Bionature, 39(2) 2019 : 59-69 ISSN: 0970-9835 (P), 0974-4282 (O)

DNA PREVALENCE OF HEPATITIS B VIRUS INFECTION ACROSS DIFFERENT DEMOGRAPHICS IN INDIAN POPULATION

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Received: 15 March 2019
Accepted: 18 May 2019
Published: 25 May 2019

Original Research Article

ABSTRACT

Introduction: Diagnosis has been the major aspects of liver-related infection, for providing early advice to patients. Data about the hepatitis B virus (HBV) DNA level among the Indian population were limited. *Objectives*: To the best of HBV diagnosis, the present study was conducted to determine the virus DNA level and the frequency of infection among the age group and genders in the Indian region.
Methods: A total of 926 suspected subjects, 520 (56.15%) male and 406 (43.84%) female, from east (n = 370), north (n = 410), south (n = 45) and west (n = 101) of ages between 6 and 75 years (mean age 39.65 ± 15.20 years) included in this study. Viral DNAs were isolated from the plasma and performed with the artus® HBV RG PCR assay for HBV specific target.
Results: HBV DNA was identified in a total of 636/926 (68.68%) subjects; 367 (70.57%) male and 269 (66.25%) female. Demographic data evaluation of viral infection among genders within different regions and age groups indicated no significant difference ($P > 0.05$). However, among females, HBV DNA infection rate & viral load exhibited decreased proportion within the southern regions and age group of 61-75 years ($P < 0.05$) compared to the male population.
Conclusions: HBV DNA infection is highly endemic among the suspected Indian population. To prevent the spread of HBV infection among Indians, it is encouraged with diagnosis procedure in the DNA level.

Keywords: Diagnosis; epidemiology; polymerase chain reaction; viral load; India.

INTRODUCTION

Hepatitis B virus (HBV), which can cause acute and chronic liver diseases is a hepato-tropic and non-cytopathic, DNA virus. Liver inflammation is characterized by different stages, during HBV infection and viral DNA replication [1]. Due to the lethal effect of HBV infection, it has been ranked tenth common concern of mortality around the world. In Indian regions, hepatitis B surface antigen carrier rate varied from 2 to 7%, along with the 400 million chronic carriers throughout the world [2,3]. In most of the parts of Asia, the Middle East, and Eastern Europe, HBV infection has become endemic, and dominantly cause liver inflammation and hepatocellular carcinoma [4,5].

HBV chronic infection, particularly in Asia, commonly leads to chronic diseases in 90% of infection [1]. Epidemiological study within different age contributed that horizontal and vertical transmission favors HBV infection. The high rate of endemic viral infection in the specific group has been suggested the association with various social-cultural practices in India [6]. Cancer registered of two decades in major metropolitan Indian urban populations; liver cancer has counted the fifth most frequent

cancer for both male and female [7]. Viral HBV DNA quantification in plasma plays the key role in the diagnosis, to monitor HBV infection, and to assess the response to treatment [8,9]. On the basis of HBV DNA viral load identification, the progression of the liver complication can be predicted for adequate treatment and management. Viral load lower than 2000 IU/ ml of HBV DNA has been associated with biochemical and histological changes in the individuals due to inflammatory activity [10], whereas high HBV DNA viral load more than 20000 IU/ ml consider a main independent risk factor as compare to HBeAg, ALT, and cirrhosis for hepatocellular carcinoma [10,11]. HBV variants may continue to increase at high DNA level without secreting HBeAg, hence quantification of HBV DNA can be a more useful measurement than HBeAg. HBV DNA viral load can also be utilized to find the capacity of antiviral therapy, by detecting viral DNA recovery with a time interval than the serologic markers [11,12].

Thus, identification of HBV infection in the countries, especially the population of Indian region and subgroup, should be considered to understand the planning and to control the disease in the possible region. To the best of HBV DNA prevalence and population dynamics of the diverse group in India, the present study was carried out to measure the virus DNA load, and the frequency of infection within the different regions, among age-groups and genders in the Indian subcontinent.

MATERIALS AND METHODS

Study Population

The study populations of 926 individuals were taken from the four major regions of the Indian subcontinent, from May 2017 to July 2018 for HBV diagnosis. The informed consent form was obtained from all subjects. The 926 suspected subjects included 520 (56.15%) male and 406 (43.84%) female, from east (n = 370), north (n = 410), south (n = 45) and west (n = 101) regions with ages ranging between 06 and 75 years (mean age ± 39.65 ± 15.20 years). The patients were divided into five age groups; 1 to15 year, 16 to 30 years, 31 to 45 years, 46 to 60 years and 61 to 75 years.

The study subjects from the east region (n = 370) were of Bengal (Kolkata = 107), Odisha (Cuttack = 52, Balipatna = 48, Bhubaneswar = 14, Lingaraj = 3), Bihar (Patna = 54, Phulwari = 7, Motihari = 5), Assam (Guwahati = 12), the adjacent country Bangladesh (Dhaka = 54, Chittagong = 9), and Nepal (Kathmandu = 5). Subjects from the north region (n = 410) were of Delhi (New Delhi = 350), Uttar Pradesh (Ghaziabad = 17, Agra = 15), Punjab (Ludhiana = 11, Faridkot = 6, Jalandhar = 5, Barnala = 2, Mohali = 01), Haryana (Ambala = 2, Gurgaon = 1). Subjects from the south region (n = 45) were of Karnataka (Mysore = 10, Mangalore = 8, Bangalore = 7), Telangana (Nampally = 9, Hyderabad = 5, Secundrabad = 2), Andhra Pradesh (Tirupati = 2), Kerala (Kochi = 1), Tamilnadu (Chennai = 1). Subjects from the west region (n = 101) were of Maharashtra (Nagpur = 59, Mumbai = 10, Dahisar = 2, Bhusawal = 1, Jalgaon = 1, Pune = 1), Gujarat (Ahmadabad = 14), Rajasthan (Baijaipur = 7, Jaipur = 6), presented the sample place.

Sample Collection and Viral DNA Extraction

Blood sample for separation of plasma was collected from all the subjects. The plasma was separated by centrifugation, aliquot, placed at -20˚ C, and processed for extraction process. Viral nucleic acid was

recovered from 500µl of plasma sample by QIAamp® DSP virus extraction procedure, following the kit manufacturer's steps.

Real-time Quantitative PCR

Quantification was performed by the Real-time PCR with provided reagents for the specific region of the HBV genome by artus® HBV RG PCR protocol. Amplification was executed while cycling in the Real-Time PCR (Rotor-gene Q) System (Qiagen, Germany), using TaqMan based PCR master mix reagents. It was programmed of thermal cycling conditions for 45 cycles: denaturation 15 seconds at 95^0C, annealing 30 seconds at 55^0C and extension 15 seconds at 72^0C, with an exponential signal of data received.

Statistically Impact

The statistical analysis adverse impact was done by Microsoft excels. The value of amplified viral DNA in IU/ ml from the blood plasma was explored to analyze the viral load distribution in this selected study population (n = 926). To evaluate finding, the one-sample Kolmogorov-Smirnov test was applied and found uneven viral load distribution ($P < 0.05$). Continuous variables were analyzed using an independent sample t-test. Chi-square test was performed to impose the associations of demographic impact and frequency for HBV infection and viral load among categorized regions, age groups, and genders. The *P*-value statistics analyzed of < 0.05 was considered to be significant.

RESULTS

Subjects detected positive from east regions (n = 253/370) were of Bengal (Kolkata = 71/107), Odisha (Cuttack = 35/52, Balipatna = 35/48, Bhubaneswar = 9/14, Lingaraj = 3/3), Bihar (Patna = 38/54, Phulwari = 6/7, Motihari = 4/5), Assam (Guwahati = 7/12), the adjacent country Bangladesh (Dhaka = 34/54, Chittagong = 7/9), and Nepal (Kathmandu = 4/5). Subjects identified positive from north region (n = 288/410) were of Delhi (New Delhi = 248/350), Uttar Pradesh (Ghaziabad = 12/17, Agra = 10/15), Punjab (Ludhiana = 10/11, Faridkot = 3/6, Jalandhar = 3/5, Barnala = 0/2, Mohali = 0/1), Haryana (Ambala = 1/2, Gurgaon = 1/1). In the south region subjects identified positive (n = 26/45) were of Karnataka (Mysore = 3/10, Mangalore = 3/8, Bangalore = 5/7), Telangana (Nampally = 6/9, Hyderabad = 5/5, Secundrabad = 2/2), Andhra Pradesh (Tirupati = 2/2), Kerala (Kochi = 0/1), Tamilnadu (Chennai = 0/1). Subjects identified positive from west region (n = 69/101) were of Maharashtra (Nagpur = 43/59, Mumbai = 6/10, Dahisar = 1/2, Bhusawal = 0/1, Jalgaon = 1/1, Pune = 0/1), Gujarat (Ahmadabad = 9/14), Rajasthan (Baijaipur = 5/7, Jaipur = 4/6), presented the positive population.

HBV DNA was detected in a total of 636/926 (68.68%) from the patients' plasma samples. The level of the viral load recorded for HBV DNA, which was measured by the Real-time PCR assay, ranged from 10.5 to > 2×10^7 IU/ ml from the plasma samples (median 881 IU/ ml). Of the DNA infected patients, 367 (70.57%) were male and 269 (66.25%) were female. The distribution of HBV DNA viral load (IU/ml) from the population was not normal ($P < 0.05$), identified by the one-sample Kolmogorov-Smirnov test. Furthermore, nonparametric tests were considered. Demographic data evaluation of viral infection among genders within different regions and age groups did not indicate a significant difference ($P > 0.05$). The different demographic and frequencies among the Indian population of

observed HBV DNA viral load infection were presented in Fig. 1 and Fig. 2. Further evaluation among females, HBV DNA positive infection rate & viral load exhibited decreased proportion within the south regions (Table 1) and age group of 61-75 years (Table 2) ($P < 0.05$). It was also not identified a significant difference in viral load between the genders in this population study ($P > 0.05$).

DISCUSSION

Although opinions for seroprevalence of HBV infection has been performed frequently [13], there is limited information about the HBV DNA prevalence among the Indians. To surge over the knowledge, this is an important aspect of HBV DNA viral level among different regions, age groups and genders in Indian population. The study population was comprised with varied numbers of subjects and positive group, from the highest in the north region (n = 288/410; 70.24%) followed by east region (n = 253/370; 68.37%), west region (n = 69/101; 68.31%) and south region (n = 26/45; 57.77%). In this study, HBV DNA was detected in a total of 636/926 (68.68%) of the participated Indian population, which reflected the high endemicity in this population. Therefore the use of HBV viral load and early identification is very important for providing therapy and for detection of hepatitis [14].

Identification of HBV DNA in the plasma can be defining the infectious stage in a particular individual. A wide range of viral load, 10.5 to $>2\times10^7$ IU/ml, was detected in plasma samples. Clinically HBV-DNA load vary greatly, from very low levels to high levels in the patients either on antiviral treatment or in those with HBV positive infection [15]. Male to female ratio of this study observed were 1.28:1. The age of presentation varied from 6 to 75 years (mean age 39.65 ± 15.20 years). The results of our study showed, 70.57% of HBV DNA positive patients were male, and 66.25% were female, which is the agreement with the earlier report in Iran and Turkey [16,17]. The use of the intravenous drug has been more common in males than females explains the high prevalent infection among male Indian population. The infection can be transmitted to young and adults during unprotected sex or with infected partners. However, the exact mode of HBV transmission was not assured and anticipated through blood and exposure to contaminated needles [18]. Prevalence of possible risk factor of HBV infection, such as marking skin tattoo, used shaving razor, and other contaminated devices can be the reason for transmission. In addition, mechanical mode of transmission has also been hypothesized within south Indian (Kerala) people, who were frequently bitten by ‘deer fly’ of genus *Chrysops*. Among them, the majority of the patients were of above 50 years old, literate, and aware of the consequences of cirrhosis and hepatocellular carcinoma [19].

The demographic frequency of subjects was identified and found fluctuation in different regions (Fig. 1) and age groups (Fig. 2). HBV infection in young and adult age fluctuated with different viral load was characterized by liver inflammatory events, and called as clearance phase [1,20]. Favored interpretation of these different viral infection patterns has been the loss of immune tolerance at the start of adulthood [20]. HBV DNA viral load difference in the northern region among male found close to the significant level, which alarms the situation and defined the lack of awareness among the group for the risk of transmission by using either intravenous drug, blood transfusion, tattooing or from infected sexual

partners [17]. Asian cohort study demonstrated that progression to cirrhosis was mainly correlated to HBV-DNA levels, where old age and male sex were important risk factors [21]. Male were more likely to get infected with HBV infection than female gender. The prevalence was found two times higher in the male in comparison with the female in HBsAg level [22]. The Higher immunity in females, comparative to males may contribute to HBsAg clearance [23].

The low HBV viral infection rate in female compared to male within the south region (Table 1) and the age groups of 61-75 years (Table 2) explains the inactive or immune-tolerant phase among them [24]. Whereas, the high viral infection rate in male population suggest more involvement in contact with contaminated household sharp items, such as razors and syringe. Population shift, because of migration or to travel among Indian male can be the other explanation to come in contact with horizontal transmission and for being the high viral load. Of the HBsAg prevalence survey in Taiwan, life expectancy was lower in males (73.5 years) than in females (79.7 years) and this was contributing to the gender difference for patients older than 60 years. Male gender was more likely to develop HBV related complications than female gender [25]. A time-series data analysis was performed for the incidence and mortality rates from hepatitis B infection and a significantly increased mortality was observed for men within the age group of 70-79 years, therefore highlighted the preventive care and treatment for an elderly people [23].

Table 1. Adverse impact of HBV DNA viral load (International Unit/ milliliter) and infection rate among genders within the major Indian subcontinent

Regions	Viral load (IU/ml)	Male (%)	Female (%)	Adverse impact
East India				
	Negative	67 (32.84)	50 (30.12)	χ^2statistic: 0.172
	$<2x10^4$	112 (54.90)	95 (57.22)	χ^2*P*-value: 0.917
	$>2x10^4$	25 (12.25)	21 (12.65)	
North India				
	Negative	52 (24.76)	70 (35)	χ^2statistic: 5.490
	$<2x10^4$	103 (49.04)	102 (51)	χ^2*P*-value: 0.064
	$>2x10^4$	55 (26.19)	28 (14)	
South India				
	Negative	11 (35.48)	8 (57.14)	χ^2statistic: 11.797
	$<2x10^4$	14 (45.16)	5 (35.71)	χ^2*P*-value: 0.003
	$>2x10^4$	6 (19.35)	1 (7.14)	
West India				
	Negative	23 (30.66)	9 (34.61)	χ^2statistic: 0.997
	$<2x10^4$	37 (49.33)	11 (42.30)	χ^2*P*-value: 0.607
	$>2x10^4$	15 (20)	6 (23.07)	

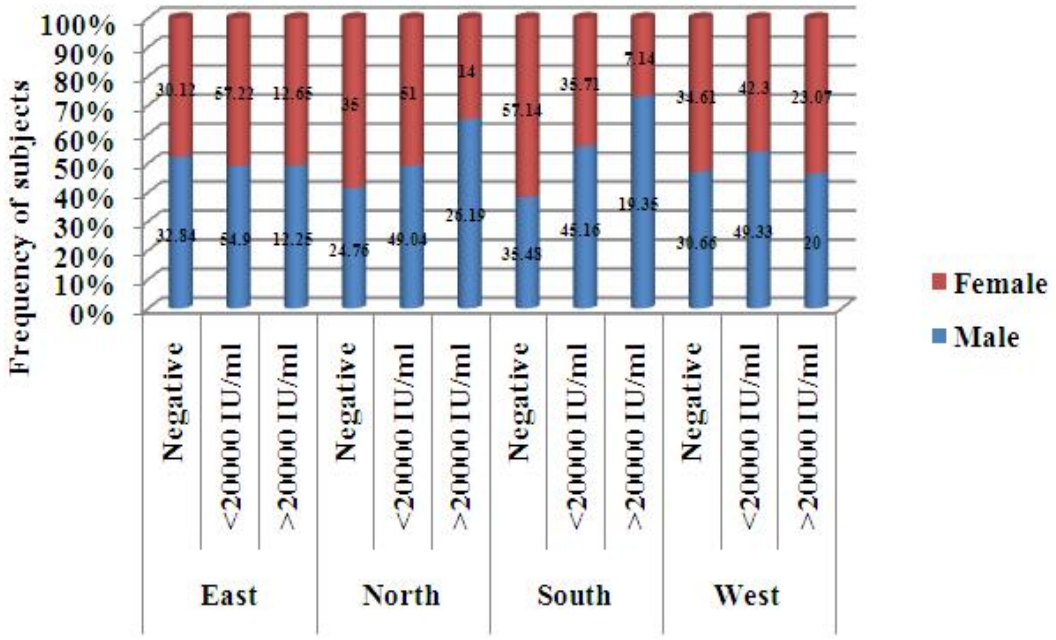

Fig. 1. Demographic frequency among male and female within Indian regions for HBV DNA viral load infections

In east region; male subjects were 32.84% negative, 54.90% with viral load <2 x 10^4 IU/ml, 12.25% with viral load >2 x 10^4 IU/ml and female subjects were 30.12 % negative, 57.22 % with viral load <2 x 10^4 IU/ml, 12.65 % with viral load >2 x 10^4 IU/ml. In north region; male subjects were 24.76 % negative, 49.04 % with viral load <2 x 10^4 IU/ml, 26.19 % with viral load >2 x 10^4 IU/ml and female subjects were 35 % negative, 51 % with viral load <2 x 10^4 IU/ml, 14 % with viral load >2 x 10^4 IU/ml. In south region; male subjects were 35.48 % negative, 45.16 % with viral load <2 x 10^4 IU/ml, 19.35 % with viral load >2 x 10^4 IU/ml and female subjects were 57.14 % negative, 35.71 % with viral load <2 x 10^4 IU/ml, 7.14 % with viral load >2 x 10^4 IU/ml. In west region; male subjects were 30.66 % negative, 49.33 % with viral load <2 x 10^4 IU/ml, 20 % with viral load >2 x 10^4 IU/ml and female subjects were 34.61 % negative, 42.30 % with viral load <2 x 10^4 IU/ml, 23.07 % with viral load >2 x 10^4 IU/ml

Patients who were in immune clearance and reactive phase need to be treated. It has also required more focus to treat patients who suffered immune-tolerant phase and with high HBV DNA load, as significant fibrosis and inflammation have been reported in infected person with persistent viral DNA levels >10000 copies/ ml. The total clearances of HBV DNA persist due to short-term antiviral treatment [26]. Reduction for the high viral load; it has created the need for an alternative form of treatment either after long-term therapy or from drug resistance infection [27,28]. Age groups, regions, genders and duration of infection possibly predict the severity of liver disease in the presence of respective HBV DNA viral loads. Thus the clinical diagnosis of HBV infection state could be improved by using accurate quantification of viral load for measuring replication rate in the infected individual.

The limitation of our study was no proper information about the clinical history of the disease in the patient, sexual behavior and sharing partner. People have been more traditional and religious and were also afraid of sharing their information, and to disclose identity because of the scare, or to be isolated from society. Even after this high prevalence, the implementation of vaccination programs has been reportedly inconsistent and limited in India [29].

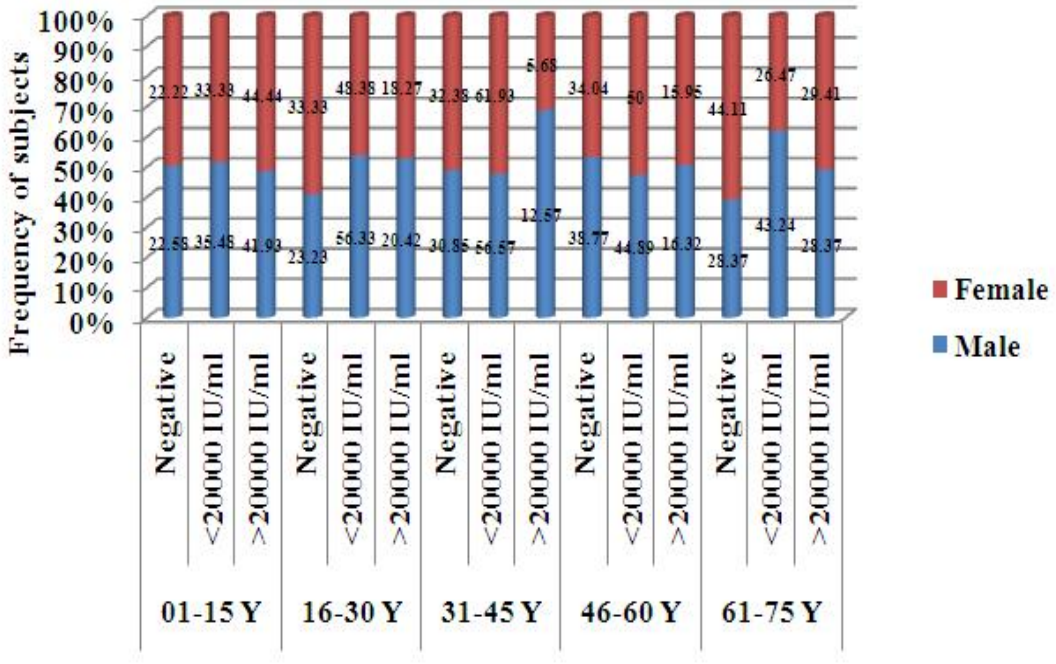

Fig. 2. Demographic frequency among male and female within age groups for HBV DNA viral load infections

In age group of 1-15 years; male subjects were 22.58 % negative, 35.48 % with viral load <2 x 10^4 IU/ml, 41.93 % with viral load >2 x 10^4 IU/ml and female subjects were 22.22 % negative, 33.33 % with viral load <2 x 10^4 IU/ml, 44.44 % with viral load >2 x 10^4 IU/ml. In age group of 16-30 years; male subjects were 23.23 % negative, 56.33 % with viral load <2 x 10^4 IU/ml, 20.42 % with viral load >2 x 10^4 IU/ml and female subjects were 33.33 % negative, 48.38 % with viral load <2 x 10^4 IU/ml, 18.27 % with viral load >2 x 10^4 IU/ml. In age group of 31-45 years; male subjects were 30.85 % negative, 56.57 % with viral load <2 x 10^4 IU/ml, 20.42 % with viral load >2 x 10^4 IU/ml and female subjects were 32.38 % negative, 61.93 % with viral load <2 x 10^4 IU/ml, 5.68 % with viral load >2 x 10^4 IU/ml. In age group of 46-60 years; male subjects were 38.77 % negative, 44.89 % with viral load <2 x 10^4 IU/ml, 16.32 % with viral load >2 x 10^4 IU/ml and female subjects were 34.04 % negative, 50 % with viral load <2 x 10^4 IU/ml, 15.95 % with viral load >2 x 10^4 IU/ml. In age group of 61-75 years; male subjects were 28.37 % negative, 43.24 % with viral load <2 x 10^4 IU/ml, 28.37 % with viral load >2 x 10^4 IU/ml and female subjects were 44.11 % negative, 26.47 % with viral load <2 x 10^4 IU/ml, 29.41 % with viral load >2 x 10^4 IU/ml

Table 2. Adverse impact of HBV DNA viral load (International Unit/ milliliter) and infection rate among genders within the different age groups population

Age Groups	Viral load (IU/ml)	Male (%)	Female (%)	Adverse impact
01-15 years				
	Negative	7 (22.58)	2 (22.22)	χ^2statistic: 0.143
	$<2x10^4$	11 (35.48)	3 (33.33)	χ^2*P*-value: 0.931
	$>2x10^4$	13 (41.93)	4 (44.44)	
16-30 years				
	Negative	33 (23.23)	31 (33.33)	χ^2statistic: 2.523
	$<2x10^4$	80 (56.33)	45 (48.38)	χ^2*P*-value: 0.283
	$>2x10^4$	29 (20.42)	17 (18.27)	
31-45 years				
	Negative	54 (30.85)	57 (32.38)	χ^2statistic: 2.88
	$<2x10^4$	99 (56.57)	109 (61.93)	χ^2*P*-value: 0.237
	$>2x10^4$	22 (12.57)	10 (5.68)	
46-60 years				
	Negative	38 (38.77)	32 (34.04)	χ^2statistic: 0.586
	$<2x10^4$	44 (44.89)	47 (50)	χ^2*P*-value: 0.746
	$>2x10^4$	16 (16.32)	15 (15.95)	
61-75 years				
	Negative	21 (28.37)	15 (44.11)	χ^2statistic: 7.471
	$<2x10^4$	32 (43.24)	9 (26.47)	χ^2*P*-value: 0.024
	$>2x10^4$	21 (28.37)	10 (29.41)	

CONCLUSIONS

In conclusion, HBV DNA infection is highly endemic among the suspected Indian population. To prevent the spread of HBV infection among Indians, it is encouraged with diagnosis procedure in the DNA level, and to sensitize the risk of HBV transmission in the different group of the population. Moreover, knowledge of HBV infection risk is able to prevent virus transmission among Indian society. It is also recommended treatment strategies in form of medicine and duration, vaccination programs for the population that could be the effective control and prevention strategy from this infection.

CONSENT AND ETHICAL APPROVAL

The written consent form was taken from all the volunteers. This study was supported by Kumaun University, Nainital and CORE Diagnostics, Gurugram and approved by its ethics committee.

AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration among all authors. Author JCK designed the study, performed the experiment and wrote the first draft of the manuscript. Author R. Kumar designed and managed the analyses of the study. Author R. Katara managed the literature searches. All authors read and approved the final manuscript.

ACKNOWLEDGEMENTS

We like to thank, the personnel of CORE Diagnostics, Gurugram, and Department of Biotechnology, Kumaun University, Nainital

for their help and support during our study. The authors also gratefully acknowledged the Founder and CEO- Zoya Brar, CORE Diagnostics, for giving permission to work and analyze data for the Hepatitis B study.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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