



Investigating the Frequency of Hepatitis B in General Population of Khyber Pakhtunkhwa, Pakistan

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Abstract: Pakistan is facing major problem of viral based hepatitis which is increasing day by day, and is considered to be the major cause of mortality and morbidity. The present study investigated the frequency of Hepatitis B patients referred to Khyber Teaching Hospital (KTH) Peshawar from the different regions of Khyber Pakhtunkhwa. The blood sample was collected from the referred 1,33,193 patients and used for *in vitro* immuno chromatographic assay (qualitative determination of HBsAg in serum or plasma), and ELISA (Enzyme Linked Immuno Sorbent Assay). Our results showed that 81(2.03%) out of 3,977 patients referred for HBsAg and anti-HCV screening presented positive test results for HBsAg. Similarly Co-infection with HCV was found in six patients (i.e. 4 females and two males). We conclude that transfusion of unscreened blood and blood products, use of contaminated syringes, lack of awareness about hepatitis and absence of hygienic conditions leads to its widespread occurrence.

Keywords: HBV, ICT (Immuno Chromatography Technique), KTH, ELISA.

1. INTRODUCTION

Hepatitis represents liver inflammation or infection which can be illustrated by the presence of inflammatory cells in the liver tissue. The infection has been caused by a group of viruses termed as hepatitis viruses all over the world. Hepatitis A, B, C, E, G and delta factor are viruses that causes hepatitis. Hepatitis may be caused by alcohol, drug, autoimmune diseases and metabolic disorder (Gilchrist, 2005). Hepatitis B virus based (HBV) infections have been demonstrated to be the major cause of this disease worldwide. According to World Health Organization (2015) report, about 2000 million people globally have been infected with HBV, and 350 million are suffering from chronic infections. It has also issued its guidelines for the prevention and treatment of this chronic infection. Among the chronically infected patients, 65 million people have been predicted to be killed from liver disease arising as a result of HBV infection (Kane *et al.*, 1995). Other studies conducted have also been focused on the screening of HBV infection and identification of preventive measures or treatments (Chou *et al.*, 2014; Lok *et al.*, 2016; Norah *et al.*, 2016). Similarly, success has also been achieved in preventing the transmission of HBV in newborns by administration of HBV vaccines and immunoglobulin to mothers but this strategy still needs to be improved (Wen *et al.*, 2015).

Hepatitis B virus is a DNA virus with approximately 3200 base pairs. According to one estimate, 350–400 million people have found to be chronically infected with HBV, and about 3 billion people exposed to HBV infection all over the world (Lavanchy, 2004; Yokosuka *et al.*, 2011).

HBV induces a variety of liver diseases, ranging from acute or fulminant hepatitis to liver cirrhosis and hepatocellular carcinoma (Michitaka *et al.*, 2010). On the other hand, hepatitis is a disease which can be fixed on its own in most of adult patients with or without medication with acute infection. There have been reports that about 1-2% of patients suffer from fulminant hepatic failure and others from chronic infection. It has also been reported that the percentage of progression from acute to chronic HBV infection is around 90% in infants and 5–10% in adults (Yim and Lok, 2005; Hoofnagle *et al.*, 2007).

Among the eight genotypes of hepatitis B virus (A–H) reported, not much is known about the prevalence of HBV genotypes. Studies have shown that genotypes B and C are the most widespread in Asia but it has also been suggested that genotypes D is major and constitute a significant health problem in Pakistan (Alam *et al.*, 2007). The rate of chronicity of genotype A infections is reported to be higher than those of other genotypes (Yotsuyanagi *et al.*, 2005; Suzuki *et al.*, 2005; Ozasa *et al.*, 2006; Yano *et al.*, 2010). The higher occurrence rate of hepatitis and subsequent mortality in Pakistan is due to liver failure and hepatocellular carcinoma. Use of contaminated needles/syringes, drug abuse, illicit sexual relations and unscreened blood transfusion are the major causes of this disease (Ali *et al.*, 2009).

Pakistan Medical Research Council (2007-08) has demonstrated that the spread rate of HBsAg is 2.5% (Farhat *et al.*, 2014). Similarly other studies have reported its spread rate as 2.28% in general population,

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1.83% in blood donors (Farooqi *et al.*, 2011) and 1.93% (Majid *et al.*, 2010), but the exact spread rate is not known (Travasso, 2014). Apart from Pakistan, HBV infection is also prevalent in our neighboring countries like India where it has been reported to be present in 40 million people. Despite several preventive measures and treatment such as vaccination, awareness programs, reuse of syringes and screening of blood before transfusion, this disease is still prevalent in Pakistan. As the treatment of this fatal disease is expensive and cannot be afforded by poor people in developing countries, the best approach would be to adopt the preventive measures and also identify the risk factors which could be easily targeted (Ashraf and Ahmad, 2015). The frequency of hepatitis B and C has been reported to be significantly higher in injectable drug users and prisoners than others (Memo *et al.*, 2012). Hepatitis B is very widespread in Asia as 75% cases have been reported from here. Various sub genotypes of HBV have been demonstrated from Indonesia but the knowledge regarding pathogen city of each sub genotype is still unknown. Knowing the exact pathogenicity of each sub genotype may help in discovering the treatment of this disease (Yano *et al.*, 2015).

In western countries, the prevalence of hepatitis B virus infection is generally low but higher in area with large immigrant population (Veldhuijzen *et al.*, 2009; Robertset *et al.*, 2016). The number of foreign born individuals having hepatitis B in the United States is significantly greater than reported by previous studies (Kowdley *et al.*, 2012). The prevalence rate of hepatitis B in this country is believed to be as high as 2.2 million.

Symptoms of Hepatitis B disease

The most common symptoms shared by hepatitis B patients include fatigue, vomiting, mild fever, dark color urine, yellow color eyes, pain in belly, joint pain, nausea, weakness and loss of appetite.

Positive effects of vaccines

Hepatitis B virus has been reported from about two billion people (WHO, 2015) including 240 million individuals as carriers (Lavanchy, 2004; Ott *et al.*, 2012). Small number of individual has been found to have positive and sustained response to large number of antiviral compounds reported. Considering this limitation, efforts should be directed more towards vaccination for enhancing immunity against HBV virus infection. The available vaccines have been found safe, showing the efficacy greater than 90% against all HBV genotypes/serotypes. The occurrence of HBV can be eliminated or reduced using worldwide vaccination (WHO, 2015; Helgi, 2015). Greater than 90% coverage

of vaccination has been demonstrated from Taiwan in 2004 as compared to low coverage countries such as Ukraine (21%), Azerbaijan (48%), France (65%), India (47%) and Indonesia (63%) (WHO/UNICEF, 2010). In United States, its coverage has been reported to be low especially in adults, however the occurrence of acute HBV has been reduced by 90% here (WHO/UNICEF, 2010). Although the occurrence of this disease has largely been reduced by vaccination but still 5-10% individuals do not show any response towards it which necessitates considering other measures for its complete eradication. The present study was arranged to find out the frequency of Hepatitis B in patients referred to Khyber Teaching Hospital Peshawar and to know about the major transmissible routes, causing hepatitis B infection.

2. MATERIALS AND METHODS

Patient's details

A total of 1, 33,193 patients visited the Khyber Teaching Hospital Peshawar in October-November, 2015, from different localities for different diseases or problems. Out of 1, 33,193 patients, 3,977 have been prescribed by their doctors for HBV screening before any treatment. Out of 3,977 patients, 1624 were screened in October and 2353 in November in Pathology laboratory of the Khyber Teaching Hospital Peshawar.

Sample collection

The blood samples were collected by the laboratory technician using disposable syringes and transferred into the collection tube (containing anti-coagulant like heparin, EDTA and Sodium citrate). The whole blood containing collection tubes were then centrifuged at given speed to get plasma specimen.

Laboratory diagnosis

The data for the study was collected on the basis of the following tests:

Imuno Chromatography Technique

The *in vitro* immuno chromatographic technique (ICT) was used for the determination of HBsAg either in human serum or plasma. The membrane strip enclosed in the test cassette has been coated with mouse anti-HBs antibody. The mouse anti-HBs particles conjugate which results in the movement of serum sample along the membrane chromatographically. As a result of this movement, visible line is formed when antibody – antigen – antibody gold particle complex is formed. The test cassette has been marked with "T" and "C" indicating test line and control line respectively. These two marks (T, C) can only be seen after the sample has been added to it. Appearance of Control line indicates that the procedure and chemicals

used are working well and enhance the validity of the test.

Enzyme Linked Immuno Sorbent Assay (ELISA) Test:

ELISA test relies on sandwich assay consisting of small sized wells coated with monoclonal antibodies (mab/mouse) supposed to be used against HBsAg. In this assay, test sample responds to immobilized mab and polyclonal anti-HBsAg antibody (guinea pig) conjugated with peroxides. The peroxides based complex link with micro wells surface if in case HBsAg is present in test sample. After incubation, it was washed to remove the unbound enzyme conjugate. After this, the Substrate solution was added which developed blue color upon more incubation. This blue color turned to yellow when the reaction is stopped by adding acidic solution. The level of intensity of the color is directly proportional to the amount of HBsAg in the test sample. According to several studies, less than cut-off value is believed to be negative for HBsAg. Specimens yielding repeatedly reactive readings by this assay are considered positive for the presence of HBsAg. These specimens must be confirmed by a confirmatory test.

3. RESULTS AND DISCUSSION

The data collection in this study was based on medically designed Performa, filled from the patients itself or from their relatives after the HBV positive test result and noted a detail disease history including the major causes or route of hepatitis B infection, its symptoms, age, sex and locality. All of the results were retrieved from the laboratory based tests recommended by doctors from different localities.

A total of 1, 33,193 patients visited the Khyber Teaching Hospital, Peshawar, from different regions such as Peshawar, Mardan, Charsadda, Nowshera, Malakand, Swat, Dir, Bajawar, Chitral, Parachinar, Hango, Kohat, D. I. Khan, Karak, Bannu, Khyber Agency, Waziristan, Miranshah etc. regarding different health problems or diseases. Some of these patients were being hospitalized.

Among the total number of patients who had visited hospital, about 3,977 patients came for the screening of HBsAg and anti-HCV, out of which 81 (2.03%) patients (Fig 1) were positive for HBsAg. Six of the patients were co-infected with HCV. The co-infected patients consisted of four females and two males.

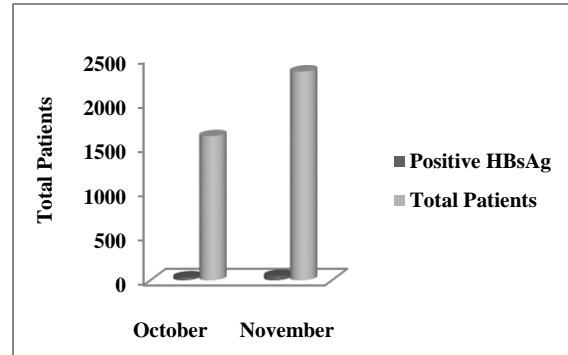


Fig 1: Distribution of Hepatitis B in selected patients at KTH. Out of prescribed/advised 3,977 patients for HBV screening, 1624 were screened in October and 2353 in November.

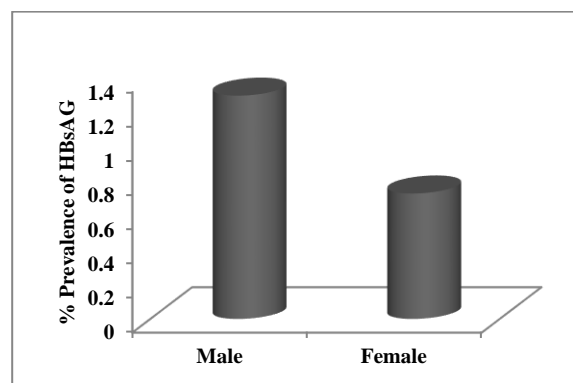


Fig 2: Average prevalence of HBsAg in males & females. It is clear from figure that the average prevalence of HBsAg in males was 1.4% and 0.8% in females.

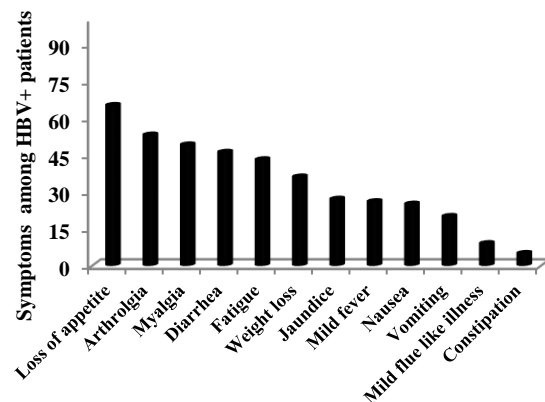


Fig 3: The Symptoms observed among HBV+ patients. The figure shows that the highest percentage symptom was loss of appetite followed by arthralgia, myalgia, diarrhea, fatigue, weight loss, jaundice, mild fever, nausea, vomiting, mild flue and constipation.

The overall prevalence of HBsAg from various regions of Khyber Pakhtunkhwa during the study was 2.03% (**Fig-1**). The average prevalence of hepatitis B virus infection in both male and female sex in the months of October and November is summarized in (**Fig-2**).

It can be noted from the Fig 2 that out of 1624 patients screened in October, 32 were HBsAg positive, including 1.17% (19/1624) males and 0.8% (13/1624) females. Similarly, in November, out of 2353 patients, 49 were HBsAg positive in which males were 1.40% (33/2353) and females were 0.68 % (16/2353).

Overall, average prevalence of HBsAg in males were 1.4% while in females 0.8%. The age group selected was 1-90 years both for males and females. Most of the cases were positive in adults, especially young people having age between 21-40 years.

The results showed the overall prevalence of HBsAg of 2.03% in the general population of Khyber Pakhtunkhwa, who visited the Khyber Teaching Hospital, Peshawar in the months of October and November, 2015. The higher prevalence was found in males (1.3%) than in females (0.73%). Reporting higher level of prevalence of HBV in males than females in our study is in strong agreement with other studies previously conducted (Ayele and Gebre-Selassie, 2013; Soomro and Mahmood, 2013; Khan *et al.*, 2013; Basit *et al.*, 2014; Walana *et al.*, 2014; Daw and El-Bouzedi, 2014). One of the possible explanations for higher prevalence of HBV in males could be that mostly males are blood donors in developing countries than females and get infected with this disease (Saberton *et al.*, 2009). Another possible explanation for getting higher frequency of HBV in males than females could be a reflection of more males coming for treatment and testing than females.

In our study, 21% of the patients were symptomless while 79% of the patients had few or more symptoms. The highest percentage symptom was loss of appetite (65%) (**Fig-3**). Co-infection with HCV was found in six patients (**Table-1**), in which two were males and four were females. The prevalence of HBV (2.03%) in our study is in strong agreement with a study conducted (Ali *et al.*, 2009), where HBV occurrence has been illustrated to be 2.4%. Our results are also comparable to some studies stating that the prevalence of the disease in military personals was 4.2% (Birku *et al.*, 2015) but in contrast to other (Walana *et al.*, 2014) where the prevalence of HBV was found to be 9.6%.

HIV co-infection reported in our study was 0% (**Table-1**) which strongly supports the findings of

Baqiet *al.*, (1999) who has also demonstrated the co-infection to be 0%. In our study, diabetes in HBsAg positive patients was 16% (Tab 1) which is comparable to other studies where the diabetes was found in 12% HBV related cases (Qureshi *et al.*, 2002). Considering the possible causes of infection amongst the 81 HBV positive patients, 35 (43%) patients gave a positive history of blood transfusion (**Tab 1**). Our results are in agreement with other results (Rezvan *et al.*, 2007; Ali *et al.*, 2009) who have claimed that unsafe blood and blood product transfusion are the major causal factor of acquiring HBV infection.

Tab 1: Associated risk factors among the HBV+ Patients.

<i>Risk factors</i>	<i>HBV Patients</i>	<i>%age</i>
History of dental treatment	57	70%
Treatment from unqualified doctor	40	49%
History of blood transfusion	35	43%
History of minor/major surgery	33	40%
HBV positive patients in family	28	35%
Shaving from street barber	26	32%
Diabetes	13	16%
Tattooing	9	11%
HCV co-infection	6	7.41%
Sharing of drug injecting equipment	1	1.2%
HIV/T.B	0	0%

The results of getting more positive HBV test in adults aged 21-40 years is comparable to recent studies (Ayele and Gebre-Selassie, 2013; Walana *et al.*, 2014; Birku *et al.*, 2015) where the prevalence of the disease was higher in patients aged below 50 years. Our results of getting more positive HBV results in age group 21-40 years is in contrast to some studies (Basit *et al.*, 2014), where higher number of such cases have been illustrated from age group greater than 60 years.

4. CONCLUSION

Among the eight genotypes of hepatitis B virus (A–H), genotypes B and C are mostly prevalent in Asia. In Pakistan, genotype D is the major one which constitutes a significant health problem. The higher occurrence of hepatitis and subsequent mortality in Pakistan is due to excessive use of contaminated syringes, drug abuse and unscreened blood transfusion. The overall prevalence of HBsAg from various regions of Khyber Pakhtunkhwa during the study was 2.03% which is in agreement with literature. Similarly, the average prevalence of HBsAg reported during the study was higher in males than females. This could be that mostly males are blood donors and visit hospitals for treatment and diagnostic tests than females in developing countries. As the treatment of this disease is expensive and cannot be afforded by poor people in developing countries, the

best approach would be to adopt the preventive measures and also identify the risk factors which could be easily targeted.

REFERENCES:

- Ayele, A. G., S. Gebre-Selassie, (2013). Prevalence and Risk Factors of Hepatitis B and Hepatitis C Virus Infections among Patients with Chronic Liver Diseases in Public Hospitals in Addis Ababa, Ethiopia. *ISRN Trop. Med.*, 563821.
- Alam, M. M., S. Z. Zaidi, S. A. Malik, S. Shaukat, A. Naeem, S. Sharif, M. Angez, J. A. Butt, (2007). Molecular epidemiology of Hepatitis B virus genotypes in Pakistan. *BMC Infectious Disease*, 7:115-120.
- Ali, S. A., R. M. J. Donahue, H. Qureshi, H. Sten, Vermund, (2009). Hepatitis B and C in Pakistan: prevalence and risk factors. *I. Jour. Infec. Disease*, 13: 9-19.
- Ashraf, S., A. Ahmad, (2015). Viral hepatitis in Pakistan: challenges and priorities. *Asian Pacific Journ. Trop. Biomed.*, 5: 190-191.
- Baqi, S., S. A. Shah, M. A. Baig, S. A. Mujeeb, A. Memon, (1999). Seroprevalence of HIV, HBV, and syphilis and associated risk behaviours in male transvestites (Hijras) in Karachi, Pakistan. *Int. J. STD-AIDS*, 10: 300-304.
- Basit, A., K. Rahim, I. Ahmad, M. Shafiq, S. Mushtaq, H. Shaheen, I. Khan, (2014). Prevalence of Hepatitis B and C infection in Pakistan. *Journ. Infection and Mol. Biolo.*, 2:35-38.
- Birku, T., B. Gelaw, F. Moges, A. Assefa, (2015). Prevalence of hepatitis B and C viruses infection among military personnel at Bahir Dar Armed Forces General Hospital, Ethiopia. *BMC Res.*, 8: 737-742.
- Chou, R., T. Dana, C. Bougatsos, I. Blazina, J. Khangura, B. Zakher, (2014). Screening for hepatitis B virus infection in adolescents and adults: asystematic review to update the U.S. Preventive Services Task Force recommendation. *Ann. Intern. Med.*, 161: 31-45.
- Daw, M. A., A. El-Bouzedi, (2014). Prevalence of hepatitis B and hepatitis C infection in Libya: results from a national population based survey. *BMC InfecDisea.*, 14: 1-9.
- Farhat, M., A. Yasmeen, A. Ahmad, (2014). An Overview of Hepatitis B and C in Pakistan. *Inter. Jour. Microbio. Allied Sci.*, 1: 98-102.
- Farooqi, J. I., R. J. Farooqi, N. Khan, Mussarat, (2011) Frequency of hepatitis B and C in selected groups of population in NWFP, Pakistan. *J. Postgrad. Medi. Insti (Peshawar Pakistan)*. Date accessed: 22 Nov. 2016. 21:165-168.
- Gilchrist, E. S., P. N. Newsome, E. Dalakas, C. Payne, P. Lee, P. Cowan. (2005) Acetaminophen-induced fulminant hepatic failure results in an SDF-1 mediated 'all or nothing' haematopoietic stem cell response. *Hepato.*, 42: 744A-745A.
- Hoofnagle, J. H., E. Doo, T. J. Liang, Fleischer, A. S. F. Lok, (2007). Management of hepatitis B: summary of a clinical research workshop. *Hepato.*, 45: 1056-1075.
- Helgi Library (2015). <http://www.helgilibrary.com/indicators/index/immunisation-hepb3-as-of-one-year-old-children>. Accessed June 10, 2016.
- Kane, M., (1995) Global programme for control of hepatitis B infection. *Vacc.*, 13: S47-S49.
- Khan, S. B., M. Alam, M. A. Khan, M. Marwat, I. Ahmad, M. Idrees, (2013). Frequency and risk factors of HB and HC virus infections in patients undergoing cataract Surgery. *Gom. J. Med. Sci.*, 11: 155-158.
- Kowdley, K. V., C. C. Wang, S. Welch, H. Roberts, C. L. Brosgart, (2012). Prevalence of chronic hepatitis B among foreign-born persons living in the United States by country of origin. *Hepato.*, 56: 422-433.
- Lavanchy, D., (2004). Hepatitis B virus epidemiology, disease burden, treatment, and current and emerging prevention and control measures. *J. Viral Hepati.*, 11: 97-107.
- Lok, A. S., B. J. McMahon, R. S. Brown, J. B. Wong, A. T. Ahmed, W. Farah, J. Almasri, F. Alahdab, K. Benkhadra, M. A. Mouchli, S. Singh, (2016). Antiviral therapy for chronic hepatitis B viral infection in adults: A systematic review and meta-analysis. *Hepato.*, 63: 284-306.
- Majid, A., M. S. Khan, S. Ullah, (2010). Rising prevalence of Hepatitis B and C and risk factors at District Headquarter Teaching Hospital Bannu, Khyber-Pakhtunkhwa. *J. Coll. Physici. Surg. Pak.*, 20(7): 492-493.
- Memon, A. R., K. Shafique, A. Memon, A. U. Draz, S. Afsar, (2012). Hepatitis B and C prevalence among the high risk groups of Pakistani population. A cross sectional study. *Arch. Pub. Heal.*, 70: 9-14.

- Michitaka, K., S. Nishiguchi, Y. Aoyagi, Y. Hiasa, Y. Tokumoto, M. Onji, (2010). Etiology of liver cirrhosis in Japan: a nationwide survey. *Journal of Gastroenterology*, 45: 86–94.
- Norah, A., Terrault, H. Natalie, Bzowej, C. Kyong-Mi, P. Jessica, Hwang, M. Maureen, Jonas, and M. Hassan. (2016) AASLD guidelines for treatment of chronic hepatitis B. *Hepatology*, 63: 261.
- Ott, J. J., G. A. Stevens, J. Groeger, S. T. Wiersma, (2012). Global epidemiology of hepatitis B virus infection: new estimates of age-specific HBs Ag seroprevalence and endemicity. *Vaccine*, 30: 2212–2219.
- Ozasa, A., Y. Tanaka, E. Orito *et al.*, (2006). Influence of genotypes and precore mutations on fulminant or chronic outcome of acute hepatitis B virus infection. *Hepatology*, 44: 326–334.
- Pakistan Medical Research Council, (2007–08). Pakistan Hepatitis B and C Prevalence Survey 2007–2008. Global Health Data Exchange, Discovers the World Health Data.
- Qureshi, H., T. Ahsan, S. A. Mujeeb, F. Jawad, I. Mehdi, W. Ahmed, S. E. Alam, (2002). Diabetes mellitus is equally frequent in chronic HCV and HBV infection. *Pak-Med-Assoc.*, 52: 280–283.
- Rezvan, H., H. Abolghassemi, S. A. Kafiabad, (2007). Transfusion-transmitted infections among multitransfused patients in Iran: a review. *Transfusion Medicine*, 17: 425–433.
- Roberts, H., D. Kruszon-Moran, Ly K. N, E. Hughes, K. Iqbal, R. B. Jiles, S. D. Holmberg, (2016). Prevalence of chronic hepatitis B virus (HBV) infection in U.S. households: National Health and Nutrition Examination Survey (NHANES), 1988–2012. *Hepatology*, 63: 388–397.
- Saberton, P. J., A. Paez, K. B. Newbold, N. M. Heddle, (2009). Geographical Variations in the Correlates of Blood Donor Turnout Rates: An Investigation of Canadian Metropolitan Areas. *I. J. Health Geogra.*, 8: 56Pp.
- Soomro, M. Z., R. Mahmood, (2013). Prevalence of Hepatitis B and Hepatitis C in elective ocular surgery (Rural Origin) at Shifa Eye Hospital, Khanpur. *Pak. J. Ophthalmology*, 29: 31–33.
- Suzuki, Y., M. Kobayashi, K. Ikeda, (2005). Persistence of acute infection with hepatitis B virus genotype A and treatment in Japan. *J. Med. Virol.*, 76: 33–39.
- Travasso, C., (2014). Indian government plans 10 regional laboratories to estimate hepatitis burden. *BMJ*, 5: 349Pp.
- Veldhuijzen, I. K., H. F. Van Driel, D. Vos, O. D. de Zwart, G. J. J. van Doornum, R. A. de Man, J. H. Richardus, (2009). Viral hepatitis in a multi-ethnic neighborhood in the Netherlands: results of a community-based study in a low prevalence country. *I. J. Infect. Disease*, 13: e9–e3.
- Walana, W., P. Hokey, S. Ahiaba, (2014). Sero-Prevalence of Hepatitis B Virus Infection among Blood Donors: A Retrospective Study in the Kintampo Municipal Hospital, Ghana. *Open Journal of Medical Micro.*, 4: 64–69.
- WHO/UNICEF. (2010) Coverage estimates revision. WHO Member States: 193Pp.
- Wan-Hsin, W., L. Ming-Wei, C. Mei-Hwei, (2015). A review of strategies to prevent mother-to-infant transmission of hepatitis B virus infection. *Expert Review of Gastroenterology & Hepatology*, 10: 317–330.
- WHO (2015). Guidelines for the prevention, care and treatment of persons with chronic hepatitis B infection. Geneva Switzerland: World Health Organization, May 12, 2015.
- Yano, K., Y. Tamada, H. Yatsushashi, (2010). Dynamic epidemiology of acute viral hepatitis in Japan. *Intervirology*, 53: 70–75.
- Yano, Y., T. Utsumi, M. I. Lusida, Y. Hayashi, (2015). Hepatitis B virus infection in Indonesia. *World Journal of Gastroenterology*, 21: 10714–10720.
- Yim, H. J., A. S. Lok, (2006). Natural history of chronic hepatitis B virus infection: what we knew in 1981 and what we know in 2005. *Hepatology*, 43: S173–S181.
- Yokosuka, O., M. Kurosaki, F. Imazeki, (2009). Management of hepatitis B: consensus of the Japan Society of Hepatology. *Hepatology Res.*, 4: 1–21.
- Yotsuyanagi, H., C. Okuse, K. Yasuda, (2005). Distinct geographic distributions of hepatitis B virus genotypes in patients with acute infection in Japan. *J. Med. Virol.* Vol. 77: 39–46.