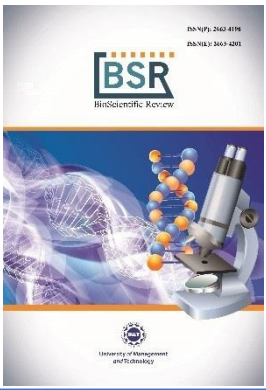

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Age-wise and Gender-wise Prevalence of Hepatitis B Virus (HBV) Infection in Lahore, Pakistan

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Abstract

The prevalence of hepatitis B virus (HBV) in the Pakistani population has been reported previously, however, studies with a city-oriented approach and focus on age and gender distribution are very limited. Therefore, the current study was designed to unravel the age-wise and gender wise prevalence of HBV in Lahore, Pakistan. A total of 350 blood samples of both male and female patients who visited National Genetic Laboratory, Lahore between February 2019 and July 2019 and who were suspected of HBV infection were screened. Sandwich based ELISA was used to detect rapid hepatitis B surface antigen (HbsAg) according to the manufacturer's instruction. Real time PCR was used to detect HBV using HBV Rotor Gene PCR kit. Out of 350 blood samples screened for HBV infection (n= 350), 180 (51.43%) were of males and 170 (48.57%) were of females. Mean age (years) with SD (standard deviation) of the screened population was 37.22 ± 12.16 years. Overall, 224 samples (64%) were found to be positive for HBV infection. In our study, the number of females with this infection (52.24%) was slightly higher than males (47.76%). However, we observed no statistically significant difference (p = 0.225) between them. Our study concludes that HBV is highly prevalent in Lahore, Pakistan. Females are slightly more susceptible to HBV infection as compared to males. This study also reports that HBV is more prevalent in the 20-40 age group.

1. Introduction

Hepatitis B virus (HBV) comprises of a partially double-stranded circular DNA surrounded by a core capsid which is enveloped by Hepatitis b surface antigens (HbsAg). The virus is a member of family Hepadnaviridae and primarily infects the liver. HbsAg particles show a varied morphology. High concentrations of HbsAg is one of the most common markers used for diagnosis and such high concentrations are found in acute infections. A positive outcome for HbsAg means a person is infected and potentially infectious [1, 2, 3]. WHO health assembly resolutions in 2010 and 2014 acknowledged hepatitis as a global health issue and declared HBV 15th highest among all causes of global mortality. Chronically infected patients have been reported to develop liver failure, cirrhosis, or hepatocellular carcinoma with a mortality rate of 15-40% [4]. HBV

infection is 50-100 times more communicable than human immunodeficiency virus (HIV**)** and 10 times more transmissible than HCV. It acts as a silent killer as many carriers may not realize that they are already infected [5].

Worldwide, about 250 million persons are infected with HBV. According to the WHO global Hepatitis report, the prevalence of HBV in general population is reportedly 3.5% and a high chronic infection rate is reported among those who were born before the availability of HBV vaccines. The highest prevalence is reported in the Western Pacific region and the African region as 6.2% and 6.1%, respectively. It has been reported also that 20% or more of those with chronic infection develop an end-stage chronic liver disease, such as cirrhosis or hepatocellular carcinoma depending on life expectancy [6]. Luckily, a highly effective vaccine against HBV is available since 1980s and its availability has remarkably altered the global HBV epidemiology [7]. This vaccine has proved to be the commercially viable combat strategy at public level against HBV epidemic. However, mortality and morbidity remains high for those with weak response to the vaccine [8]. Moreover, the mortality rate due to liver cirrhosis or hepatocellular carcinoma for perinatally acquired HBV is as high as 40% for men and 15% for women [9].

HBV endemicity is intermediate in Pakistan with an estimated 3-5% ratio. Interior Sindh, Karachi, Southern Punjab, Kurrum agency, Northern Waziristan and some parts of Lahore are reported to have HBV prevalence of >5% [10, 11, 12, 13, 14, 15]. In Pakistan, HBV reportedly shows 44.45% association with chronic liver diseases [16]. The unsafe administration of therapeutic injections in health care facilities has been identified as a consistent risk factor for HBV [14, 16, 17].

Although scattered reports of HBV prevalence in Pakistan are available; however, the number of studies about Lahore, one of the most populous cities of Pakistan, is meagre. Therefore, we conducted this study with the purpose of finding out the prevalence of HBV in Lahore.

2. Methodology

For this cross-sectional retrospective study, a total of 350 patients suspected of HBV (including both male and female patients) who were between 10 to 70 years of age and visited National Genetic Laboratory, Lahore from February 2019 to July 2019, were screened for HBV seropositivity. The tests were carried out using the method previously described by Khan, F et al. 2011, with little modifications [14]. ALT level was measured using reagents (Diagnotest; Paris, France) and Anti-HBV II kit (Cobas, Roche, Switzerland) based on sandwich based ELISA, according to the manufacturer's instructions. This was performed to detect rapid hepatitis B surface antigen (HbsAg).

2.1. ALT (Alanine Amino Transferees) Test

This test is based on photometric analysis and the procedure was adopted according to the manufacturer's (Diagnotest; Paris, France) instructions. OD was taken using semi-automated clinical chemistry analyzer Microlab-300.

2.2. Anti-HBV Screening

Patients with elevated levels of ALT were further screened for HBV antibodies through ELISA. HBV antibodies were analyzed using Anti-HBV II kit (Cobas,

Table 1. Seroprevalence of HBV

	HBV Positive							
	Overall Prevalence		S.P		M.P		W.P	
	n	%	n	%	n	%	n	%
Male	107	47.77	5	14.29	22	6.3	35	10
Female	117	52.23	54	15.4	21	6	42	11.9
	HBV Negative							
Male	73	15						
Female	53	21						

Roche, Switzerland) based on sandwich based ELISA. Readings were measured on Cobas e411 Analyzer. In this assay, results are determined automatically by the software which associates the electro-chemiluminescence signal gained from the reaction product of the sample with the signal of the cutoff value.

2.3. Real Time PCR

HBV Rotor Gene (HBV RG) PCR was used in this study which is a kit of HBV DNA detection for real time PCR. All the enzymes and the reagents which are required for the amplification of 110 bp sequence of the HBV genome were present in the HBV RG Master except Mg2+. It also has control over inhibition and regulate the amplification efficacy of the system by detecting possible PCR inhibition when HBV PCR product is absent.

3. Statistical Analysis

Data analysis using descriptive statistics was carried out through excel and SPSS (version 21). Chi-square test was used to measure the association of HBV prevalence with gender and age. Mean ± SD was calculated for age. Data was stratified for age groups and gender. Prevalence was calculated in terms of percentage of the number of positive patients for this study population.

4. Results

A total of 350 blood samples of suspected HBV positive patients were screened for hepatitis B virus (n= 350), out of which 180 (51.43%) patients were male and 170 (48.57%) patients were female. Mean age (years) with SD (standard deviation) of the screened population was 37.22 ± 12.16 years. A total of 224 samples were found seropositive for HBV infection as summarized in Table 1. The overall seroprevalence of HBV for the screened population was 64% (Figure 1).

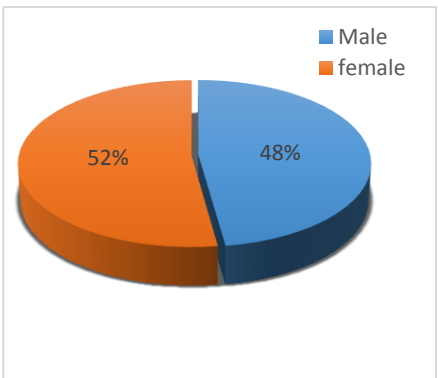

Figure 1. The graph shows overall seroprevalence of HBV based on gender

In this study group, HBV prevalence was slightly higher in females (n= 117, 52.24%) as compared to males (n= 107, 47.76%). However, no statistically significant association was found between

Table 2. Gender Wise Prevalence of HBV

Gender	Hepatitis B status			p-value*
	Positive n (%)	Negative n (%)	Total N	
Male	107(47.77)	73(15)	180	0.225
Female	117(52.23)	53(21)	170	

***** The chi-square statistics is significant at p=.05

gender and prevalence (p= 0.225). The results showed no significant interaction (p= 0.279) between gender and age regarding the prevalence of HBV (Table 2). The population of the study was stratified into five major groups based on the parameter of age as shown in Table 2. Most of the people in the population were young and were part of the age group 21-40. However, age seemed to have a connection with HBV. There was a marked increase in HBV prevalence towards second decade peaking at 31% and 42% for males and females, respectively (Figure 2).

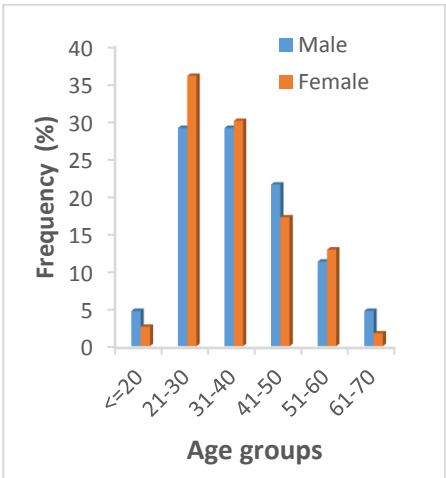

Figure 2. Gender wise frequency of HBV positive cases in age groups

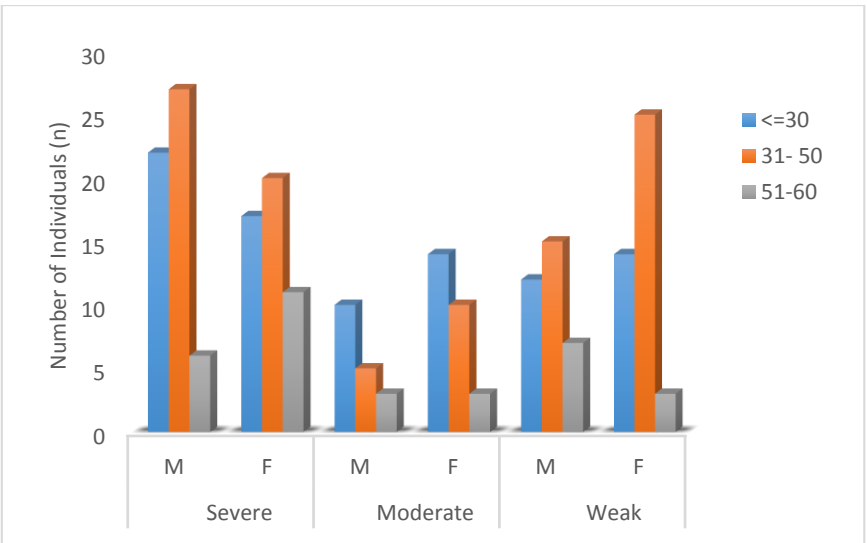

Figure 3. The graph shows distribution of severity of HBV in age groups based on gender

Table 3. HBV Prevalence in Gender * Age groups

Gender	Age Groups												Total (N)	p-value*
	10 - 20		21 - 30		31 - 40		41 - 50		51 - 60		61 - 70			
	n	%	n	%	n	%	n	%	n	%	n	%		
Male	5	4.68	31	28.97	31	28.97	23	21.45	12	11.22	5	4.68	107	
Female	3	2.57	42	35.89	35	29.91	20	17.09	15	12.82	2	1.7	117	0.279
Total	8	3.57	73	32.59	66	29.46	43	19.19	27	12.05	7	3.13	224	

*The Chi-square statistic is significant at α = .05

The severity of the disease was related to age groups and it showed high severity in population aged between 31-51 years (27 females and 20 males). The population in age group 51-60 showed the lowest rates of severity for both males and females (Table 4, Figure 3).

Table 4. Age and Gender based Distribution of Severity of HBV (N=224)

Age Groups	Severe		Moderate		Weak	
	M	F	M	F	M	F
<=30	22	17	10	14	12	14
31- 50	27	20	5	10	15	25
51-60	6	11	3	3	7	3
Total	55	48	18	27	34	42

5. Discussion

In our study, we used the serological method to evaluate HBV seroprevalence in patients who were suspected of HBV infection. An overall seroprevalence of 64% is very high and suggests the lack of efficient combatting programs. This becomes apparent when we compare the rates with developed countries with efficient vaccination programs [18, 19]. As compared to HBV prevalence found in the current study, the results of previous studies reported an overall prevalence of 6.39% for KPK [20, 21] and 2.45% for Rawalpindi. General prevalence was reported to be between 2.11% and 5.46% across different parts of the country [18, 19, 22]. This difference can be attributed to many reasons such as different study designs, different population groups, variations in epidemiology, demography and diagnostic assays. Furthermore, most of the studies reported prevalence among general population using a large sample size, whereas the sample of this study comprised only those people suspected with HBV infection. A similar study conducted in the Malakand Division of Pakistan reported 21.05% seroprevalence of HBV in the population suspected with HBV infection [23]. In injectable drug addicts, 22.4% were found seropositive for HBV [24].

We observed a difference in HBV prevalence across gender. Unlike most previous studies that reported high prevalence for males [19, 21, 23], our study showed a higher rate of infection in females, although the total number of samples (n) did not show a wide variation (170 males and 180 females). We found a report from Iran stating high prevalence in females [25]. This difference can be attributed to increase in risk behavior in females as they adopt a metropolitan lifestyle. Another reason could be the transmission of HBV from males to females as marital partners. Marital status plays a significant role in HBV transmission, particularly in heterosexual communities [26, 27]. There should be studies focused on this aspect of transmission to evaluate its possible contribution in spreading the disease. Health care workers at health centers are

mostly females. Also, females are usually the caretakers of sick family members at home. So, they remain the more exposed population.

In the stratified age groups, higher prevalence was recorded among the younger population as compared to older people and/or children. These results are comparable to another study which also reported a similar trend of higher prevalence among younger people as compared to children and/or older age groups [14]. This might suggest that the risk of exposure to HBV at a young age is more common in Pakistan. A similar study stated a high prevalence of HBV antigen in chronic cases resulting from exposure at a young age [28]. A study from India also reported high prevalence (32.7%) for the population between 15-20 years [29, 30] of age. Another study from Iran reported high prevalence for age group 25-34 [31].

The intermediate prevalence of HBV in the Asian region shows a distinct pattern of prevalence. This might be the result of ongoing vaccination programs. The variation in the global prevalence of HBV and the increasing rate of morbidity and mortality due to chronic liver diseases caused by it ask for a targeted approach [32] for its treatment and extermination. The understanding of region and age specific HBV prevalence is very important for disease control. Moreover, efficient management of disease and the evaluation of vaccination programs is essential since chronic HBV infection has been reported as an outcome of exposure to HBV as an infant [33]. So, it is very important to target pregnant women in control and immunization strategies. HBV is asymptomatic, hence it is considered to be the most dangerous of all types of hepatitis. It is very important to timely diagnose HBV to control its rapid spread [20]. A survey should be conducted to evaluate the rate of HBV infection in the general population at a large scale with the help of authorities to design future prevention programs.

6. Conclusion

This study showed the seroprevalence of HBV in various age groups. The infection was found to be more common in females than males and younger people suffered from a greater severity of infection than the older population. Timely diagnosis of HBV is crucial to control its spread. The high seroprevalence of HBV is alarming. Similar studies will help to devise surveillance and control programs for HBV prevention. All stakeholders should take drastic measures to control the spread of HBV and devise public health policies.

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