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Original Article

Microbiology Section

Burden of Hepatitis B Virus at a Tertiary Care Hospital, Doda, Jammu and Kashmir, India: A Cross-sectional Study

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ABSTRACT

Introduction: Hepatitis B Virus (HBV) causes most frequent chronic liver disease of infectious origin in human beings worldwide, with more than 600,000 deaths caused by end-stage liver disease complications per year. The most used test for identifying acute HBV infections and carriers is the detection of HBsAg. Immunochromatography assays have been suggested for routine use in clinical microbiology laboratories for the detection of HBsAg since they are easy to use, affordable, don't need specialised equipment, and are straightforward to run. Compared to commercially available HEPA card kit for the detection of the same markers, Enzyme Linked Immunosorbent Assay (ELISA) was shown to be more sensitive for the detection of HBsAg. This study is first of its kind in District Doda, Jammu and Kashmir, India.

Aim: To know the burden of HBV in a Tertiary Care Hospital, Government Medical College, Doda using HEPA card kit and ELISA method.

Materials and Methods: The present hospital-based cross-sectional study was carried out in the Department of Microbiology, Government Medical College and Hospital, Doda Jammu and Kashmir, India during the period from January 2020 to December 2020. The study comprised blood samples from all age groups referred by clinical departments for testing HBsAg. Tests were performed using an immunochromatographic technique (HEPA card Diagnostic enterprises) for the qualitative detection of HBsAg, and results were interpreted in accordance with the manufacturer's guidelines. The collected data was analysed in Microsoft excel sheet using Chi-square test to know the burden of HBV infection.

Results: Among total number of 5,448 samples tested, 50 (0.92%) were positive for HBsAg which comes under low epidemicity (<2%) as per World Health Organisation (WHO) guidelines. The number of positive females and males were 30 (0.84%) and 20 (1.07%), respectively. Females were predominate over males and majority of the positive patients (N=29) were younger than 40 years though prevalance (2.1%) was higher in age group above 40 years. All samples which shows positive by rapid test were also shown positive by ELISA test.

Conclusion: Overall prevalence of HBV was 0.92% which comes under low epidemicity (<2%) as per WHO guidelines. It can be an alternate option for community based studies and also helps to improve the public health and to prevent the spreading of disease in the local population.

Keywords: Enzyme linked immunosorbent assay test, Low epidemicity, Rapid test

INTRODUCTION

The HBV is one of the major global health problem. Annually more than two billion people are infected worldwide [1] among which more than 240 million [2] are HBV carrier cases and more than 7,86,000 deaths with liver cirrhosis and hepatocellular carcinoma [3]. According to WHO, from the estimated 257 million people infected with HBV, about 89% are oblivion of their carrier status because of the absence of symptoms [4] thereby creating a "silent epidemic" [5]. Hepatitis B may cause Acute Hepatitis (AHB) or Chronic Hepatitis (CHB) [6] having an incubation period of about 75 days on average, but can be upto 180 days [7].

The countries are categorised into zones of High prevalence (8%), Moderate prevalence (2-8%), and Low prevalence (2%) based on the prevalence of chronic HBV infection worldwide [5]. High HBV endemicity is defined as the presence of HBsAg in populations in Sub-Saharan Africa, East Asia, and Alaska, while intermediate endemicity is defined as the presence of HBsAg in populations in the Amazon basin, Middle East, and Indian Subcontinent, and low endemicity is defined as the presence of HBsAg in populations in western and northern Europe, North America, and Australia [8].

India harbours 10%-15% of the global pool of HBV, with the prevalence rate of 2%-4%, falls in the intermediate HBV endemicity group. Transmission is believed to be mostly occur during early childhood by close physical contact (horizontally transmission), although upto 30% of cases are due to vertical transmission [9]. At community level, researchers reported the pravalance of HBV in Tamil Nadu, Tripura, West Bengal and North India as 5.7%, 3.6% [10], 2.97% [11] and 2.1% [12], respectivey.

In Kashmiri population prevalence of HBV has been reported as 0.56%-1.1% [13] and 1.2% [14]. In Ladakhi population high pravalance of 7.86% [15] and 4.2% [16] has been reported. In Jammu prevalence of 2.44% has been reported [5]. Kadla SA et al., reported overall prevalence of HBV in south Kashmir as 2.4% with Anantnag, Kulgam, Shopian and Pulwama having prevalance of 2.25%, 2.5%, 2.61% and 2.19%, respectively [17]. The probability of HBV in Jammu and Kashmir is now more likely, necessitating a thorough regional epidemiological assessment, which is currently lacking as a result of subpar study results from various districts. In order to identify hotspots of HBV endemic areas, it is crucial to research and quantify the prevalence of HBV in various regions of the state. HBV seroprevalence research has never been conducted in the Doda district of Jammu, Jammu and Kashmir, India. Hence, it is important to estimate the prevalence of HBV in Doda district of Jammu province. This was the first study conducted in Doda District of Jammu and Kashmir, India. Doda is the largest district (Geographical Area-2758.95 Sq Km) in the Jammu province having population of 409,936 (Male: 213,641, Female: 196,295), located at *33.13°N 75.57°E*, at an altitude of 5000 feet above the sea level. The district shares border with Anantnag, Ramban, Kishtwar, Udhampur and Chamba district of Himachal Pradesh, India. The

entire district is hilly, being divided into 02 assembly constituencies viz. Doda and Bhaderwah. The distict is predominantly rural and has agricultural and pastoral economy [18].

Unawareness of an ongoing infection of HBV delays the diagnosis of HBV-related liver disease and favours the spread of the virus. Therefore an attempt was made to know the seropositivity of HBV at tertiary care centre which helps to improve the public health and to prevent the spreading of disease in the local population. HBV can be detected by serological methods like rapid test (card method) and ELISA test as the most common, fast and economic methods to detect different virus markers such as HBsAg, anti-HBsAg, anti-HBcAg, HBeAg and anti-HBeAg [19].

ELISA is a biochemical assay that uses antibodies and an enzyme-mediated colour change to detect the presence of antigen with high sensitivity [20]. Rapid diagnostic test strip is a lateral flow one step immunoassay based on the antigen capture, or "sandwich" principal [19]. HEPA card is a one step immunoassay based on the antigen capture, or "sandwich" principle. The method uses anti-HBsAg antibodies conjugated to colloidal gold and anti-HBsAg antibodies immobilised on a nitrocellulose strip in a thin line [16,13]. This study was conducted since the presence of HBsAg in serum or plasma is the most important indicator for the diagnosis of HBV infection [20]. Hence, present study was conducted to know the burden of HBV in Tertiary care Hospital, GMC Doda using HEPA card kit and ELISA method.

MATERIALS AND METHODS

The present hospital-based prospective study was carried out in the District Doda, which falls under the jurisdiction of Government Medical College and Hospital, Doda, Jammu and Kashmir, India. The duration of study was one year from January 2020 to December 2020. Samples were collected by various clinical departments (OPD and IPD) from preoperational, antenatal and haemodialysis patients and dispatched to Microbiology laboratory for testing. Demographic details of the patients were recorded. Results were shared with the patients and the concerned clinicians.

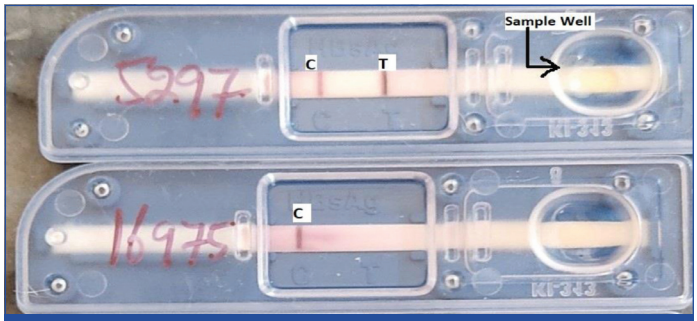
Sample size calculation: Sample size for the study was determined using formula, $n=4\ pq/l^2$ where, 'n' is the sample size, 'p' was the estimated prevalence of HBsAg based on neighbouring region of Ladakh with reported prevalance of 4.2% [16]. 'q'=(1-p) and 'l' is the allowable error, and l=1% (absolute error). Based on the findings of pilot study, a non response rate of 15% was taken into consideration [14].

Specimen Collection

Blood samples of all age groups and both sex were sent by various clinical departments (OPD and IPD) for testing HbsAg was included in the study. Blood samples (5 mL) were collected from the total of 5,448 patients. The blood sample was poured into a red top tube without anticoagulant and centrifuged at 3000 rpm for five minutes. The serum was separated and it was used for the present study. Samples were immediately tested for the presence of HBsAg. If positive, the serum was stored for ELISA (-20ºC) and if negative the sample were discarded as per Helsinki Guidelines.

1. HBV rapid test by HEPA card: These tests were done as part of preoperative screening, antenatal screening, screening on haemodialysis patients. The sera was screened for the presence of HBsAg by a rapid test kit based on the principle of one step immunoassay (HEPA card-Diagnostic enterprises). Those found positive on screening were confirmed by third generation ELISA kits (Bioelisa HBsAg Kit, Spain) according to the manufacturers protocol.

For rapid card test, about 50 µL of serum was transferred to the specimen well and timer was started for 15 minutes. The results were read according to the manufacturer's instructions. The positive result is, when two coloured lines appeared, one in the control region (C) and the other in the test region (T). The negative result, shows one coloured line should appear in the region (C) only [Table/Fig-1].



[Table/Fig-1]: HEPA card method.

2. ELISA screening test: Samples positive for HBV were confirmed by ELISA (Bioelisa HBsAg Kit, Spain) at Department of Microbiology, Government Medical College and Hospital, Doda Jammu and Kashmir, India. This test was based on a one-step "Sandwich" principle. In brief, HBsAg coupled with Horseradish Peroxides (HRP) serves as the conjugate, Tetramethyl Benzedrine (TMB) and peroxide as a substrate. Upon completion of the test, a colour develops which is directly proportional to the amount of HBsAg in the sample. The method was followed according to the manufacturer instructions. The absorbance was read for each blank well at 450 nm within 30 minutes. It is recommended to read positive samples using a 620-630 nm reference filter.

STATISTICAL ANALYSIS

Data was analysed in Microsoft excel Sheet. Continuous variables were analysed in the form of mean and standard deviation while categorical variables were summed up as frequency and percentages. Chi-square test was used to obtain results. A p-value <0.05 was considered statistically significant.

RESULTS

Total 5,448 patients were screened for HbsAg with 3,578 (65.68%) female and 1,870 (34.32%) male. Fifty samples were positive on screening by HEPA card method, all 50 positive samples were confirmed by ELISA method. Seropositivity of HBsAg was 0.92% [Table/Fig-2]. Out of 3578 female patients 30 (0.84%) were positive and out of 1870 male patients 20 (1.07%) were positive for HbsAg [Table/Fig-2]. Among 50 positive patients, females (30) were more affected than males (20), though proportion wise males (1.07%) were more affected than females (0.84%). No seropositive patient was affected in age group of 0-20 years. The majority of the studied population (75.5%) and majority of the seropositive patients (N=29) were younger belonged to the age group of 21-40 years though the infection rate was more prevalent (2.1%) in the age group of above 40 years. Highest positivity was seen in the month of April (1.55%) [Table/Fig-3].

Males			Females		
Tested	Positive	Percentage	Tested	Positive	Percentage
1870	20	1.07%	3578	30	0.84%
Age (years)	Total no. of samples tested		Total no. of positives		Total positive (%)
0-10	100		00		0.00
11-20	235		00		0.00
21-40	4113		29		0.70
Above 40	1000		21		2.1

[Table/Fig-2]: Gender and age-wise distribution of patients tested and seropositive for HbsAg.

Month	Total no. of samples screened	Total no. of HBsAg positive samples	Total positive %
January	352	05	1.42
February	239	03	1.25
March	784	09	1.14
April	257	04	1.55
May	427	06	1.40
June	427	05	1.17
July	496	02	0.4
August	505	04	0.79
September	590	02	0.33
October	402	02	0.49
November	402	03	0.71
December	567	05	0.88
Total	5448	50	0.91

[Table/Fig-3]: Total number of samples tested and positive for HBsAg (month wise data).

DISCUSSION

This was the first study of its kind in District Doda to estimate the prevalence of HbsAg amongst general population. In this study, the overall seroprevalence of HbsAg of 0.92% was observed. The prevalence was lower than the national average of 2-4% [21,22], and lower than many adjoining regions with Anantnag, Kulgam, Shopian and Pulwama having prevalance of 2.25%, 2.5%, 2.61% and 2.19%, respectively [17], 0.56%-1.1% [13] and 1.2% [14] in Kashmiri population and 2.44% [5] in Jammu. A total 5448 blood samples were tested. Out of these, 50 (0.92%) were positive for HBsAg, which comes under low epidemicity (<2%) as per WHO guidelines. The present study has reported higher positive rate among male (1.07%) as compared to females (0.84%). These findings were in consonance with Shashi SS et al., [5], who observed seropositivity of 2.44% with 1.58% in males and 0.86% in females, respectively. In the present study, higher prevalence rate was seen in the age group above 40 years. These findings were in consonence with the studies by Ingale H et al., [23], Sood S and Malvankar S, [24], Dutta S et al., [25] and Shashi SS et al., [5] who observed highest prevalance in the age group of 40-60 years in Jammu. Another community based cross-sectional study conducted in Ladakh region found prevalence of HbSAg of 4.2% (Kargil district-7.40%; Leh district 1.96%) with higher prevalence in males (4.86%) than females (3.78%) and more positivity within 21-40 years age group [16]. Naqshbandi I et al., reported that the prevalence of HBsAg in Srinagar region was 1.2%, with significantly higher positivity among males (14-4.2%) and only 1 (0.1%) was female [14]. The reported prevalence of HbsAg in neighbouring countries of Pakistan, China, Sri-Lanka, Bangladesh and Iran is 3-5% [10], 6.89% [11], less than 2% [12], 5.4% [26] and 1.7% [27], respectively [Table/Fig-4].

Author name, [reference no.]	Year of publication	Place	HBV (serological)
Naqshbandi I et al., [14]	2016	District Srinagar	1.2%
Rabyang S et al., [16]	2021	Ladakh region	Ladakh 4.2% Kargil district-7.40%; Leh district 1.96%
Shashi SS et al., [5]	2018	District Jammu	2.44%
Ali M et al., [10]	2011	Pakistan	3-5%
Wang H et al., [11]	2019	China	6.89%
Noordeen F et al., [12]	2015	Sri-Lanka	Less than 2%
Al-Mahtab M, [26]	2016	Bangladesh	5.4%
Poorolajal J et al., [27]	2009	Iran	1.7%
Present study	2022	Doda	0.92%

[Table/Fig-4]: Previous studies showing trend of HBV prevalence [5,10-12,14,16,26,27].

Month-wise highest cases were recorded during first four months (January, Febuary, March and April) as 21 positive cases followed by 17 positive cases during next four months (May, June, July and August) and 12 positive cases during last four months (September, October, November and December) of the year. Highest and lowest number of positive cases were recorded during the month of March and July/September/October as nine and two positive cases, respectively. However, proportion-wise highest and lowest cases were recorded during the month of April and September as 1.55% and 0.33% respectively.

Although India lies in intermediate endemicity zone, the prevalence of HBV infection is low in Nanded region. Effective childhood immunisation programe is likely to further reduce the burden of infection in our country. The similar study in Maharashtra and costal Karnataka reported the prevalence of <2% (1.57%) and 0.62%, respectively [28].

Serological testing can identify patients with persistent HBV infection based on their HbsAg levels. Infants born to HBsAg-positive mothers, pregnant women, sex partners of HBV infected people, homosexuals, household contacts, people born in areas with an HBsAg prevalence of less than 2%, injection drug users, and people who have been exposed to blood and body fluids due to sexual assault and needle stick injuries among healthcare workers are all groups for which the Centres for Disease Control and prevention (CDC) has advised HBsAg testing (CDC, 2008) [29]. According to Sandhu R and Sharma G practices like having several sexual partners, engaging in unprotected sexual activity, sharing needles with IV drug users, and getting tattoos may be the cause of the increased frequency among men [9]. However, in females, a strong immune response aids in the faster and more effective removal of HBV. Furthermore, Ingale H et al., reports high prevalence of infection in adults may be due to higher chances of exposure to HBV due to sexual activity [23].

Limitation(s)

The topography of the district Doda is hilly mountainous region with the general population having limited access to healthcare facility, for that reason resampling of any suspected patients was a very difficult task.

CONCLUSION(S)

In present study, the prevalence of HBV infection in this region is 0.92%, with higher positive rate among male (1.07%) as compared to females (0.84%). Therefore, as per WHO classification present study area is a low prevalence area. Effective childhood immunisation programme might reduce the burden of infection in our area. Hospital-based studies like this can be an alternative option for improve the public health and to prevent the spread of the disease in the local population.

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