Vol 4 (2), July 2013; 56-63

Prevalence of Transfusion Transmitted Infection in Replacement and Voluntary Blood Donor

Syeda Kanwal Zehra[1], Saira Bano[1], Shazia Tabassum Hakim[1], Sayyada Ghufrana Nadeem[1]*

[1]Department of Microbiology, Jinnah University for Women, Karachi-74600, Pakistan.

ABSTRACT

The aim of this study is to present the status of transfusion –transmitted infections among the apparently healthy donors so as to increase the awareness of complications of blood transfusion and make the clinicians more vigilant with regard to judicious use of blood. Screening of blood is mandatory for providing safe blood. The magnitude of transfusion transmitted infections (TTI) varies from country to country depending on TTI's load in that particular population. Transfusion transmitted infections create significant burden on health care system. Donor selection is of paramount importance because infected individuals serve as an asymptomatic reservoir and a potential source of transmission. This retrospective study was carried out in healthy blood donors in the age group of 18-60 years; study was done on blood units collected from replacement and voluntary donors. The serum samples were screened for Hepatitis B Surface Antigen (HBsAg); antibodies against HIV I and II, Hepatitis C Virus (HCV) by Chemiluminescent microparticle immuno assay (CMIA) method. Screening for Syphilis was carried out by RPR Rapid plasma reagent. Seropositivity of transfusion transmitted disease in replacement donors was 1.93% in hepatitis B surface antigen, 2.41% in hepatitis C virus, 0.09% in HIV and 1.15% in syphilis. Voluntary donors had low infectivity rate as compare to replacement donor.

Key words: CMIA, HBsAg, Replacement donors, Syphilis, TTI, Voluntary donors.

INTRODUCTION

Blood is life. Blood transfusion is the transfer of biological material from man to man. Blood has been used in 1930s for the various indications (Zafar, 2000). Blood transfusion, a fundamental part of medicine and surgery, also carries the risk of transfusion-related infections like Hepatitis B and C, HIV and Syphilis, malaria and infrequently toxoplasmosis, brucellosis and viral infections like CMV, Epstein Barr Virus and Herpes (Widmann FK, 1985). Transfusion of blood and blood products is a lifesaving fact. Measuring their sternness, WHO has recommended pre-transfusion blood test for HIV, HBV, HCV and Syphilis as obligatory (World Health Organization). All these diseases are competent of causing major mortality, morbidity along with an economic load for both the affected person and the country. With every one unit of blood transfusion there is 1% chance of transfusion related complications including Transfusion transmitted infections (Widmann FK, 1985) the safety of blood and its products has gained tremendous importance since the documentation of blood born viral infections in regularly transfused patients. These may be thalasemic children, oncology patients or aplasticanemia patients (Naseem and Haq, 2009). Detection of hepatitis B surface antigen (HBsAg) in blood is diagnostic for infectivity with HBV and in blood bank screening for HBsAg is approved routinely to detect HBV infection (Bhattacharya *et al.*, 2007). Similarly antibodies to hepatitis C virus (anti-HCV) are used to detect HCV infectivity

*Corresponding author: juskz_110@hotmail.com

(Olokoba *et al*., 2008). Safe blood transfusion is the expression which refers to legal and coherent healing use of blood and blood products. World Health Organization (WHO) recommendation of safe blood transfusion is provision of well-matched blood which are cross matched and screened at least for five WHO suggested transfusion transmitted infections, human immunodeficiency virus (HIV), hepatitis C (HCV), hepatitis B (HBV), syphilis and malarial parasite (Dipta & Rahman, 2009). Objectives of the study were to guesstimate occurrence of Hepatitis B and C in blood donors of local area and recommend measures for safe blood transfusion. In this study, we intended to estimate the prevalence of HIV, HBV, HCV and Syphilis among apparently healthy blood donors. It would also reflect on the blood safety dimensions and can be carefully extended to provide estimation about the disease burden in the community.

HIV: HIV is the contraction used for the Human Immunodeficiency Virus. HIV attacks the body's immune system. Normally, the immune system produces white blood cells and antibodies that attack viruses and bacteria. The infection combating cells are called T-cell lymphocytes. Months to years after a person is infected with HIV, the virus destroys all the T-cell lymphocytes. This disables the immune system to shield the body against diseases and tumors. Various infections will be able to develop; the opportunistic infections take benefit of the body's destabilized immune system. These infection which normally won't cause severe or lethal health problems will eventually cause the death of the HIV patient (Rombauts, 1997). Untreated HIV disease is a continual progressive process that begins with infection, is often followed by a "primary HIV syndrome," and progresses in adults over a median period of more than 10 years to the late stage: AIDS. From the time of infection, the virus continuously and swiftly replicates, mutates, and as a result diversifies and evolves in response to selective pressure. Immune system damage also begins upon infection. The viral load and the volume of this process occur in lymphoid tissue, and the immune system struggles to seize the process in check. Slowly, but persistently, the process destroys vital components of the host immune system. Progression is often accelerated in infants with prenatal HIV infection. Eventually the host becomes increasingly liable to and eventually dies as a result of complications of opportunistic infections and malignancies resulting from immune system dysfunction (Cohen, 1998).

Epidemiology of HIV: The viruses that are the source of acquired immune deficiency syndrome (AIDS), human immunodeficiency virus types 1 and 2 (HIV-1 and HIV-2), are lentiviruses (Hahn, 2000). The global prevalence of HIV-1 has stabilized at 0.8%, with 33 million people breathing with HIV/AIDS, 2.7 million new infections, and 2.0 million AIDS deaths in 2007 (The official site of the National AIDS Program of Pakistan NACP)During the last three decades, the HIV pandemic has entered our realization as an inexplicable disaster. HIV and AIDS has already taken a dreadful human toll, lying assert to millions of lives, inflicting pain and grief, causing dread and doubt and threatening economic devastation. While Sub-Saharan Africa remains the nastiest affected region in the world, there is mounting concern about the emergence of HIV and AIDS in Asia, which is home to more people than any other constituency of the world. Though the epidemic in Asia is less severe than in other parts of the world and rates of infectivity in the general population remain comparatively low, it is clear that HIV has ongoing spreading swiftly through the region and enormous Asian population is gradually more at risk. (USAID, 2004)

Pakistan is the second largest country in South Asia that stands only a few steps at the back of India and Nepal in terms of HIV epidemic. In spite of many efforts, the HIV infection rate has enlarged considerably over the past few years and infect, the country has moved from a low occurrence to intense epidemic with HIV prevalence of more than 5% among injecting drug users (IDUs) in at least eight major cities (NACP-HASP, 2007).Other high-risk groups, such as men who have sex with men (MSM), hijra sex workers (HSWs) and female sex workers

(FSWs), also look set to reach this threshold level. Many bridging populations, totaling almost five million persons, are in direct sexual contact with these groups and are exposed to HIV infection through unprotected sexual activity. The heterogeneity and interlinking of high risk injecting and sexual behavior, combined with low levels of HIV knowledge and prevention, and high levels of other sexually transmitted infections (STIs), indicates that HIV could spread rapidly to marriage partners or sex clients and result in generalized epidemic.HIV is not currently a dominant epidemic in Pakistan. However, the number of cases is growing (The official site of the National AIDS Program of Pakistan NACP) Recent variation in the epidemiologic data regarding HIV prevalence in Pakistan suggests alarm. Unpublished data indicate a recent increase in HIV prevalence from surveys of IV drug users (IDUs) all around the nation. According to Sindh AIDS control program HIV seroprevalence rate of IDUs in Karachi is about 9%. In 2002-2003 Family Health International sponsored cross-sectional studies suggest 23% seroprevalence among IDUs in Karachi. HIV/AIDS Surveillance Project (HASP) of Canadian International Development Agency results in 2004 suggested 26% seroprevalence among IDUs in Karachi. Now HIV is reported routinely among IDUs in other areas of county,including Rawalpindi, Lahore and Sargodha (The official site of the National AIDS Program of Pakistan NACP)There is no doubt that Pakistan has moved from a nascent epidemic into one with a intense epidemic, with high rate of prevalence in at least one major high risk population.

Hepatitis B and C: The Hepatitis B and C virus infections are known to occur in the general population and because of blood and blood products transfusion, it has made the stipulation of safe blood difficult, that's why screening of blood completely necessary (Olokoba, *et al*, 2008). These viruses infect mankind from centuries but in 1970s hepatitis B virus was discovered and hepatitis C in 1990s. Both viruses can cause acute illness where patients have acute jaundice and very high serum Alanine transaminase (a part of liver function tests). In legal period 90% of adults, incisive infectious disease B resolves within II-tierce calendar month with entire improvement. The assumption is different in new born and up to quintet geezerhood of age where full recovery is seen only in 10% while xc% develop chronic liver disease. In acute liver disease C full recovery appear in only 20-thirty% of cases while70-eighty% require treatment for clearance of disease as they may go on to develop chronic liver disease. If the mother is HBsAg empiricist philosophy and is also positive for HBeAg (a marker for infectivity) than there is a large integer% encounters that she will transmit the disease to her newborn. If the mother is HBsAg positive and not HBeAg positive, than the probability goes down to 20-30%. It is therefore recipient to equal pregnant parent for these 2 viruses. For the former scenario the newborn has to receive 2 immunizations within large integer hours of biological process, the hyper immune globulin at one internet site and the infectious disease B vaccine at another site. For the latter forgather only hepatitis B vaccine is recommended.

Epidemiology of HBV & HCV: HBV linguistic process come alongs all over the world. The WHO has estimated that there are more than two 1000000000 HBV infected folk and about 37digit million chronic vectors worldwide. There are approximately 620 000 HBV related killing each year. In addition, approximately 4-5 million new HBV infections occur worldwide each year, of which a quarter progresses to liver unwellness. In high endemic region, like key Asian republics, Sou'-east Asia, Sub-Saharan Africa and the Parrot sink, the HBV carrier rate is over 8%. In low endemic body part, like the United States, Northern Europe, Land and surroundings of South America, HBsAg ratio is less than 2%. The Middle East, some Eastern Dweller countries and the Mediterranean basin are considered areas of liaise endemicity with a carrier rate between 2% and 8% (WHO. Department of Communicable Diseases Surveillance and Response). In many countries, after the introduction of mass immunization operation, the generality of HBV notably changed, resulting in a reduction of the HBsAg carrier place

and HCC incidence (Zanetti, 2008). It was estimated that somebody cancer transpose approximately 4% of all new cancer cases diagnosed worldwide and that more than 50% of viscus person were attributable to HBV. The highest epoch-adjusted incidence rate (> 20 per C 000) was reported from Southeast Asian and Sub-Nilo-Saharan language Individual countries that are endemic for HBV linguistic process. Up to 90% of child septic during the first year of life and 30%-50% of children infected between one to four years of age develop chronic linguistic process and about 25% of someone who become chronically infected during childhood die from HBV-related liver cancer or cirrhosis (WHO-Expanded Programme on Immunization Hepatitis B Vaccine). Pakistan is endemically high in HBV (Noorali *et al*., 2008) with nine million people infected with HBV (Hepatitis prevention & control program Sindh 2009) and its infection rate is on a steady rise (Hakim *et al*., 2008). The reason may be the deficiency of proper upbeat facilities, poor system status and less public consciousness about the transmission of major communicable diseases including HBV, HCV and HIV (Alam *et al*., 2008).

Globally, an estimated 130-170 million persons (2%-3% of the populace\'s population) are beingness with hepatitis C virus (HCV) infection (WHO, 2004). This corruptness, particularly in its chronic form, is associated with sizable morbidity and mortality. More than 350 000 destruction are attributed to HCV infection each year, most of which are caused by inhabitant cirrhosis and hepatocellular malignant neoplastic disease. (Perz *et al*., 2006). An estimated 27% of liver unwellness and xxv% of HCC can be attributed to hepatitis C planetary, and disease revenue enhancement can be even more square in countries with a high burden of communication. For example, in Japan, up to 90% of all reported cases of Hepatocellular carcinoma caused by HCV infection (Perz *et al*., 2006). In development countries where resources and facilities may be significantly limited, the ratio of HCV is higher as compared to the developed homo. Roughly 10 million make full have been septic with HCV in Asian nation. The majority of patients have acquired their infection through unsafe injections, apply of syringes and needles and community barber patronise used for face up and armpit shaving. More than two-thirds of HCV patients were 40 to fifty years old. Development of a vaccine against HCV is more problematic fixed cost to the genetic nonuniformity of the representation. However, with large integer% of HCC in processing countries attributable to HCV (approximately 93,000 cases per class) a vaccine would make a major contribution to cancer hindrance. (Wild and Hall, 2000)

Syphilis: Spirochetes are spiral-shaped, motile bacteria that are divided into the families Spirochaetaceae, Brachyspiraceae, and Leptospiraceae (Paster and Dewhirst. 2006). Spirochaete taxon, which are members of the family Spirochaetaceae, are fastidious anaerobiotic or microaerophilic host associated spirochetes. While the majority of Spirochete taxonomic group are found in the ?ora of humans and animals, a few species are infective for humans. Treponema globus pallidus subspp. pallidum, endemicum, and pertenue, the functionary of reproductive organ syphilis, endemic syphilis, and framboesia, respectively, together with Treponema carateum, the agent of pinta, are primary pathogens of humans that have eluded in vitro cultivation (Stamm, 2001). Spirochete denticola and certain other oral Treponema species that are associated with man periodontal illness are arable, opportunistic pathogens (Holt and Ebersole, 2006). Syphilis causes symptoms in three different stages (primary, secondary, and tertiary), separated by periods when no symptoms go on (latent stages). Syphilis is extremely transmittable during the primary election and secondary traveling. Infection is usually beddispersed through intimate contact. A single sexual encounter with a material assemblage who has early-represent sexually transmitted disease statement in unhealthiness about one third of the time. The bacterium enters the body through mucous animal tissue, such as those in the channel or mouth, or through the life. Within hours, the bacteria reach nearby liquid body substance nodes, and then spread throughout the body through the bloodstream.

Syphilis can also be spread in other construction. It can infect a fetus during pregnancy, causing birth defects and other trouble. It can also be spread through contact with skin. However, the bacteria cannot go retentive outside the homo body. People with syphillus often have other pathological process, including other sexually transmitted diseases (STDs).Each stage of indication (primary, secondary, and tertiary) is progressively worse. If not tempered, syphilis can persist without grounds for many years and may price the heart or brainiac, maybe leading to death. If detected and treated former, syphilis can be cured, and there is no permanent alteration.

Epidemiology of Syphilis: Syphilis is a multistage disease that is usually genetic through contact with active lesions of a intimate soul or from an infected meaning woman to her craniate (Paster and Dewhirst, 2006). Efforts to eliminate syphilis have met with only mild success (Hook and Peeling, 2004). The World Health Organization (WHO) estimated that there were 12 large integer new caseful of venereal disease in 1999, with more than xc% of the cases occurring in developing countries Congenital syphilis is a leadership cause of stillbirth and prenatal mortality in many of these countries (Schmid *et al*., 2007; Tichonova *et al*., 1997). Despite the accessibility of new diagnostic tests and antibiotic medical care, venereal disease has reemerged in several developed countries. While the widespread epidemics of syphilis that occurred in Soviet Union in the 1990s and more new in Nationalist China mostly mired heterosexuals, smaller occurrent in the United Suggest, Canada, and European country predominately involved men who have sex with men (Schmid *et al*., 2007). However, late amount in syphilis rates for U.S. women and infants suggest that heterosexually transmitted syphilis may be an emergent problem in the Cooperative States (Martin *et al*., 2009.). A major concern associated with increased rates of Venus's curse is that someone, early venereal infection (i.e., primary and secondary initiate) improve transmission of human immunode?ciency virus (Virus infection) by 2- to 5-fold, thus promoting the spread of HIV (Douglas, 2009). By comparing international studies with our study on seroprevalence of syphilis in IDUs, our resolution are quite heights (25.89%). When compared with the study results of 2005 in IDUS of Karachi (18.2%) (Government of Pakistan. 2005. However a significant ($P<0.05$) decrease in syphilis was observed in Karachi. When the same study results (3.9%) in Lahore. syphilis in IDUs were compared with our study results (25.89%), a decrease in seroprevalence of syphilis was noted, which was highly significant ($P<0.001$).

MATERIALS AND METHODS

Total 13170 donors who were recruited in this study and all are males. We collected the blood from voluntary and replacement blood donors. Replacement donors are donors who donate blood on behalf of their patient and family members. Voluntary blood through blood donation camps organized by man organizations and student bodies including the pupil of Medical Colleges, commercial enterprise organization in Metropolis. Name, age (eighteen-60 years), Sex, edible fruit of bear, address and contact confine were recorded for each donor, while giving them a unique identification number. Donors with history of any febrile illness in the late past, free weight loss, uncontrolled looseness of the bowels, Recent distort, liver disease, cardiovascular disease, pulmonary disease, evilness, epilepsy, malaria, strange or overweening bleeding, recent donation of rakehell, receipt of descent, and winning contraindicated medicine were excluded. Detailed history of immunization was taken. Weight, pulse, blood somaesthesia and somesthesia were recorded for each donor. Screening for fern genus was done clinically along with copper sulfate specific gravitation method. Scrutiny was made for any marks of drug abuse or any skin lesions/ infections at the vein puncture information processing system. A written informed consent was taken from each donor before the blood donation. Proper sterilization and other care were taken during the blood collection and blood units were stored by appropriate method acting. After collection all samples are screened for Human immunodeficiency virus (HIV) by HIV

Ag/Ab Combo Kit on Abbott's (I-2000 and I 2000 SR) Chemiluminescent microparticle immuno assay technology (CMIA).Hepatitis B by HBsAg Qualitative and HBsAg Qualitative II kit on Abbott's (I-2000 and I 2000 SR) Chemiluminescent microparticle immuno assay technology (CMIA).HCV by Anti - HCV kit on Abbott's (I-2000 and I 2000 SR) Chemiluminescent microparticle immuno assay technology (CMIA).

Treponema Pallidum: Detection of Treponemal Antibodies (Reagin) by Rapid Plasma Reagin Test (RPR) with Spinreact and TECO kits. Calibrators and Controls are run before samples all controls are satisfactory.

RESULTS AND DISCUSSION

Most of the bestower, who were recruited in this study, came from public sentience. It was to be noted that maximum public presentation of donors came from get on group eighteen-60 years. It may reflect proper awareness among the young population about blood donation. Blood donors are of various type voluntary blood donors, replacement or family blood donors, plasma and platelet donor and commercial donors. Form the above donors the voluntary donor are the safest donor because they donate blood regularly and know their medical status. Replacement donor and family donors are at risk because they donate blood when they need blood, they are the donors which can transmit the infectious agent which they have in family. They don't know anything about their medical status. Commercial donors are highly at risk because they sell their blood to fulfill their needs they mostly are IV drug user because of hygiene and close contacts with each other. In this study we work on two types of donor replacement and voluntary donors. This is the comparative study of infective rate between these two types of donors. According to our data which we collected from the Husaini Blood Bank Karachi. We screened 13170 donors from which 6585 are replacement donors and 6585 are voluntary donors. These donors are screened for Hepatitis C, B HIV and syphilis. We observe that in voluntary blood donors 260 donors are reactive and in replacement blood donor is 368 donors. This observation shows that the replacement donor show high reactive rate as compare to voluntary as shown in Table I. Highest reactive rate among voluntary blood donors was observed of hepatitis B while highest reactive rate among replacement blood donors was observed of HCV.

All Donors were screened for HIV replacement donor show more HIV positive then voluntary donors infective. Voluntary donors are 0.02% infected among them and replacement donors are 0.09% infected. (Table II). In replacement donation 127 donors and 115 voluntary donors are HBsAg reactive. In our data the ratio of reactivity in both types of donor is very similar because in our region Hepatitis B is the major disease which is growing very fast and lack of awareness about this disease is also a reason of less difference in a reactive numbers. Anti HCV only 99 persons are found reactive of Hepatitis C in voluntary rather 159 individual are reactive in replacement blood donor. In syphilis 76 donors are R.P.R positive in replacement donor but 45 donors are reactive.

Table I. Reactive rate of voluntary blood donors and replacement blood donors.

Test	vol.donor	rep.donor	vol.reactive rate	rep.reactive rate
Hepatitis B	6585	6585	115	127
Hepatitis C	6585	6585	99	159
HIV	6585	6585	1	6
Syphilis	6585	6585	45	76
Total Reactive			260	368

Table II. Reactive Rate of Hepatitis B, C.HIV and syphilis.

Donors	Total Screened	HIV +ve	%	HBsAg +ve	%	HCV +ve	%	RPR +ve	%
Replacement	6,585	6	0.09	127	1.93	159	2.41	76	1.15
Voluntary	6,585	1	0.02	115	1.75	99	1.50	45	0.68

In Pakistan Hepatitis C is the major disease which is spread by transfusion of blood and its products

and needle stick injuries. We also perform Hepatitis C PCR for these donor and we found one (1) donor is RNA positive among 6585 donors in replacement but voluntary donors are all are RNA negative. RPR done for syphilis but this test have shown false positive result when in some conditions such as IV drug use, Lyme disease, Certain types of pneumonia, Malaria, Pregnancy, Systemic lupus erythematosus (SLE) and some other autoimmune disorders, Tuberculosis (TB) but these donors are also screened for malaria as well because it is also a mandatory test for screening as per WHO recommendation. That's why the chance of false positive result is less in these donors.

CONCLUSION

In Pakistan the ration oaf replacement donor is 7 % and voluntary donor is 2.7 % .But according to our data it very from the donor survey in our country because we collect the specific data of specific range but the survey is done on yearly basis. The reactive rate is increased in voluntary donor because of less awareness about the blood donation and bias also occurs in verbal screening or donor recruitment because some people tell lie when we ask these question. We have to promote the voluntary blood donation and also give awareness to our young youth because some have a doubt in blood donation that if they donate blood they get infection or weakness. Voluntary blood donation is the safest donor for the recipient. In our country we have less blood donation but the need of blood is high.

REFERENCES

Alam MM, Zaidi SZ, Malik SA, Naeem A, Shaukat S, Sharif S, Angez M, Khan A, Butt JA.2007.Serology based disease status of Pakistani population infected with Hepatitis B virus. BMC Infect Dis, 7:64.

Bhattacharya P, Chandra PK, Datta S, Banerjee A, ChakrabortyS, Rajendran K *et al*. 2007. Significant increase in HBV,HCV,HIV and Syphilis among blood donors in west Bengal, Eastern India. World J Gastroenterol, 13:3730–3733.

Cohen PT. 1998. Understanding the HIV disease: hallmarks, clinical spectrum, what we need to know. The AIDS knowledge base http://hivinsite.ucsf.edu/akb/1997/04over/index.html.

Dipta TF, Md. Rahman T. 2009. Safe blood transfusion: past, present and future. Bangladesh J Pathol, 24(1):1–2.

Douglas JM. 2009. Penicillin treatment of syphilis-clearing away the shadow on the land. JAMA 301:769–771.

Hahn BH, Shaw GM, De Cock KM & Sharp PM. 2000 AIDS as a zoonosis: scienti¢c and public health implications. Science 287:607-614.

Hakim ST, Kazmi SU, Bagasra O. 2008. Seroprevalence of Hepatitis B and C Genotypes among Young Apparently Healthy Females of Karachi-Pakistan. Libyan J Med, 3:66-70.

Holt SC, and Ebersole JL. 2006. The oral spirochetes: their ecology and role in the pathogenesis of periodontal disease, p. 323–356. In J. D. Radolf and S. A. Lukehart (ed.), Pathogenic Treponema molecular and cellular biology. Caister Academic Press, Norfolk, England.

Hook EW, and Peeling RW. 2004. Syphilis control—a continuing challenge. N. Engl. J. Med. 351:122–124.

Martin IE, Tang RS, Sutherland K, Tilley P, Read R, Anderson B, Roy C and Singh AE. 2009. Molecular characterization of syphilis in patients in Canada: azithromycin resistance and detection of Treponema pallidum DNA in whole-blood samples versus ulcerative swabs. J. Clin. Microbiol. 47:1668–1673.

NACP-HASP (2007), Summary Report – Integrated Biological and Behavioural Surveillance Study: HASP, Islamabad

Naseem L, Haq A. 2009. Predonation testing of the potential blood donors- A pilot study at a tertiary care hospital. Int J Pathol, 7(1):25–30.

National AIDS Program of Pakistan (NACP). 2007. Available at: http://www.nacp.gov.pk/

Noorali S, Hakim ST, McLean D, Kazmi SU, Bagasra O. 2008. Prevalence of Hepatitis B virus genotype D in females in Karachi, Pakistan. J Infect Developing Countries, 2:373-378.

Olokoba AB, Olokoba LB, Salawu FK, Danburam A, Desalu OO, Midala J, *et al*. 2008. Hepatitis C virus and Human Immunodeficiency virus co-infection in North Eastern Niger Res J Med Sci, 2:217–219.

Paster BJ, and Dewhirst FE. 2006. The phylogenetic diversity of the genus Treponema, p. 9–18. In J. D. Radolf and S. A. Lukehart (ed.), Pathogenic Treponema molecular and cellular biology. Caister Academic Press, Norfolk, England.

Perz JF, Armstrong GL, Farrington LA, Hutin Y, Bell B. 2006. The contributions of hepatitis B virus and hepatitis C virus infections to cirrhosis and primary liver cancer worldwide. J Hepatol, 45:529–38.

Rombauts B. 1997. Farmaceutische Microbiologie (met inbegrip van de farmaceutische technologie van steriele geneesmiddelen). Cursus 1ste graad apotheker VUB, 11:14-16.

Schmid GP, Stoner BP, Hawkes S and Broutet N. 2007. The need and plan for global elimination of congenital syphilis. Sex. Transm. Dis. 34(Suppl.):S5–S10.

Stamm LV. 2001. Treponema pallidum, p. 1795–1808. In M. Sussman (ed.), Molecular medical microbiology, 1st ed. Academic Press, London, United Kingdom.

Tichonova L, Borisenko K, Ward H, Meheus A, Gromyko A, and Renton A. 1997. Epidemics of syphilis in the Russian Federation: trends, origins, and priorities for control. Lancet 350:210–213.

USAID. 2004. Family Health International Pakistan Quarterly Report: http://www.fhi.org/NR/rdonlyres/eh3rk74b4h72wbdebspbxuen2ag3hdb42rlpg7riuv5ytxzq3xm64uiz3vsewaucdhppx6czyi7bpd/PakistanQuarterlyReportDec2004.pdf.

WHO. Department of Communicable Diseases Surveillance and Response. Hepatitis B. Available from: http://www.who.int/csr/disease/hepatitis/HepatitisB_whocdscsrlyo2002_2.pdf.

WHO. Expanded Programme on Immunization Hepatitis B Vaccine. Available from: http://www.who.int/vaccines-documents/DoxNews/updates/updat31e.pdf.

Widmann FK. 1985. Technical manual American Associations of Blood Banks. Anglington USA: 325-44.

Wild CP, Hall AJ. 2000. Primary prevention of hepatocellular carcinoma in developing countries. Mutat Res, 462:381-393.

World Health Organization (WHO). 2009. Screening Donated Bloods for Transfusion-Transmissible-Infections. Available at: http://www.who.int/bloodsafety/ScreeningTTI.pdf+&cd=1&hl=en&ct=clnk&gl=pk&client=firefox-a.

World Health Organization. 2004. Global burden of disease (GBD) for hepatitis C. J Clin Pharmacol, 44:20–9.

Zafar N. 2000. Survey of blood transfusion practices. J Coll Physicians Surg Pak, 10(3):90–92.

Zanetti AR, Van Damme P, Shouval D. 2008. The global impact of vaccination against hepatitis B: a historical overview. Vaccine, 26:6266–6273.