Sero-Prevalence of Hepatitis B Surface Antibodies (IgG) Among Nigerian and Indian Students of Jodhpur National University, India

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Abstract: Hepatitis B virus is highly infectious and a major global health problemas it causes chronic liver diseases such as cirrhosis and hepatocellular carcinoma.The study was carried out to assess and compare the prevalence of HbsAb among Nigerian and Indian students of Jodhpur national university India, in an effort to identify the vulnerable individuals and enlighten the public about the danger and risk factors associated with the sero negative status of HbsAb individuals. Total of 202 samples were studied using enzymes linked immunosorbent assay (ELISA). Antibody index was obtained and result wasanalyzed according to standard procedure. Result shows that 37/202 (18.32%) of the total subjects were positive to HbsAb. High prevalence of 23/101 (22.77%) HBsAb was found among Indian students while only 14/101 (13.86) of Nigerians werefound to be positive. Also,17/69(24.64%) of total females students werepositive while 20/133 (15.03%) of the male students were positive. Studentsaged17-22 years had high prevalence of HbsAb (22.44%) followed by those aged 23-28years(17.39%), 29-34years(11.11%) then 35 years andabove with (0%) prevalence. According to vaccination status 25/68 (36.76%) of vaccinated subjects were positive while only 12/134 (8.95%)were positive for non-vaccinated. 37/194 (19.07%) of unmarried students were positive while among 8 married students none of them waspositive.Additionally, 33/169 (19.52%) of the individuals that live in urban area were HbsAb positive while only 4/33 (12.12%) of those living in rural area were positive. This research clearly shows that Indian students have high prevalence of HbsAb than Nigerian students, due to the fact that majority of students tested positive already vaccinated with hepatitis B vaccine and majority of them were Indians. Total of 18.32% prevalence of HbsAb among the subjects also questioned the effectiveness of hepatitis B vaccination program in both two countries. Therefore there is need for health promotion awareness campaign to educate the general public about importance of HBV vaccination.

Keywords- Antibodies, Hepatitis B, Seroprevalence, Vaccination

INTRODUCTION

Hepatitis B virus (HBV), a DNA virus of the family *Hepadnaviridae* is the causative agent of hepatitis B infection. Hepatitis B is one of the most common infectious diseases in the world and a major health problem. According to the most recent World Health Organization estimate, 2 billion people worldwide have serologic evidence of past or present HBV infection, and 350 million are chronically infected and at risk for HBV-related liver disease (Willey *et al.,* 2011). It is 50 to 100 times more infectious than HIV and 10 times more infectious than hepatitis C virus (HCV) with many carriers not realizing they are infected with the virus. It is an important cause of liver diseases such that chronic infection with HBV is a common cause of death associated with liver failure, cirrhosis and liver cancer (Belo *et al.,* 2010).

The virus has caused severe endemic in parts of Africa and Asia (Opalaye *et al.,* 2014). The prevalence of HBV varies between 2% in developed countries where the prevalence is low to about 8% in developing countries where infection is endemic with sex, age and socio-economic status as important risk factors for infection (Fairley *et al.,* 2012). The degree of HBV endemicity often correlates with predominant mode of transmission. The disease has an enormous impact on health and national economy of many countries and the severity of the disease is highly variable and often unpredictable.

The minimum infectious dose is so low that such practices like sharing of tooth brush or a razor blade can transmit infection (Odusanya *et al.,* 2015).

Hepatitis B virus is a blood borne and sexually transmitted pathogen that is spread through contaminated blood or other body fluids (saliva, sweat, semen,vaginal secretions, breast milk, urine, and feaces). Transmission can occur when using the same syringe, having tattoos or body piercing, mother to child during childbirth, during medical procedures, occupational exposure, during sexual intercourse. Hepatitis B virus also shares similar routes of transmission with HIV but is highly infectious about 100 times than HIV (Chang, 2007). Currently, there are four recognized modes of transmission (Viral Hepatitis Prevention Board, 1996) which are; from mother to child at birth (prenatal), by contact with infected person (horizontal), by sexual contact and by exposure to blood or other infected fluids. Because HBV can remain stable and infectious on environmental surfaces for at least 7 days, transmission may occur indirectly via contaminated surfaces and other objects such as tooth brush, baby bottles, razors, eating utensil, hospitals equipments, by contact with mucous membranes or open skin breaks (Willey *et al*., 2011).

The disease affects people of all age groups, but in most studies conducted recently HBV infection is predominant in young adults and are acquired sexually or through injecting drug used (Shepard *et al.,* 2006*).* Most people who become infected with HBV are able to clear the virus from their blood stream within 6 months of post infection and develop immunity. Those who have not cleared the virus after 6 months are considered to have chronic hepatitis B infection. The risk of death from HBV related liver cancer or cirrhosis is approximately 25% for persons who acquire chronic infection at Childhood. Moreover, 8% to 10% of people in the general population in developing countries become chronically infected and most acquire infection with HBV at childhood (Weinbaum *et al.,* 2008; WHO, 2009).

The clinical manifestations of HBV infection in acute infection are either prodromal, or icteric and recovery (Luka *et al.,* 2008). After the incubation period which varies depending to the type of virus, patients clinically present with chills, headache, nausea, vomiting and may precede jaundice (Roche *et al.,* 2011).The liver becomes tender and enlarged with a right upper quadrant pain. Splenomegaly and adenopathy may also occur in 10% to 20% of cases (Ola *et al*., 2008). The recovery to normality clinically and biochemically is a rule in almost all HBV infections (Belo, 2000; WHO, 2009). However, some do remain chronically infected especially with HBV and may progress to liver cirrhosis and, or to hepatocellular carcinoma (Muhammed, 2010).

HBsAg which is the first antigen to appear, and appears during the incubation period, the prodromal, as well as during acute disease (Weinbaum *et al., 2008*; Uneke *et al.,* 2005). It appears after infection and disappears after one to two months following jaundice. During convalescence it falls to undetectable levels and if it persists for more than 6 months then this indicates a carrier state and a risk for chronic hepatitis and hepatocellular carcinoma (Chang, 2007; Ola *et al* 2008). Presence of HBsAg means that the patient is potentially infectious. Antibodies to HBsAg (Anti-HBs) replace HBsAg as the acute infection resolves, and this indicates immunity in almost 80% of cases after the acute infection (Taura *et al.,* 2008). These Anti-HBs also appears after HBV vaccination. Some lose these antibodies that are acquired after acute HBV infection and may become susceptible to disease HBV (Ndams *et al.,* 2008). During the window period, and when the HBsAg has disappeared and HBsAb has not yet appeared, Hepatitis B core antibody (HBcAb) is detectable and can be used for diagnosis. Acute or chronic infection can be differentiated by the presence of Immunoglobulin M (IgM) to HBcAg in

acute infection, and Immunoglobulin G (IgG) in chronic infection (Taura *et al.,* 2008; Daura, 2009). After the appearance of HBsAg in one or two weeks, the HBcAb starts to be detectable. Very rarely HBcAg will be detected since it is within the HBsAg (Maikudi, 2011).

Nigeria is classified among the group of countries highly endemic for HBV infection. About 75% of the Nigerian population is reportedly likely to have been exposed to HBV at one time or the other in their life. Although hepatitis B vaccination is highly effective in preventing infection with HBV and consequent acute and chronic liver disease, this infection is still a major problem in Nigeria as reported by various workers (Weinbaun *et al*., 2008; Adoga *et al.,* 2010; Luka *et al.,* 2008).

India is in the intermediate zone of endemicity with prevalence of 4.7%, account for a total pool of approximately 36 million carriers contributing to 10-15% of the total infected population worldwide (Thomas *et al.,* 2013). In India 250,000 infant get infected every year and 90% of them, develop chronic infection. There is wide variation in social, economic, and health factors in different regions of India, which may explain the difference in HBV carrier rate, reported by investigators in different parts of the country. Professional blood donors constitute the major high-risk group for HBV infection in India, with hepatitis B surface antigen positivity rate of 14% (Maikudi*et al.,* 2011).

The aim of this study is to determine the Seroprevalence of Hepatitis B surface antibodies (IgG) among Nigerian and Indian students of Jodhpur National University India.

Study site: This study was conducted at Jodhpur National University, Rajasthan India. Jodhpur National University (JNU) is a state private university created under the state government private university act by state government. The University is situated in the suburbs of Jodhpur and is housed in a campus of 30 acres; along Jhanwar road Boranada. The University has more than 13,000 students. It is recognized by UGC under Section 2(f) of UGC Act 1956. The university is offering various courses under the discipline of faculties, include faculty of engineering and technology, faculty of science faculty of medicine and health as well as the dental collage.

Study population: The study population targeted only registered students of Jodhpur national university, includenative Indian students and foreign Nigerian students. Both two sexes' male and female students were enrolled in the study between the ages of 17 to 35 years.

Ethical clearances

An approval for the study was obtained from Jodhpur medical college and hospital research ethics committee. The aim of the study was explained clearly to the clients and Informed consent was obtained before proceeding to the study.

All participants voluntarily signed consent forms intheir own handwriting as evidence of willingness to provide samples for the tests. While responses to structured questionnaire administered in English and Hindi were used to obtain demographic data, history of HBV infection and HBV vaccination.

Interview/Data Collection:

All post graduate and undergraduate students, who consented, were recruited for the study. The socio-demographic characteristics of respondents such as age, sex, marital status, religion, level of education, immunization history, nationality, economic status, etc were obtained using an interviewer administered questionnaire. Each questionnaire have sample identification number to enable tracing the subject with specific blood sample.

Sample collection and processing:

About 3-4 ml of blood sample was collected from each individual enrolled in this study aseptically by venipuncture using a sterile disposable needle and syringe. Blood sample collected was put in to a plain container labeled with unique sample identification number correspond to the number contain on

the respective questionnaire.The collected blood samples transported immediately to the microbiology laboratory (Opalaye*et al.,* 2014).

Blood samples received in the microbiology laboratory, department of microbiology Jodhpur National University India. All samples were allowed to clot and centrifuged for serum separation prior to testing. All sera sample were stored at 4^0c until tested using enzymes linked immunosorbent assay qualitative techniques.

The ELISA kit manufactured and produce by Vialeenrico Fermi 1/9 20090 opera in Milan, Italy. Various performance tests conducted on this product with 100% sensitivity and 99.8% specificity.

Assay procedure

1 hour before use all necessary reagent for this test, as well as the samples were brought to a room temperature (18-26^{0}C) and mixed gently.

100µl of samples, and controls were distributed to the appropriate wells on the microtitre plate according to the scheme.

50µl of conjugate was also added to all wells, except to blanking well A1.

Microplate incubated for 120 minutes at 37^{o}C, sealed with cardboard sealer in dark.

The plate sealer removed and liquid from all wells was drained and washed with washing buffer and blotted on absorbance paper.

100µl of chromogen/substrate 1:1 ratio was also added to each well, blanking well included. Microplate incubated for 20 minutes in dark at a room temperature, sealed with a cardboard sealer.

Enzymatic reaction was stopped by adding 100µl of stop solution to all wells.

Optical density (O.D) was read at 450, 620 and 630nm using ELISA reader within 30 minute after dispensing the stop solution.

Calculation of result for qualitative test

Cut-off value calculated through the following formula:

Cut-off = cut off control mean

Negative control (O.D 450nm)0.049

Positive control (O.D 450 nm)2.853

Cut off control mean (O.D 450nm)0.193

Cut-off = 0.193

Samples with an OD value high than the Co value are consider positive for anti HBs with immunity level protective against HBV infection.

Samples with an OD value lower than Co value must be consider negative or weak positive for anti HBs.

Antibody index was obtained and resultan alyzed according to standard procedure.

RESULTS

Total number of 202 samples tested whereby 37 (18.32%) positive and 165 (81.68%) were negative (Table 1). In relation to nationality of the subjects, equal number of sample (101) tested from each side whereby 14 (13.86%) of the Nigerian students were positive and 23 (22.77%) of Indians tested positive (Table 2). Base on the gender of the subjects, out of 133 male students tested 20 (15.03%) were positive while among 69 females 17 (24.64) tested positive (Table 3). With respect to age, out of 98 students between 17-22 age group 22 (22.44%) were positive. Among 23 -28 age group, 69 students tested 12 (17.39%) were positive. Students with age group 29 - 34 were 27 and only 3 (11.11%) positive. Those above 35 years 8 were tested, non of them was positive (Table 4).According to the vaccination status of the subjects 68 students were already vaccinated out of which 25 (36.76%) tested positive. And 134 not vaccinated, in which 12 (8.95%) of them shows positive (Table 5). Out of 194 single students 37 (19.07%) tested positive while among 8 married students non of them were positive (Table 6). Among 33 students live in rural area 4 (12.12%) tested positive. And out of 169 students of urban area 33 (19.52%) shows positive (Table 7).

Table-1 Shows the total distribution of seroprevalence of HBsAb (IgG) among all subjects.

No. of samples tested	Hepatitis B surface antibody (IgG)	
	Positive	Negative
202	37 (18.32%)	165 (81.68%)

Table 2- Shows distribution of HBsAb in relation to nationality of the subjects.

Nationality	Number tested	HBsAb (IgG) +	% prevalence
Nigerian	101	14	13.86
Indians	101	23	22.77
Total	202	37	

Table 3- Shows distribution of HBsAb in respect to gender of the subjects.

Gender	Number tested	HBsAb (IgG) +	% prevalence
Male	133	20	15.03
Female	69	17	24.64
Total	202	37	

Table 4- Shows distribution of HBsAb among all subjects in respect to age.

Age group	Number tested	HBsAb (IgG) +	% prevalence
17 - 22	98	22	22.44
23 - 28	69	12	17.39
29 - 34	27	3	11.11
35 - above	8	0	0
Total	202	37	

Table 5- Shows distribution of HBsAb in respect to vaccination status of the subject.

Subject type	Number tested	HBsAb (IgG) +	% prevalence
Vaccinated	68	25	36.76
Non vaccinated	134	12	8.95
Total	202	37	

Table 6- Shows distribution of HBsAb among all subjects in respect to marital status.

Marital status	Number tested	HBsAb (IgG) +	% prevalence
Single	194	37	19.07
Married	8	0	0.00
Total	202	37	

Table 7- Shows distribution of HBsAb in respect to area in which the subject live.

Area live	Number tested	HBsAb (IgG) +	% prevalence
Rural	33	4	12.12
Urban	169	33	19.52
Total	202	37	

DISCUSSION

In this study 18.32% of the subjects tested positive for HBsAb indicating that these subjects had protective antibody against HBV infection. Perhaps this could beby the virtue of previous exposure to the virus or as a result of seroconversion following vaccination. The result of thisstudywas in

agreement with the findings of Opaleye which shows that about 17.65% of the individuals in their study were positive to HBsAb (Opaleye *et al.,* 2014).The fact that about 81.68% of the individual tested in this study were HBsAb negative, signifies that these individuals are at risk of contracting HBV infection especially among those in high prevalence area like Nigeria.

The study findings further indicated that14/101 (13.86%) of Nigerian students were positive to HBsAb, While 23/101 (22.77%) were positive amongtheIndian students. This research shows that Indian students had high percentage prevalence of HBsAb than Nigerians, and may be due to the fact that majority of the Indian students that tested positive to HBsAb were vaccinated against HBV. It also indicated that the level of awareness about hepatitis B vaccination program is poor in Nigeria especially the northern part where most of the Nigerian students came from. Information collected from the research questionnaire shows that out of 101 students tested from Nigeria only 19 of them received hepatitis B vaccine. Though WHO adopted hepatitis B vaccination as part of the expanded program on immunization (EPI) in 1991, but it was not incorporated in national immunization program (NIP) until 2003, and still it was not available until around 2008 (Mbaawuaga *et al.,* 2008). It was also noted that hepatitis B infection was not commonly perceive as a problem~~s~~ in Africa. This is because the infection is often sub-clinical and there is long interval before the This was in contrast with the research of Chuwuka *et al*, Which reported that vaccinated and non-vaccinated children tested had HBsAb seroprevalence of 11.78% and 7.8% respectively (Chukwuka *et al*., 2004). However it was surprised that only (36.76%) of vaccinated individual were positive to HBsAb. Therefore this might be due to administration of incomplete dosage of the vaccine to the individuals.The 8.95% positivity rate among the non-vaccinated subjects in this study indicated that those individuals have been infected with hepatitis consequences of the infection ~~to~~ manifest. That was why many people were not having much concern about hepatitis B vaccination in most of African countries including Nigeria (Roche and Samuel, 2011).

Distribution of HBsAb in respect to gender of the individuals tested shows that 20/133 (15.03%) of the male students were positive while 17/69 (24.64%) of the female students were positive. This is ~~in~~ contrary to the findings of SudhaBhat *et al*., where male health workers had the highest seroprevalence of HBsAb of 25% while females have 17% seroprevalence (SudhaBhat *et al*., 2007). Female students have the highest percentage seroprevalence of HBsAb in this study, because majority of those that received hepatitis B vaccine were females. So itmight be as a result of seroconversion due to vaccination.

According to the distribution of HBsAb on the basis of age, result shows that age group 17-22 years had the highest percentage prevalence of 22.44% observed in this age group was in agreement with the findings of Emmanuel *et al*, in which majority of the individuals that tested positive to HBsAb were within the age group of 19-23 years (Emamanual *et al.,* 2006).The high rate of HBsAb among the 17-22 years age group in this study correlate with the fact that majority of those vaccinated belong to this age group.

About 25/68 (36.76%) of vaccinated individuals were sero positive to HBsAb while only 12/134 (8.95%) of non-vaccinated were positive.
B virus in the past, and acquired protective immunity.

Marital status was also among the socio-demographic factor considered in this study, where 37/194 (19.07%) of the unmarried individuals were HBsAb positive while among 8 married individuals tested all were HBsAb negative with (0%) prevalence.

In terms of place of residence where the individuals live, 4/33 (12.12%) of the rural inhabitants tested positive to HBsAb while 33/169 (19.52) of the urban individuals tested positive. This may be because of the

high level of immunization awareness in the urban than rural areas. Majority of the subjects shows positive after been vaccinated, were also from urban areas.

CONCLUSION

The study shows that the prevalence of HBsAb among the studied subjects was 22.42%. This research clearly shows that Indian students had high prevalence of HBsAb than Nigerian students, and this could be due to the fact that majority of the students that tested positive to HBsAb have already been vaccinated with hepatitis B vaccine and most of them were Indians. Therefore there is need for health promotion awareness campaign to educate the general public about importance of taking HBV vaccination. The study recommends that the Government should create HBV clinics in all government owned hospitals and health care centers for the enlightenment of communities about the danger, risk factors associated with hepatitis B infection and importance of hepatitis B vaccination.

REFERENCES

Belo, A. C. (2010): Prevalence of hepatitis B virus markers in Surgeons in Lagos, Nigeria. *East Afr Med J,* (77): 5

Chang, M. (2007). Hepatitis B Virus infection. Seminars in Fetal and Neonatal Medicine, 12:160-167

Chukwuka, J., Ezechuku, C. and Egbunonu I. (2004).Seroprevalence of hepatitis B makers in primary school children in Nigeria. *Journal of Cinical Practice* 7(1):8-10.

Cunninghan F., Leveno K., Sily L. B. (2005). Obstetrics 22 ed: Mcgraw-hill.

Custer, B., Sullivan, S.D., Hazlet, T.K., Iloeje, U., Veenstra, D L. and Kowdley, K.V. (2004): Global epidemiology of hepatitis B virus. *Journal of Clinical Gastroenterology* 38 (103).158–168.

Dane, D.S., Cameron, C.H. and Briggs, M. (2007). Virus-like particles in serum of patients with Australia-antigen-associated hepatitis.*Lancet J*. (7649): 695–698.

Daura, L. A. (2009). Screening for hepatitis B Virus in pregnant women attending main campus clinic, Ahmadu Bello University, Zaria.UnpublishedM.Sc thesis, Department of Microbiology, Faculty of Science, Ahmadu Bello University, Zaria.

Drostein, C., Nippraschk, T., Maregold, C., Meisel, H., Brixner, V., Roth, W. K., Apedjinou, A.and Gunther S. (2004).Prevalence of hepatitis B virus DNA in anti-HBV-Positive/HBS Ag negative sera correlates with HCY but not HIV Serostatus,*Journal of Clinical Virology,* 29:59-68.

Emmanual, M., John, K. and David, M. (2006).Seroprevalence of hepatitis B makers in healthy individuals.*International Journal of Applied Microbiology,* 5(6):56-60.

Luka, S. A., Ibrahim, M. B. and Iliya, S. N., (2008). Seroprevalence of hepatitis B surface antigen among pregnant women attending Ahmadu Bello University Teaching Hospital, Zaria, Nigeria. *Nig J Parasite* 29 (1):38-41.

Maikudi, S. (2011). Prevalence of hepatitis B surface antigen (HbsAg) among pregnant women attending University Health Services (Sick-bay), Ahmadu Bello University, Zaria. Unpublished M.Sc. thesis Department of Microbiology, Faculty of Science, Ahmadu Bello University, Zaria.

Mbaawuaga, E. M., Enenebeaku, M. N. and Okopi, J. A. (2008). Hepatitis B virus infection and control in Nigeria. *African Journal of Biomedical Research.* 7(2):45-47.

Muhammed, I. A. (2010).Seroprevalence of hepatitis B surface antigen in HIV infected patients attending Ahmadu Bello University Teaching Hospital, Shika, Zaria. Unpublished B.Sc. thesis. Department of Microbiology, Faculty Science Ahmadu Bello University, Zaria.

Ndams, I. S., Joshua, I. A., Luka, S.A. and Sadiq, H. O.(2008).Epidemiology of

hepatitis B infection among pregnant women in Minna, Nigeria. *SciWld J,* (3): 3.

Odusanya, O. O., Alufohai, F. E., Maurice, F. P., Wellens, R., Weil J. and Ahonkhai V. I. (2005).Prevalence of hepatitis B surface antigen in vaccinated children and controls in rural Nigeria.*Intl J Infect Dis* 9(3):139-143.

Okwori, E., Egwu, C. and Audu, F. (2009).Seroprevalence of HBsAb among health care workers in tertiary hospitals Benue Nigeria. *International Journal of Hematology*5(2):12-20.

Ola S. O., Olegbayo, J. A., Yakubu, A., Odaibo, G. N.andOlaleye, O. O. (2008).Nigerian butchers and hepatitis B virus infection. *Official Journal of Research Forum for Digestive System, Gastroenterology research and education.* 7 (4).90-96.

Opalaye, O., Salami, S. and Funmulayo, F. (2014).Seroprevalence of HBsAb and HBsAg among pregnant women in southern Nigeria.*Journal of Dental and Medical Science* 4(3):67-71.

Otebayo, J. A.,Taiwo, B. U.,Akingbola, T. S.,Odaibo, G. N.,Adedapo, K. S.,Penugonda, S.,Adewole, I. F.,Olaleye, D. O., Murphy, R. and Karki, P.(2008). Prevalence of hepatitis B and C seropositivity in a Nigerian cohort of HIV-Infected Patients. Ann Hep 7:152-156.

Prevention of hepatitis B in India. (2002). An over view of WHO, New Delhi 38:18-23.

Redd, J.T., Baumbach, J., Kohn, W., Nainan, O., Khristova, M. and Williams, I. (2007). Patient-to-patient transmission of hepatitis B virus associated with oral surgery. *J Infect. Dis.* 195 (9): 1311–4.

Roche, B. and Samuel, D. (2011).The difficulties of managing severe hepatitis B virus reactivation.*Official Journal of the International Association for the Study of the Liver* 31 (1): 104–107.

Shepard, C. W., Simard, E. P., Finelli, L., Fiore, A. E.and Bell, B. P. (2006). Hepatitis B virus infection, Epidemiology and Vaccination. *Journal of Infectious disease* 5(7):78-98.

Sudha, B., Shamee, S. and Karandeep, S. (2007). Seroprevalence of HBsAb among health care workers in Karnataka. *Indian journal of pathology and microbiology* 7(4):477-479.

Taura, D. W., Oyeyi, T. I.and Hafsat, M. N. (2008).Prevalence of hepatitis B surface antigen (HBsAg) among HIV patients attending Aminu Kano Teaching Hospital, Kano, Nigeria.*Intl J Biomed Sci* (4): 1.

Tse, K.Y., Ho, L.F. and Lao, T. (2005). The impact of maternal HBsAg carrier status on pregnancy Outcomes: a case-control study. *Journal Hepatol.* 43(5):771-5.

Weinbaum, C. M., William, I., Mast, E. E., Wang, S. A., Finelli, L., Wesle, A., Neitzel, S. M. and Ward, J.W. (2008). Recommendations for identification and public health management of persons with chronic hepatitis B virus Infection. Morbidity and Mortality Weekly Report 57:1-20.Willey, J. M., Sherwood L. M. and Woolverton C. J. (2011). The History and scope of Microbiology 8th Edition, New York. The McGraw Hill Companies. International Edition. Pp 1-72.

World Health Organization. (2009). Hepatitis B Fact sheet No. 204. Accessed10.Available; http://www.who.int/csr/diseases/hepatitis.