 minutes for the separation of the serum for each sample.

The separated sera were transferred into sterile Laxbro vials and were kept at 2-8^0 C in the refrigerator. All the 75 samples were tested by ELISA method for HBsAg detection in the sera by using SD HBsAg ELISA SD 3.0 BIOLINE diagnostic kit.

Re-constitution of reagents in the test kit

The test kit which was used for the detection of HBsAg was the SD HBsAg ELISA SD 3.0 BIOLINE. This kit was stored in the sterile condition at 2-8^0 C as guided by the kit literature. The kit was brought to room temperature before performing the test.

The necessary articles like micropipette, glass ware for preparing reagents etc. were brought to the place where the test was to be performed.

Materials in the kit and preparation of the reagent kit

- Coated micro-plates: 96 well coated with anti-hepatitis B surface antigen (anti-HBS) were kept at 2-8^0 C in the provided aluminum bag and accurately sealed.
- Enzyme conjugate: Anti-HBS conjugated to horseradish peroxide (HRPO) the preservative used was proclin (300 qs) which is ready to use.
- Positive control: HBsAg positive human serum {the preservative used is proclin (300 qs)}
- Negative control: Normal healthy human serum {the preservative used is proclin (300 qs)}
- Substrate: Tetramethyl benzidine with citrate-phosphate buffer and DMSO 101X concentrated was provided which were diluted in a sterile glass ware just before use. Making 1:100 mixtures with substrate concentrate and substrate diluents.
- Substrate diluents: Citrate-phosphate buffer and hydrogen peroxide
- Washing solution: PBS TWEEN 20, 20 X concentrations was provided. 25 ml of was concentrate was taken and filed up to 500 ml with distilled water in the washing tank

- Stop solution: 1.6 N sulphuric acids which were ready to use was provided.

Performance of the test as per standard procedure

- The number of strip well and other components were calculated which were required for the test and were placed at room temperature.
- The strip wells were taken as negative controls 3 well, positive controls 2 well and blank well 1 well. The remaining well were for samples.
- 100 ml of negative control to 3 wells, positive control to 2 well and a blank well (nil) were added and samples to each well was added.
- 25 ml of enzyme conjugate to each well was added
- The micro-plate was sealed with pate sealer and the wells were mixed well on vibrating mixer. Mixing is very important to get the reproducible results.
- The wells were incubated at 37 ± 1^{0}C for 90 minutes.
- The wells were washed for 5 times with 350 ml diluted washing solution with ELISA washer and liquid was aspirated from the well.
- 100 ml of diluted substrate solution pipette and added to each well.
- The micro-plate was covered with adhesive plate sealer and the plate was incubated for 30 minutes at room temperature.
- 100 ml of stop solution was added to each well
- The wells were read at 450 nm with reference wavelength at 620 nm. The reading was completed with 30 minutes from the end of assay

Data was entered and analyzed using proportions and the appropriate statistical test.

RESULTS

Maximum subjects 40 (53.3%) belonged to the age group of 21-30 years overall as well as among the voluntary blood donors i.e. 60%. Among voluntary blood donors, 96% were males. 60% belonged to urban areas. Among rural subjects, maximum (53.3%) were having liver disorders. Only 28% were illiterate.

Liver disorder was more common among illiterates i.e. it was 76.2%. Overall only 24% were having high risk behaviour and all of them were heterosexuals. 76% were not having any high risk behaviour. Liver disorder was more common (66.7%) among heterosexual high risk behaviour subjects compared to only 22.8% with no high risk behaviour.

Maximum (20%) seropositivity was found among doctors followed by nurses (16.7%). No one was found seropositive among dentists, lab technicians and housekeeping staff. No one was positive among patients with cirrhosis of liver. But the seropositivity was 69.5% among patients with chronic hepatitis.

Table 1: Age and sex wise distribution of study subjects.

Age group (years)	Voluntary blood donors		Patients with liver disorders		Health care workers		Total
	Male	Female	Male	Female	Male	Female	
< 20	01 (04%)	00	02 (08%)	02 (08%)	00	01 (04%)	06 (08%)
21-30	15 (60%)	00	05 (20%)	03 (12%)	09 (36%)	08 (32%)	40 (53.3%)
31-40	07 (28%)	01 (04%)	00	02 (08%)	02 (08%)	01 (04%)	13 (17.3%)
41-50	01 (04%)	00	02 (08%)	01 (04%)	02 (08%)	00	06 (08%)
51-60	00	00	03 (12%)	02 (08%)	01 (04%)	00	06 (08%)
61-70	00	00	02 (08%)	00	01 (04%)	00	03 (04%)
71-80	00	00	01 (04%)	00	00	00	01 (1.4%)
Total	24 (96%)	01 (04%)	15 (60%)	10 (40%)	15 (60%)	10 (40%)	75 (100%)

Table 2: Distribution of study subjects as per various demographic factors.

Demographic factors		Voluntary blood donors	Patients with liver disorders	Health care workers	Total
Locality	Rural	10 (33.3%)	16 (53.3%)	04 (13.4%)	30 (40%)
	Urban	09 (20%)	15 (33.3%)	21 (46.7%)	45 (60%)
Literacy	Illiterate	05 (23.8%)	16 (76.2%)	00	21 (28%)
	Primary education	13 (68.4%)	05 (26.3%)	01 (5.3%)	19 (25.3%)
	Secondary education and higher	07 (20%)	04 (11.4%)	24 (68.6%)	35 (46.7%)
High risk behaviour	Homosexual	00	00	00	00
	Heterosexual	05 (27.8%)	12 (66.7%)	01 (5.5%)	18 (24%)
	No high risk behaviour	20 (35.1%)	13 (22.8%)	24 (42.1%)	57 (76%)

Table 3: Seropositivity among voluntary blood donors.

Sex	Seropositive		Seronegative		Total	
	Number	Percentage	Number	Percentage	Number	Percentage
Male	00	00	24	100	24	96
Female	00	00	01	100	01	04

No one was seropositive among voluntary blood donors.

Table 4: Seropositivity among health care workers.

Category	Seropositive		Seronegative		Total	
	Number	Percentage	Number	Percentage	Number	Percentage
Doctors	02	20	08	80	10	40
Dentists	00	00	01	100	01	04
Nurses	01	16.7	05	83.3	06	24
Lab technicians	00	00	07	100	07	28
Housekeeping staff	00	00	01	100	01	04
Total	03	12	22	88	25	100

Table 5: Seropositivity among patients with liver disorders.

Liver disorder	Seropositive		Seronegative		Total	
	Number	Percentage	Number	Percentage	Number	Percentage
Chronic hepatitis	16	69.5	07	30.5	23	92
Cirrhosis of liver	00	00	02	100	02	08

Table 6: Comparison of seropositivity among three groups.

Groups	Seropositive		Seronegative		Total	
	Number	Percentage	Number	Percentage	Number	Percentage
Voluntary blood donors	00	00	25	100	25	33.3
Health care workers	03	12	22	66	25	33.3
Patients with liver disorder	16	64	09	36	25	33.4
Total	19	25.4	56	74.6	75	100

Seropositivity was found highest among patients with liver disorder (64%) followed by among health care workers (12%). No one was seropositive among voluntary blood donors. Overall the seropositivity was 25.4%.

DISCUSSION

Maximum subjects 40 (53.3%) belonged to the age group of 21-30 years overall as well as among the voluntary blood donors i.e. 60%. Among voluntary blood donors, 96% were males. 60% belonged to urban areas. Among rural subjects, maximum (53.3%) were having liver disorders. Only 28% were illiterate.

Liver disorder was more common among illiterates i.e. it was 76.2%. Overall only 24% were having high risk behaviour and all of them were heterosexuals. 76% were not having any high risk behaviour.

Liver disorder was more common (66.7%) among heterosexual high risk behaviour subjects compared to only 22.8% with no high risk behaviour. Seropositivity was found highest among patients with liver disorder (64%) followed by among health care workers (12%). No one was seropositive among voluntary blood donors.

Overall the seropositivity was 25.4%. Gupta N et al found a positivity rate of 0.66% among 44064 voluntary blood donors screened by them.[6] Patel Y et al reported a seroprevalence of 1.7% among voluntary blood donors.[7]

Elavia AJ et al observed a 10% seroprevalence among health care workers which is close to that reported in the present study of 12%.[5] Present study found a seroprevalence of 64% among patients with liver disorder which is similar to that reported by Vaiphei K et al of 70%.[8]

Shivananda PG et al conducted a study among voluntary blood donors, health care workers and patients with liver

disorders and observed that the overall seroprevalence was 58.58% which is almost double reported in the present study of 25.4%.[9] Chakravarthi A et al found a seroprevalence of 60.6% among patients with liver disorder which is similar to that observed in the present study of 64%.[1] Dharmadhikari CA et al screened 5606 blood samples (4900 of voluntary blood donors and 706 of patients with liver disorders) and found that seropositivity was 0.8% among voluntary blood donors and 20% among patients with liver disorders.[3] Thakur et al [10] found a seropositivity of 8% among health care workers, 3.26% among voluntary blood donors.

Hence it should be made customary that all patients with liver disorders should be screened for HBsAg attending the hospital for control of HBV infection, as most of the patients with liver disorders were found positive.

Vaccinations with recombinant DNA vaccine to prevent HBV infection should be advised and implemented for all medical and paramedical personnel including all departments of the hospital.

Effective awareness should be spread among the people with high risk behaviour to follow safe sex methods to get protection not only against HBV but also HIV. Attention should focus more among 21-30 years age group as well as males for health education and screening as these two groups are found to be high risk in the present study.

CONCLUSION

Patients with liver disorders especially chronic hepatitis were mostly seropositive. Among health care workers, being a doctor was an important risk factor for acquiring hepatitis B infection. Hence all health care workers should be immunized for hepatitis B.

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Ethical approval: The study was approved by the Institutional Ethics Committee

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