

Full Length Research Paper

Prevalence of hepatitis B in the blood donors of N-W.F.P and FATA regions and the current scenario of HBV infection in Pakistan

Najib Ullah Khan¹, Lubna Siddique¹, Ijaz Ali^{1*}, Aqib Iqbal¹, Iqbal Munir¹, Farzana Rashid², Sajid Ali³, Fazal Raziq⁴ and Zahoor Ahmad Swati¹

¹Institute of Biotechnology and Genetic Engineering N.W.F.P Agricultural University Peshawar, Pakistan.

²Lahore College for Women University, Lahore, Pakistan.

³Department of Biotechnology, Peshawar University, Pakistan.

⁴Hayat Abad Medical Complex, Peshawar, Pakistan.

Accepted 5 May, 2010

Hepatitis B is a fatal liver disease caused by the hepatitis B virus. In this study, blood donors from various districts of the North-western frontier province and the federally administered tribal area (FATA) of Pakistan were tested for HBsAg and HBV DNA by ICT (Immuno-chromatographic test), ELISA and RT-PCR. Out of the 7148 blood donors, 244 (3.41%) were positive for HBsAg by ICT, 147 (2.05%) by ELISA while 132 (1.85%) were positive by PCR. Our data indicates that the incidence of hepatitis B has decreased in these regions in recent times.

Key words: HBV, HBsAg, Pakistan.

INTRODUCTION

Hepatitis B is caused by the hepatitis B virus (HBV); an enveloped virus containing a partially double stranded, circular DNA genome and classified within the family hepadnaviridae (Ganem and Schneider 2001). Hepatitis B virus infects the liver and causes an inflammation called hepatitis, originally known as serum hepatitis (Barker et al., 1996). The acute illness causes liver inflammation, vomiting, jaundice and sometimes death. Chronic hepatitis B may eventually cause liver cirrhosis and liver cancer (Chang, 2007). The infection is preventable by vaccination (Pungpapong et al., 2007).

Although replication takes place in the liver, the virus spreads to the blood where virus specific proteins and their corresponding antibodies are found in infected people.

Blood tests for these proteins and antibodies are used to diagnose the infection (Bonino et al., 1987). Transmission of hepatitis B occurs by percutaneous and permucosal exposure to infective body fluids (Hollinger and Liang, 2001).

It is estimated that nearly 2 billion people around the world have serologic evidence of past or present HBV infection, while 350 million people are chronically infected (Alter, 2003). The prevalence of HBV is highest among the developing countries of Asia, Africa and the Pacific Islands and lowest among the developed countries of America, Europe and Australia. Pakistan being part of the developing world, viral hepatitis is a major public health problem (Hasnain, 1994).

With the purpose of investigating the prevalence of HBsAg or active HBV infection among the blood donors from N-W.F.P and FATA regions, and understanding the overall scenario of HBV prevalence among the blood donors from all the four provinces of Pakistan, we tested 7148 blood donors for HBsAg or HBV DNA by ICT, ELISA and RT-PCR, and have also analyzed data published in this respect from the entire country since 1996. Our results indicate that the prevalence of HBV has decreased among the blood donors from various parts of N-W.F.P and FATA regions. Moreover, the data indicates

*Corresponding author. E-mail: bachakhan35@yahoo.com. Tel: 0092-3339544062. Fax: 0092-9218102.

Abbreviations: FATA, Federally administered tribal area; ELISA, enzyme-linked immunosorbent assay; PCR, polymerase chain reaction; RT-PCR, reverse transcriptase-PCR; HBV, hepatitis B virus; HBsAg, hepatitis B antigen; ICT, immuno-chromatographic test.

Table 1. Prevalence of HBV among the blood donors from FATA and N-W.F.P as revealed by Immuno-chromatographic test (ICT), ELISA and RT-PCR.

S/N	Month	Donors	HBsAg (ICT)	HBsAg (ELISA)	RT-PCR
1	JANUARY	974	25	18	17
2	FEBRUARY	1013	30	21	18
3	MARCH	972	32	22	20
4	APRIL	938	25	17	17
5	MAY	936	30	13	12
6	JUN	1095	42	13	10
7	JULY	673	23	23	20
8	AUGUST	547	27	19	18
Total	8	7148	244 (3.41%)	147 (2.05%)	132 (1.85%)

that over the past few years, the prevalence of HBV has decreased among the blood donors of Pakistan.

MATERIALS AND METHODS

Blood donors

Blood was taken from the voluntary blood donors and examined either at Hayat Abad Medical Complex (HMC) or at the Institute of Biotechnology and Genetic Engineering, N-W.F.P Agricultural University Peshawar.

Immuno-chromatographic test (ICT)

Initially, all the blood donors were tested for HBsAg by immuno-chromatographic test. Strips used were from Determine Abbot. Samples positive by ICT were furthered for next step evaluation.

ELISA

Sera positive by ICT were tested for HBsAg antibodies by ELISA (BIOKIT, S.A, Barcelona-Spain) according to the manufacturer's instructions. All the ELISA positive samples were processed for DNA extraction.

DNA isolation and real time PCR

DNA isolation from the HBsAg positive ELISA samples and subsequent RT-PCR was carried out with the help of DNA extraction and RT-PCR kit from Sacace (Sacace, Biotechnology, Italy) according to the manufacturer's instructions, inside the Cepheid smart cyler (Nasdaq: CPHD, California, US).

Comparative study of online data

Studies published with respect to the prevalence of HBsAg among the blood donors of Pakistan were downloaded from the World Wide Web (www) and the data given was analyzed. Average prevalence of HBsAg was calculated for all the four provinces followed by taking average of the studies revealing the prevalence of HBsAg among the blood donors from around the country along with the average calculated in the case of individual provinces. Graphs were drawn using Microsoft Excel.

RESULTS

HBV prevalence among the blood donors in N-W.F.P and FATA

A total of 7148 voluntary blood donors were initially screened for HBsAg by ICT. 3.41% of the volunteers were detected positive for HBsAg (Table 1). All the samples positive by ICT were further processed by ELISA which indicated that out of the total number of volunteers, 2.05% were positive for HBsAg (Table 1).

Samples positive by either ICT or ELISA were used for HBV DNA extraction and subsequent RT-PCR. The real-time PCR assay revealed that 132 (1.85%) donors had active HBV DNA in their blood (Table 1).

HBV prevalence among the blood donors in Pakistan

After gathering information on the prevalence of HBV in various provinces of Pakistan from the published studies available on the World Wide Web (www), we analyzed the data for the trend of HBV prevalence among the blood donors since the first publication appeared in 1996. Our analysis indicated that a total of 27 publications have appeared on HBsAg prevalence among the blood donors from various regions of Pakistan since 1996. In all of the studies, HBsAg has been detected either by ICT, ELISA or MEIA (Table 2). The average prevalence of HBV in all the four provinces and the Capital of Pakistan from 1996 to 2009 was 2.12, 4.8, 4.51, 3.09 and 3.28% from N-W.F.P, Baluchistan, Sindh, Punjab and the capital city Islamabad, respectively (Figure 1, Table 2). Analysis of the entire data indicated that the average prevalence of HBV was 3.54% during 1996 - 2000 while it was 3.26% during 2006 - 2009 (Figure 2).

DISCUSSION

In the first part of our study, we have investigated 7148 voluntary blood donors for the prevalence HBsAg or HBV

Table 2. Published studies revealing the prevalence of hepatitis B among blood donors belonging to all the four provinces and the capital city of Pakistan since 1996. Technique used in each study has also been mentioned.

S/N	Reference	Location	Prevalence	Technique
1	Ahmad et al. (2007)	Balochistan	4.8%	ICT
2	Ishtiaq et al. (2007)	Rawalpindi	2.45%	MEIA
3	Ahmad et al. (2004)	Peshawar	1.9%	MEIA
4	Mumtaz et al. (2002)	Rawalpindi	5.86%	ICT
5	Khattak et al. (2002)	Rawalpindi	3.3%	ELISA
6	Mahmood et al. (2004)	Multan	3.37%	ICT
7	Asif et al. (2004)	Islamabad	2.51%	ICT
8	Muhammad, S. (2006)	Skardu	8.4%	ELISA
9	Faisal et al. (2000)	Abbottabad	1.55%	ICT
10	Alia et al. (2008)	Peshawar	1.40%	ELISA
11	Asad et al. (2007)	Lahore	1.52%	ICT
12	Akhter et al. (2005)	Karachi	2%	ICT
13	Fayyaz et al. (2006)	Bahawalpur	2.69%	ICT/ ELISA
14	Syed and Mark (2009)	Pakistan	2.4%	ICT
15	Syed, 1998	Karachi	3.5%	ICT
16	Kakepoto et al. (1996)	Karachi	2.28%	ICT
17	Yosaf et al. (1998)	Bahawalpur	1.1%	ICT
18	Rehman et al. (1996)	Pakistan	5%	ICT
19	Mujeeb. (2008)	Sindh	6.2%	ICT
20	Farhat et al. (2006)	Pakistan	2.16%	ICT
21	Sulthan et al. (2007)	Pakistan	1.46 - 2.99%	ICT
22	Hashim et al. (1999)	Faisalabad	2.06%	ICT
23	Mujeeb et al. (2006)	Karachi	4.7%	ICT
24	Maqbool et al. (2007)	Northern areas	3.66%	ICT
25	Ayub et al. (2006)	Liaquetpur	5.96%	ICT
26	Zeeshan et al. (2007)	Rawalpindi	2.31%	ICT
27	Waqas et al. (2005)	Punjab	4.93%	ICT

DNA by antibody-based tests and RT-PCR. Four studies have earlier reported prevalence of HBsAg among the blood donors of N-W.F.P (Faisal et al., 2000; Ahmed et al., 2004; Maqbool and Mohammad, 2007; Alia et al., 2008). These studies have focused on the antibodies-based tests alone in order to examine the prevalence of hepatitis B among the blood donors in the N-W.F.P province including FATA. In our study, we have coupled the antibodies-based test with modern RT-PCR based HBV DNA detection in order to exactly figure out the prevalence of active HBV infection among the blood donors from N-W.F.P and FATA. As compared to the average pre-valence of HBsAg among the blood donors of N-W.F.P and FATA as documented by earlier studies (2.12%), this study reveals a decreasing trend (2.05%) in the prevalence of HBsAg in our subject areas. None of the studies mentioned above have used DNA based detection in order to figure out the active infection but our study reveals that the prevalence of active HBV infection is even lower (1.85%) than the average HBsAg prevalence recorded in the case of all previous studies

including the present one. The decreasing trend of HBV infection indicates improvement in health care facilities and aware-ness among the general population over the past few years.

According to previous studies, from the year 1996 to 2009, high prevalence of HBsAg among the blood donors (8.4 and 6%) has been reported from Sindh province (Muhammad, 2006; Syed et al., 2008) followed by Rawalpindi, Punjab (5.86%), (Mumtaz et al., 2002) while the lowest (1.1%) prevalence of HBsAg was seen in Bahawalpur, Punjab (Yousuf et al., 1998). From the year 1996 to 2009, the average HBsAg prevalence among the blood donors reported by various studies from N-W.F.P, Baluchistan, Sindh, Punjab and the capital city Islamabad (Figure 1, Table 2) shows high prevalence of HBsAg among the blood donors from Baluchistan and low prevalence in the Punjab province. Analysis of the entire data on HBsAg from all over Pakistan also indicates that the HBV infection is on a declining trend since the year 1996 (Figure 2). The average prevalence of HBsAg among the blood donors of Pakistan, as reported by various

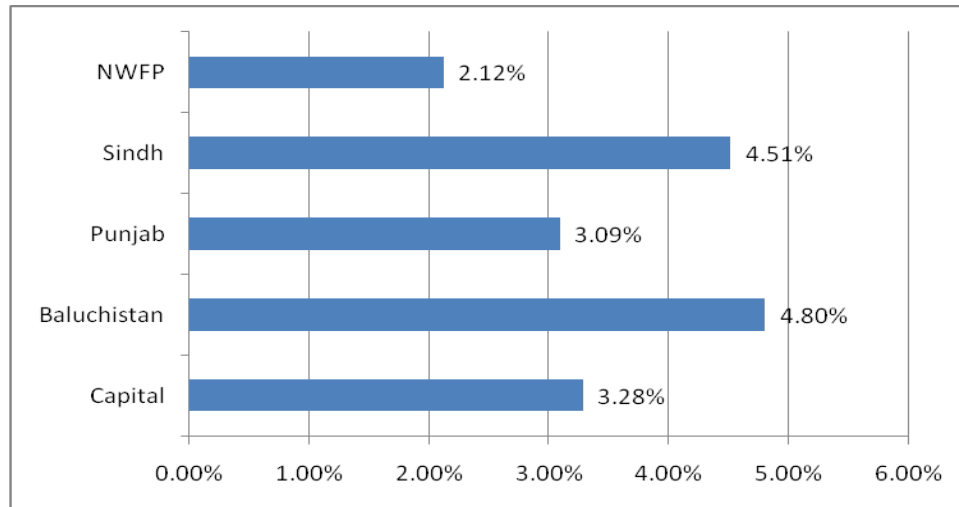


Figure 1. Average prevalence of HBsAg among the blood donors from various Provinces and the capital city (Islamabad) of Pakistan from 1996 - 2009.

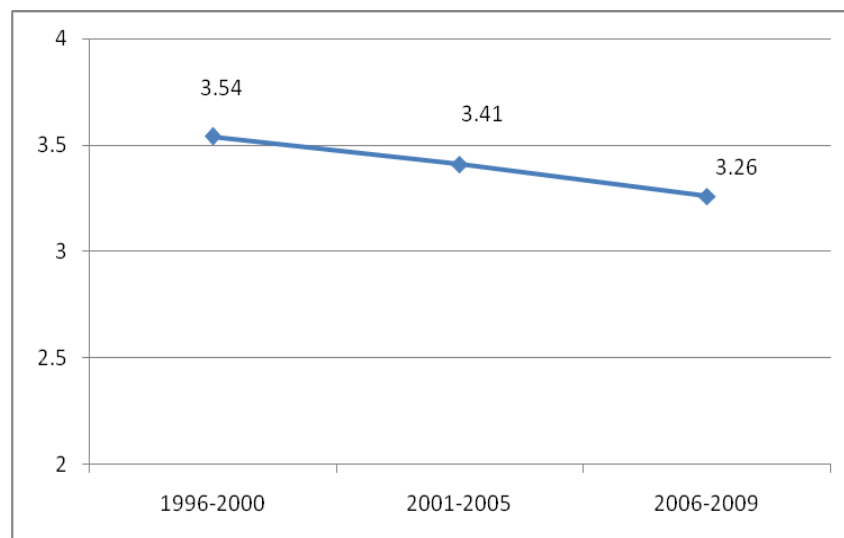


Figure 2. Average prevalence of HBsAg among the blood donors of the entire country (Pakistan) from 1996 - 2009. Prevalence (%) is shown along y-axis while x-axis shows duration.

studies from 1996 to 2000, is 3.54, which dropped down to 3.41% during 2000 - 2005. The data published with respect to prevalence of HBsAg among the blood donors of Pakistan since the year 2005 to date indicates that the average prevalence of HBsAg has further decreased to 3.26% (Figure 2).

Our study reveals that the prevalence of HBV infection among the blood donors of N-W.F.P and FATA as well as in the case of the entire country (Pakistan) has decreased over the past couple of years. This could well be attributed to the awareness created among the health professionals and general public about hepatitis B

around the country.

REFERENCES

- Ahmad J, Taj AS, Rahim A, Shah A, Rehman M (2004). Frequency of Hepatitis B and Hepatitis C in healthy blood donors of NWFP, a single center experience. *J. Postgrad. Med. Inst.* 18(3): 343-52.
- Ahmad Z, Aslam MI, Ali S (2007). The frequency of hepatitis B and C among volunteer blood donors in Balochistan. *Hepat. Monthly*, 7(2): 73-76.
- Akhter S, Younus M, Adil S, Hassan F, Jafri S (2005). Epidemiologic study of chronic hepatitis B virus infection in male volunteer blood donors in Karachi, Pakistan. *BMC Gastroenterol.* 5(1): p. 26.

- Alia Z, Waheed ZT, Kashan A, Liaqat A, Farah F, Saeed R (2008). Seroprevalence of Hepatitis B, C and HIV in healthy blood donors in Northern Pakistan. *Pak. J. Pathol.* 19(3): 101-106.
- Alter MJ (2003). Epidemiology of hepatitis B in Europe and worldwide. *J. Hepatol.* 39: 64-69.
- Asad UI, Fuad S, Naveed A, Mahmood NM, Riaz Q (2007). Hepatitis B and Hepatitis C in blood donors. *Ann. King Edward Med. Coll.* 13(1): 59-61.
- Asif N, Kokhar N, Ilahi F (2004). Seroprevalence of HBV, HCV and HIV infection among voluntary non remunerated and replacement donors in Northern Pakistan. *Pak. J. Med. Sci.* 20(1): 24-28.
- Barker LF, Shulman NR, Murray, Hirschman RJ, Ratler F, Diefenbsch WC, Geller HM (1996). Transmission of serum hepatitis. 1970. *JAMA*, 276(10): 841-844.
- Bonino F, Chiaberge E, Maran E, Piantino P (1987). Serological markers of HBV infectivity. *Ann. Ist. Super. Sanita*, 24(2): 217-23.
- Chang MH (2007). Hepatitis B virus infection. *Semin. Fetal Neonatal. Med.* 12(3): 160-167.
- Faisal A, Shah SH, Tariq M, Khan JA (2000). Prevalence of Hepatitis B carrier and HIV in healthy blood donors at Ayub Teaching Hospital. *Pak. J. Med. Res.* 39(2): 91-2.
- Farhat AB, Zia U, Salamat N, Ayub M, Ghani E (2006). Anti-hepatitis B core antigen testing, viral markers, and occult hepatitis B virus infection in Pakistani blood donors, implications for transfusion practice. *Transfusion*, 47(3): 74-79.
- Fayyaz M, Khan MA, Chaudhary GM, Qazi MA, Ahmed G (2006). Hepatitis B, C & HIV, seroprevalence of infection in blood donors. *Prof. Med. J.* 13(4): 632-36.
- Ganem D, Schneider R (2001). Hepadnaviridae: the viruses and their replication. In *Virology*. (eds) Knipe DM, Howley PM, Philadelphia PA, Lippincott, Williams, and Wilkins. pp. 2923-2969.
- Hashmi ZY, Chaudry AH, Ahmed M, Ashraf M (1999). Hepatitis B virus antigenemia in healthy blood donors in Faisalabad. *The Professional*, 6(4): 547-550.
- Hasnain SS (1994). Hepatitis Prevention, vaccination, treatment. *Med. Herald*, 1: 15-19.
- Hollinger FB, Liang T (2001). Hepatitis B virus. In *Fields Virology*. (eds) Knipe, Howley PM, Philadelphia PA. Lippincott Williams, Wilkins. pp. 2971-3036.
- Ishtiaq A, Samiullah S, Sattar K, Rehan M, Muhammad A, Ashraf A (2007). Seroprevalence of Hepatitis B and C among the healthy blood donors at Fauji Foundation Hospital, Rawalpindi. *Pak. J. Med. Sci.* 23(1): 64-67.
- Kekepotto GN, Bhally HS, Khaliq G, Kayani N, Burney IA, Iddiqui T, Khurshid M (1996). Epidemiology of blood borne viruses. A study of healthy blood donors in Southern Pakistan. *Southeast Asian J. Trop. Med. Public Health*, 27: 703-706.
- Khattak MF, Salamat N, Bhatti FA, Qureshi TZ (2002). Seroprevalence of Hepatitis B, C and HIV in blood donors in Northern Pakistan. *J. Pak. Med. Assoc.* 52(9): 398-402.
- Mahmood MA, Khawar S, Anjum AH, Ahmed SM, Rafiq S, Nazir I, Usman M (2004). Prevalence of Hepatitis B, C and HIV infection in blood donors of Multan region. *Ann. King Edward Med. Coll.* 10(4): 459-61.
- Maqbool A, Mohammad AN (2007). Frequency of Hepatitis B surface antigen and anti-Hepatitis C antibodies in apparently healthy blood donors in Northern areas. *Pak. J. Pathol.* 18(1): 11-14.
- Muhammad AK, Muhammad A, Abdul R, Azam A, Muhammad A, Allah D (2006). Prevalence of HBV, HCV and HIV in blood donors at Liaquetpur. *Professional Med. J.* 13(1): 23-26.
- Muhammad SA (2006). Prevalence of anti Hepatitis C antibodies and Hepatitis B surface antigen in healthy blood donors in Balistan. *Pak. Armed Forces Med. J.* 56: 189-191.
- Mumtaz S, Rehman M, Muzaffar M, Hassam M, Iqbal W (2002). Frequency of seropositive blood donors for Hepatitis B, C and HIV viruses in Railway Hospital Rawalpindi. *Pak. J. Med. Res.* 41(2): 51-53.
- Pungpapong S, Kim WR, Poterucha JJ (2007). Natural history of hepatitis B virus infection, an update for clinicians. *Mayo Clin. Proc.* 82(8): 967-975.
- Rehman K, Khan AA, Haider Z (1996). Prevalence of Seromarkers of HBV and HCV in health care personnel and apparently healthy blood donors. *J. Pak. Med. Assoc.* 46: 152-154.
- Sulthan F, Mehmood T, Mahmood MT (2007). Infectious pathogens in volunteer and replacement blood donors in Pakistan. *Int. J. Infect. Dis.* 11(5): 407-412.
- Syed AM (2009). Prevalence of HBV, HCV and HIV infections among family blood donors in Karachi, Pak. *Int. Conf. AIDS*. 12: 39.
- Syed AM, Mark S (2008). Temporal trends in hepatitis B and C infection in family blood donors from interior Sindh, Pakistan. *BMC Infect. Dis.* 8: 43.
- Waqas J, Farooq A, Usman S, Tehseen I, Hamid JQ (2005). Prevalence of Hepatitis B, C and HIV in blood donors of South Punjab. *Sims J.* 100(1): 13-16-2.
- Yosaf M, Hasan SMA, Kazmi SH (1998). Prevalence of HBsAg among volunteer blood donors in Bahawalpur division. *The Prof. Med. J.* 5: 167-71.
- Zeeshan T, Shiba A, Zubair T, Imran AE, Rizwan AM, Beenish A, Abbas H (2007). The prevalence of transfusion transmitted infections in healthy blood donors in Rawalpindi district. *Int. J. Pathol.* 5: 21-25.