



Research Article

Molecular Prevalence and Causes of Hepatitis B Virus Infection in District Bannu Khyber Pakhtunkhwa, Pakistan

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Abstract

Hepatitis B Virus (HBV) infection is a major health problem in the developing countries counting Pakistan. This study aimed to examine causes and molecular prevalence of HBV in district Bannu Khyber Pakhtunkhwa province, Pakistan. For the diagnosis of anti HBV antibodies a total of 500 blood sample were collected from gender patients, (300 males and 200 females) were screened, directed by clinicians of HBV in the Khalifa Gull Nawaz Teaching Hospital Bannu. All the individuals were aged between 01-60 years categorized into three groups. Out of which 78 (26%) males and 48(24%) females were reported positive for anti HBV. Individuals including 75 (25%) males and 47 (23.5%) females were found positive for HBV DNA while the remaining 4patients including 2 males and 2 female were found negative. Sex-wise distribution of HBV infection shows that as compared to female population 47 (23.5%) male population was more infected 75 (25%), the higher prevalence rate was found in female as compare to male. The present study also observed prevalence of HBV age wise from 1 to 60 years. The lowest HBV rate is found in age group of 1-20. This may be due to the reason that they have a strong immune system and also have a minimum contact with a factors transmitted HBV such as a barber contact, drug abuse, tattooing and piercing, surgery, and blood transfusion. Moderate rate is found in age group 21-40. This is due to the fact that these people have weak immune response and high contact with risk factors associates with HBV than children. Highest prevalent rate found in age group of 41-60 due to weak immune system and lack of awareness. Extensive vaccination and other preventive measures should be taken to stop the spread of this dreadful disease in the general population of District Bannu KP Pakistan.

Introduction

Viral Hepatitis is a diseases of liver in which inflammation of liver cells occur. The word Hepatitis is the combination of two words, according to the Greek word hepar means the "liver" and the Latin word means the "inflammation" so the liver have an effect on and hinder its ability to renew. Liver is a soft and elastic organ that complete different function such as blood filtration (break down of harmful substance, remove bacteria and injure Red blood cells) and production of bile that is store in a gall bladder and then release in small intestine for fat digestion. Hepatitis caused liver cells failure and then inflammation of the liver also. Hepatitis B virus (HBV) is a hepadnavirus. Hepatitis B is a viral disease that causes liver inflammation [1,2]. Hepatitis B is caused by the hepatitis B virus (HBV), the HBV make attacks the liver cells and causing acute as well as chronic hepatitis. Acute hepatitis produces simple sign and symptoms from which people get well soon, while chronic hepatitis symptom are more subtle and treatment is focused on to check long term liver injure and spread of the disease. When

infection is appeared, it may be acute or chronic (long-term) [3]. Acute hepatitis B (affecting approximately 20% of people infected) is usually a low grade sickness from which people totally recover. The chronic form of hepatitis B (affecting approximately 20% of people infected) is more serious and mainly lead to complications if not treated at proper time, such as liver cirrhosis (scarring of the liver), liver malfunction and liver cancer [4]. Hepatitis B infection is the main health problem all over the world About 2 billion people are infected with Hepatitis B Virus (HBV) worldwide of which 350 million are chronic HBV transporter. Each year approximately 1 to 2 million people die from HBV related complications such as chronic hepatitis, cirrhosis and hepatocellular carcinoma [5,6].

HBV transmission has been observed by mucosal contact with to infected blood and body fluids. HBV spread through blood, serum, body fluids, semen, and saliva. The HBV can survive for numerous days in dry blood and on table surfaces, needles, syringes and razors. The use of non sterilized dental and surgical apparatus, cut off from barber, reuse of needle for

nose and ear stabbing/shrill, reuse of non-refundable syringes and distribution needles with drugs abusers, sharing personal things such as razors, toothbrushes, and sexual and delayed close up personal contact with infected person are also the widespread ways of HBV transmission [7].

Pakistan is highly endemic (9 million people infections across the country), with 3% chronic HBV carriers, its transmission and the speed of this disease is increasing day to passing day [8].

High frequency of HBV was observed in ecological areas of low financial group, which underscores the importance in controlling this disease because approximately, 67.5% of the Pakistani population belongs to rural areas of low financial group. This study was planned with the main aim to determine the causes and molecular prevalence of HBV infection in district Bannu Khyber Pakhtunkhwa Pakistan, as limited data is available about the HBV infection in this province of the country.

Material and methods

Study/ Experimental area

The research study was conducted in the District Bannu Khyber Pakhtunkhwa province, Pakistan, as all the research's experimental work was conducted in Hematology lab and PCR section in Khalifa Gulnawaz Teaching hospital Bannu. Blood samples and investigational data were collected from patients attending the Institute of Khalifa Gull Nawaz. Patients belonging to different areas of Bannu were included in this study.

Study sample

For the diagnosis of anti HBV antibodies a total of 500 blood sample were collected from gender patients, (300 males and 200 females) were examined, directed by clinicians of HBV in the Khalifa Gull Nawaz Teaching Hospital Bannu. All the persons were aged between 01-60 years arranged into three groups including aged, adults and children. Informed permission was collected from all the people included in this study and the most considerable and specific was the permission from children parents for permit us to do experiments and collect data for completion of our work without taking any risk. Out of which 78 (26%) males and 48(24%) females were reported positive for anti HBV antibodies and then these were additional processes for the finding of HBV DNA. Individuals including 75 (25%) males and 47 (23.5%) females were found positive for HBV DNA while the lasting 4patients including 2 males and 2 female result were negative. Took blood from patient and then centrifuged at 10,000 RPM for 15 mints to separate serum. Testing performed directly after the sample has been collected. Sample may stored at 2-8 °C for up to 3 days. For long term storage, specimens should be kept below -20°C.

HBV screening

HBV screening was carried out with ICT (Immunochromatographic Technique) for finding anti HBsAg and other such as HBsAb, HBeAb, and HBe AB.

HBs Ab

It is also called as Hepatitis B surface. "Positive" or "reactive" anti-HBs test result indicates that a person is protected against the hepatitis B virus. When HBsAb is positive (antibodies are present) it generally means that persons have recovered from a hepatitis B infection and have some resistance. This protection can be the effect of getting the hepatitis B vaccine or effectively improving from a earlier hepatitis B infection.

HBe Ab

Is one of the antibodies formed by the body when it defend itself against hepatitis B. These antibodies are aimed at against the "soluble" or extractable piece of the "core" antigen of the Hepatitis B Virus (HBV).

HBc Ab

It is also known as the Hepatitis B core antibody. The attendance of anti-HBc indicates preceding or existing infection with hepatitis B virus in an approximate point in time structure. IgM antibody to hepatitis B core antigen (IgM anti-HBc): Positivity indicates the current illness with hepatitis B virus (<6 mos). Its existence indicates sensitive infection. HBc Ab (Hepatitis B core antibody) positive mean - A "positive" or "reactive" antiHBc (or HBc Ab) test result indicates a past or current hepatitis B infection. The core antibody does not offer any defense alongside the hepatitis B virus.

HBV ELISA

Micro-well based ELISA (Enzyme linked Immunosorbent Assay) for detection of Antibodies to HBV in human serum.

HBV PCR

PCR (polymerase chain reaction) are used to identify HBV in the serum of patient blood. PCR is a test on molecular stage to identify presence of HBV and Viral load.

Results

The epidemiological study of Hepatitis B was conducted in district Bannu from January, 2018 to April, 2018. Total 500samples of males (n=300) and females (n=200) were studied in a Khalifa Gull Nawaz Teaching Hospital of Bannu. The Data was collected Gender-wise and Age- wise. For detection of HBV all the Samples were examined by ELISA, ICT and PCR Tests.

Sex-wise distribution of HBV in general population of district Bannu

For the identification of anti HBV antibodies total 500 patients (300 males and 200 females) were examined, out of which 78 (26%) males and 48(24%) females were reported positive for anti HBV antibodies and then these were additionally processed for finding HBV DNA. Individuals including 75 (25%) males and 47 (23.5%) females were found positive for HBV DNA while the remaining 4patients including 2 males and 2 female were found negative (Table 1). Sex-wise distribution of HBV infection shows that as compare to female

population 47 (23.5%) male population was more infected 75 (25%) (Figure 1).

Age-wise distribution of HBV in general population of Bannu

The patients of 01–60 years of age were categorized into three groups Aged, Adults and Children’s. After demonstration, it was found that the rate of HBV infection was directly related to age i.e. higher the age higher was the rate of HBV infection (Figure 2).The highest HBV infection was found in the age-group of 41–60 years (32%) categorized as Aged, lowest in the age-group of 01–20 years (10%) as the Children and moderate in the age-group of 21–40 years which is the Adults is (13.33 %) (Table 2).

The above graph shows the sex-wise distribution of HBV +ve in the general population of District Bannu KP Pakistan. The green color cone shape strip shows the total samples, the blue color cone shape strip shows the males and the red color cone shape strip shows the females. The total sample contains the five hundred, in which the male are about three hundred and the female are about two hundred in numbers. In the above graph it is shown that the HBv anti +ve males are about 78 shown by blue color cone shape strip and the anti HBv +ve female are about 48 shown by red color cone shape strip. The percentage of HBv +ve males is about 26 shown by blue color cone shape strip and females are about 23.5 shown by red color cone shape strip.

Graph 2.2.shows the sex-wise distribution of the HBv +ve male shown by blue color cone shape strip and the HBv +ve female shown also by the red color cone shape strip. In this graph the HBv +ve is shown in both sexes i.e. male and female and also the percentage of HBv +ve found in male and female in total percentage at total sample found in a required data. there found about 25% HBv +ve male shown by blue color cone shape strip and about 23.5% HBv +ve female shown by red color cone shape strip. Furthermore there are 75 HBv +ve male and 47 HBv +ve female shown by the corresponding colors Figure 3.

In the above graph there shown the age-wise distribution of HBv +ve in total sample with a percentage in general population of district Bannu KP Pakistan. The patients of 01–60 years of age were categorized into three groups. After demonstration, it was found that the rate of HBV infection was directly related to age i.e. higher the age higher was the rate of HBV +ve infection, as shown by the above mentioned graph. The highest HBV infection was found in the age- group of 41–60 years which was 64 and is 32%, shown by red colour cone shape strip as compare to other age-groups. There shown a moderate percentage found in the age-group of 21–40 years

Table 1: Sex-wise Distribution of HBV in General population of District Bannu.

Gender	Total Sample	Anti HBV +ve	% of Anti HBV +ve	HBV DNA +ve	% of HBV DNA +ve
Male	300	78	26	75	25
Female	200	48	24	47	23.5
Total	500	126	50	122	48.5

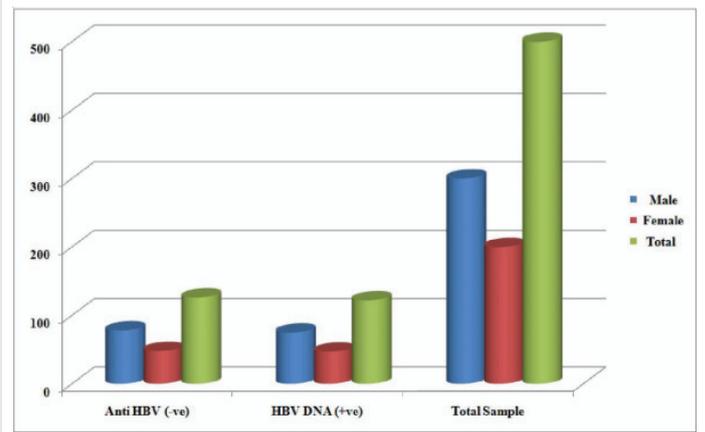


Figure 1: Sex-wise distribution of HBV in General population of District Bannu.

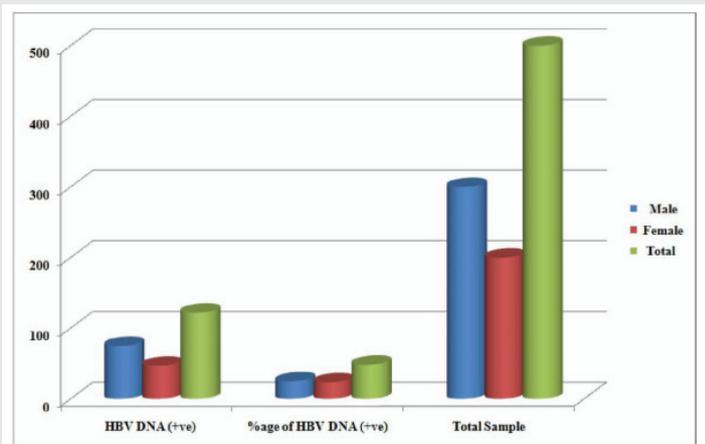


Figure 2: Percentage of Sex-wise Distribution of HBV in General population of District Bannu.

Table 2: Distribution of HBV on the basis of age in General population in District Bannu.

Age, groups	Categories	Total Samples	HBv +ve	%age
01-20	Children	80	8	10%
21-40	Adults	120	16	13.33%
41-60	Aged	200	64	32%
	Total	500	88	55.33%

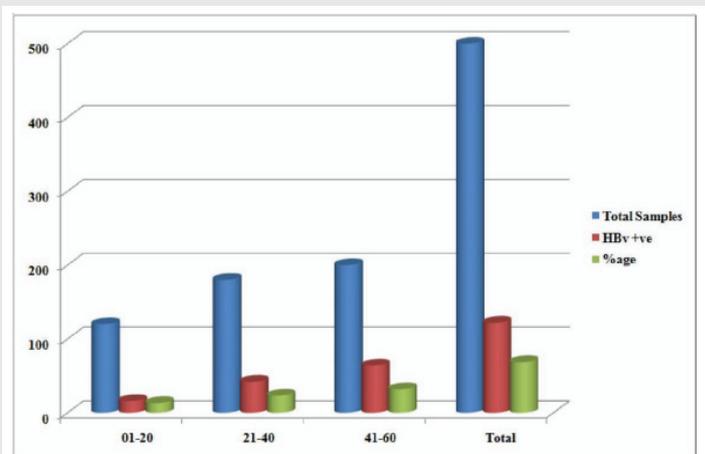


Figure 3: Age-wise Distribution of HBV in General population of District Bannu.



which is 23.33% and shown this moderate percentage by a green cone shape strip. While from the above graph at the starting point it is shown also the patients having the HBV +ve of about 16 and percentage is shown by green cone shape strip which is very low 13.33%.

Discussion

The word Hepatitis is the combination of two words, according to the Greek word hepar stand for the "liver" and the Latin word stand for the "inflammation" so liver is affected and stop its capacity to reform Hepatitis B is a viral infection that causes the liver infection [1,2]. Infection of Hepatitis B is caused by the hepatitis B viruses, which attack the liver cells and causes acute or chronic hepatitis acute hepatitis produces clear and simple symptoms from which people get well soon, while chronic hepatitis symptoms are more subtle and causes liver damage and spread of disease For the year 2000, the model estimated 620000 persons died worldwide from HBV-related causes: 580000 (94%) from chronic infection-related cirrhosis and HCC and 40000 (6%) from acute hepatitis B. In the surviving birth group for the year 2000, the model expected that without immunization, 64.8 million would become HBV-infected and 1.4 million would die from HBV connected disease. Infections acquired during the prenatal period, in early childhood (5 years old), and >5 years of age accounted for 21, 48, and 31% of deaths, respectively. Routine infant hepatitis B vaccination, with 90% coverage and the first dose administered at birth would prevent 84% of global HBV-related deaths [4,5,9,10-25]. Chronic hepatitis B virus infection is one of the most serious infections and a major risk factor for deaths from cirrhosis and liver cancer. We estimate age-, sex- and region-specific prevalence of chronic HBV infection and calculate the absolute number of persons being chronically infected. Of the 17 029 records screened, 1800 report on the prevalence of HBsAg Infections covering 161 countries were included. HBsAg sero prevalence was 3.61% (95% CI 3.61-3.61) worldwide with highest endemicity in countries of the African region (total 8.83%, 8.82-8.83) and Western Pacific region (total 5.26%, 5.26-5.26). Within WHO regions, prevalence ranged from 0.20% (0.19-0.21; Mexico) to 13.55% (9.00-19.89; Haiti) in the Americas, to 0.48% (0.12-1.90; the Seychelles) to 22.38% (20.10-24.83; South Sudan) in the African region. We estimated that in 2010, globally, about 248 million individuals were HBsAg positive. The pooled prevalence of HBV in Nigeria was 13.6% (95% confidence interval 11.5, 15.7%). The pooled prevalence (% [95% CI]) among subgroups was: 14.0% (11.7, 16.3) for blood donors; 14.1% (9.6, 18.6) for pregnant women attending antenatal clinics; 11.5% (6.0, 17.0) for children; 14.0% (11.6, 16.5) among adults; and 16.0% (11.1, 20.9) for studies evaluating adults and children. HBV prevalence in Nigeria varied by screening method [% (95% CI)]: 12.3% (10.1, 14.4) by using enzymelinked immune sorbent assay; 17.5% (12.4, 22.7) by immune chromatography; and 13.6% (11.5, 15.7) by HBV DNA polymerase chain reaction. HBV infection is hyper endemic in Nigeria and may be the highest in SubSahara Africa. Our results suggest that large numbers of pregnant women and children were exposed to HBV from 2000 to 2013. Increased efforts to prevent new HBV infections are urgently needed in

Nigeria. Realizing the burden of the disease, in 2007-2008 the first national survey was conducted to estimate the prevalence of hepatitis B in Pakistan [15,26]. A total of 47,043 individuals were tested and the overall prevalence of hepatitis B was 2.4%. The highest prevalence of hepatitis B was found in the province of Baluchistan (4.3%) followed by the province of Sind (2.5%). Pakistan is highly endemic (9 million people infections across the country) with 3% chronic HBV carriers and the infection rate is rising day by day High prevalence of HBV was observed in geographical areas of low economic status, which underscores the importance in controlling this disease because approximately, 67.5% of the Pakistani population belongs to rural areas of low economic status With 3% chronic HBV carriers and the infection rate is rising day by day [2,8,27]. High prevalence of HBV infection was reported from Malakand division (16.53%) and D.I.Khan division (14.63%) as compared to Hazara division (14.15%), Peshawar division (14.15%), Kohat division (13.67%), Bannu division (13.5%) and Mardan division (13.35%). Gender-wise prevalence among the HBV DNA positive samples showed that males were more affected than females. In this study the PCR positive HBV samples included 443 (70.43%) males and 186 (29.57%) females [26]. Male to female ratio was found to be 2.38:1. Age-wise was observed in all the PCR positive samples which were categorized into five age groups. The highest infection rate of 39.27% was observed in the age group of 16 to 30 years while a lower infection rate of 4.93% was observed in the age group of more than 60 years.

The result rates of all of these above mention studies was lowered than the result of present study conducted in Bannu. In the current research work the occurrence of HBV observed gender wise and males (25%) were found more effected than females (23%). Maximum infection rate in males than females remained also described in the earlier research work conducted by Alam *et al.*, (2007a). Similarly in another study of hepatitis B virus infection among different sex and age groups in Pakistani Punjab, Khan *et al.* (2011b) reported high prevalence in male than female Nwokediuko (2010) also reported a significantly higher infection rate in male as compared to the female).

Males mostly highly infected with HBV may be because of to their maximum contact with different HBV transmitting factors like drugs use, blood transfusion and barber contact because the barbers have no sufficient information about HBV infection and have unclean condition in shop. Jobs and business that is a different social activity may be another main reason because of which the rate of HBV infection is higher in males than females. For business, jobs and other activities male often remain outside their home while female mostly performed their activities inside a home, restricted to the home and thus safe up to some extent from HBV infection as they have a low chances of association with HBV risk factors [13,26].

The present study also observed prevalence of HBV age wise from 1 to 60 years is the aged people with less immune response. The lowest HBV rate is (10%) found in age group of 1-20 which is considered the most important part of research which was assigned by the full support to me toward their parents/guardian for shows, that its due to the reason that they



have a strong immune system and also have a minimum, even no specific and common contact with a factors transmitted HBV such as a barber contact, drug abuse, tattooing and piercing, surgery, and blood transfusion as this category is the immature stages people. But my aim was to shows that how the HBV affect on the different stages of age. Moderate rate is (13.33 %) found in age group 21-40 which is Adults. This is due to the fact that these people have weak immune response and high contact with risk factors associates with HBV than children. Highest prevalent rate (32%) found in age group of 41-60 due to weak immune system and lack of awareness [23].

Conclusion

HBV is a major health trouble internationally affects more than a few people all over the world and increase gradually with the increase in age i-e direct proportional to the age factor. Similarly the percentage and amount of Hepatitis B was highest in male as comparison to female population which may be due to the fact that male has dominancy in the society, performs different activities The HBV has a high prevalence rate in Bannu due to the lack of education, poverty, unawareness about HBV risk factors, and absence of proper blood screening facilities. It is suggested that the vast preventative measure should be applied during operating measures and proper viewing during blood transfusion. Against Hepatitis B infections further effort should be made to train the public about possibility factors of HBV and their way of spread and also confirm the untrained health workers working in different parts of district Bannu in order to decrease the rate of infection. It is accomplished that HBV infection is still prevailing in the Khyber Pakhtunkhwa Province.

Recommendation

Government of Pakistan, conducted the high scale survey by investing million rupees resources, so, they should utilized these data by publishing to help the health sector in policy making, reporting only descriptive are not enough. It is suggested that the enormous preventative measure should be applied during working events and accurate viewing during blood transfusion. Huge knowledge programs, high level vaccination and other preventive measures should be taken to stop the spread of this disturbing disease in the Khyber Pakhtunkhwa, Pakistan. In short the results of this work suggested that for providing health education on Hepatitis B, infinite and more awareness should be given to both male and female individuals as Knowledge about a problem is usually the first step towards risk reduction and improvement in the quality of life.

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