Detection and determination of hepatitis B using molecular and serological methods in patients with hepatitis B in Al-Diwaniya Iraq

Mohsin Abdulkhudhur Al-Zubaidi - Iraq
Masoumeh Bahreini - Assistant Professor Microbiology - University of Ferdowsi - Faculty of Science - Iran
Mohammad Reza Sharif moghadam - Assistant Professor Microbiology and Molecular Biology - University of Ferdowsi - Faculty of Science - Iran
Wissam Saleh Abood Al-Rubaye - Assistant Professor Microbiology (medical viruses) - University of Qadisiyah - Faculty of Medicine – Iraq

Abstract

Hepatitis B virus *(HBV*) is one of the most prevalent pathogens in the world and infection with this virus is a serious threat for public health. The pathogenesis of HBV depends on the critical interplay between viral and host factors .

Test 88 samples for the detection of serological markers using ELISA and PCR technique for positive HBsAg. Regarding population group (HBsAg) positive, the seroprevalence rate of HBsAg, anti-HBs, anti-HBc IgM, HBc Total HBeAg , anti-HBe and HBV DNA were 25%, 5%, 12%, 10%, 9%, 17% and 22% respectively. In this study, hepatitis was diagnosed with the following tests HBsAg, anti-HBs, anti-HBc IgM, anti-HBc total using the ELISA device. HBV DNA was also tested using PCR. To determine the stage of the disease, the HBeAg, anti-HBe and ALT test was added and the stages of the disease were Chronic HBV, Acute HBV, Incubation period, Recovery stage, Window stage, false positive and carrier stage 36.36%, 25%, 17.07%, 15.90%, 2.27%, 2.27 % and 1.13% respectively.

Keywords: Hepatitis B, HBs Ag, Anti HBs, Anti HBe, HBV DNA, Diwaniya – Iraq.

Introdaction

Hepatitis B Viruses, an enveloped DNA virus of *Hepadnaviridae* family, has genome size of 3.2kb (1). Hepatitis B virus belongs to the *Hepadnaviridae* family, genus *Orthohepadnavirus* and has a partially double-stranded circular DNA genome of 3.2 kb, with four overlapped open reading frames (ORF) (2). Hepatitis B virus (HBV) is a global public health problem. It is reported that 2 billion people have exposed and 350 million people chronically infected with HBV. The chronic infection further lead to cirrhosis and hepatocellular carcinoma (HCC), resulting into one million deaths worldwide annually (3).In human, HBV infection can be influenced by some of major factors such as existence of various genotypes, mutant species and immune status of the host (4). The major route of transmission of HBV is perinatally from hepatitis positive chronically infected mothers or via early horizontal transmission from close contact with immediate family members or sexual contact. HBV is survival for 6 months at room temperature and 7 days at 44°C. Genotype of Hepatitis B virus appears to play an important role in host viral interactions influencing clinical symptoms progress (5).The virus uses reverse transcription to copy its DNA genome during HBV replication. However, this *HBV* polymerase lacks proof

reading ability, allowing mutations to occur which leads to a heterogeneous population of *HBV* (6). *HBV* genome seen to play a vital role in different outcome of this infection (7). Mutations in *HBV* surface (S), pre core (PC) and basal core promoter (BCP) genes are observed frequently in HBV infected patients and these mutations are associated with the clinical outcomes of HBV disease (8).Hepatitis B virus has been classified as having 10 genotypes (A-J), and subgenotypes have been reported for four of these, i.e., A-D, F and I (9). The geographical distribution of HBV genotypes is as follows: A (subgenotypes A1-A6) in Asia, Africa, Europe and America; B (subgenotypes B1-B9) in Asia, Oceania and Canada; C (subgenotypes C1-C16), prevalent in Asia and Oceania. Genotype D (subgenotypes D1-D9) has a global distribution(10). Occult HBV infection is defined as the persistence of *HBV* DNA (primarily in the liver tissue or, in some cases, in the serum) in HBsAg-negative individuals (11). HBV infection is generally diagnosed when the circulating HBsAg is detected serologically. However, recent progress in molecular-based technologies, such as PCR-based methods,(12).Furthermore,in *HCV*-infected patients, occult *HBV* infection can worsen the course of *HCV* infection (13).

Materials and Methods

This study included of individuals sample collection

-Samples were collected from patients and healthy patients to Diwaniyah Teaching Hospital in Diwaniyah, Iraq before surgery and before marriage, It also collected from blood donors.

Patients Group

HBsAg was examined for (23123) of patients who be referred to the Diwaniyah Teaching Hospital prior to performing surgical procedures ,pre marriage investigation and blood donors, which is followed as prophylactic program against hepatitis B in Iraq. for the period January 2017 to July 2017 and included ages of 15-82 years. The number of male (15344) and female (7779).

Laboratory tests

A volume of 5 ml of fresh blood was drawn from every theme, collected in a sterile plastic tube, left to clot at room temperature (20-25°C) for clotting. then centrifuged at 2000 rpm for 10 minutes, then serum was collected in sterile and separated in to (2 tube for each sample) and stored at -20°C until use. The rest serum was kept frozen at -20°C for determination of ELISA tests (HBsAg, anti-HBsAb, HBeAg, anti-HBeAb, anti-HBcIgM and anti-HBcAb total) and DNA extraction for PCR technique.

every part of material (reagent and sera) were allowed to stand at room temperature before use. every one other samples were subjected examination by:-

Bioelisa HBsAg kit 96 test, Bioelisa anti- HBc IgM kit 96 test, Bioelisa anti-HBc total kit 96 test, Bioelisa HBe Ag and HBe Ab kit 96 kit and Bioelisa anti-HBs kit 96 test (Foresight USA)

Stripe for liver enzyme (ALT Germany),Kit for detection of HBV DNA (Ab Analitica Italy)and DNA Extraction Kit 100 test(Geneaid USA).

Results

This study attempted to determine detection of HBsAg as a diagnostic test of HBV infection in Al- Diwaniya-Iraq. And determination of different stages the disease . As a risk group selected from many attendants for hospitals and health centers before surgery, before marriage, and blood donors.

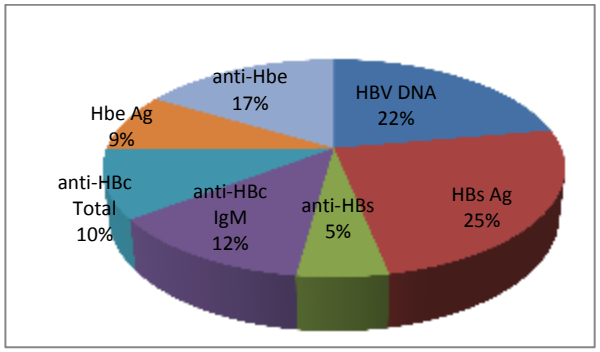
Total test of 23123 serum samples were collected from patients and blood donors.

The number of positive samples to examine HBsAg (88 sample) of the total samples examined, males (45) and females (43) ranged between 15-82 years, (the study sample). Retest 88 sample of positive HBsAg tested for the detection of serological markers using ELISA and PCR technique. Regarding population group HBsAg positive,the seroprevalence rate of HBs Ag, anti-HBs, anti-HBc IgM, anti-HBc Total, HBe Ag, anti-HBe and HBV DNA were 25%, 5%, 12%, 10%, 9%, 17% and 22% respectively from total test positive marker (format 1).The HBs Ag found in 15-82 years ages. Moreover males had higher positive HBs Ag marker than female (male: female ratio1.04-0.96), (format2). Regarding seroprevalence of HBs Ag, a non-significant. The high incidence of hepatitis B was observed for the middle age groups from 15 years to 44 years and the seroprevalence of HBs Ag in age groups 45-54, 55-64, 65-74 and 75-84 years old were decreased 11.36%, 6.81%, 5.68%, and 4.54% respectively (table 1). These results of seroprevalence of HBV markers among population in present study categorized Iraq under low endemicity area 0.38% (table 2). anti-HBs the highest age group 45-55 years and the highest anti-HBc IgM was observed in the age group 35-44 years and lowest found in the age group of 75-84 years. While the anti-HBc total was 6.81% found in two age groups 25-34 and 45-54 years and the lowest ratio was in the age group 65-74 years. The highest rate of anti-HBe in the age group 35-44 years was 13.63% and the lowest rate is 3.40% in the age group 55-64 years . Shows that high positive anti-HBe in the average population of Al-Diwaniyah-Iraq aged between 25 to 54 years. The virus activation HBeAg Positive , the highest rate in two age groups 35-44 and 75-84 years was 10.22% and the lowest was in the age groups 55-64 and 65-74 years .The positive sera HBV DNA for 66(74%) patients from 88 Positive sera HBsAg. The highest HBV DNA percentage in the age group 35-44 years was 18.18% and the lowest in the age groups 65-74 years and 75-84 years were

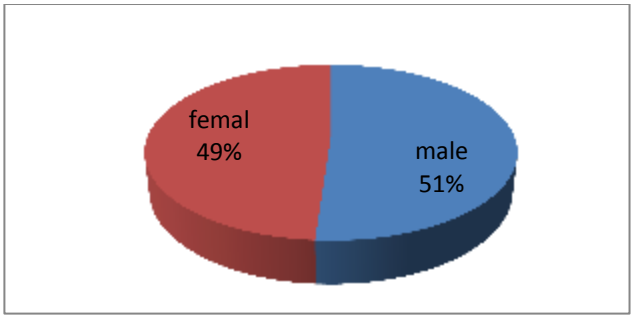
4.54% for both of them (table 1).The married patients 68 (77.27%) showed high significance than single patients 22 (22.73%) in acquiring the infection and the Higher percent 48 (54.54%) of both phases of patients have blood transfusion, which amounted to 39 (44%) (Table 3).

In this study, hepatitis was diagnosed with the following tests (HBs Ag, anti-HBs, anti-HBc IgM, anti-HBc total) using the ELISA device. HBV DNA was also tested using PCR. To determine the stage of the disease, the HBe Ag, anti-HBe and ALT test was added and the stages of the disease were Chronic HBV, Acute HBV, Incubation period, Recovery stage, Window stage, False positive and carrier stage (37%,25%,17%,16%,2%,2% and 1%) respectively, (Format 3)

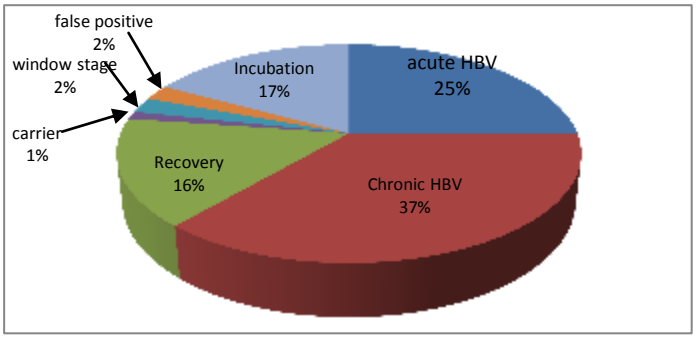
history of surgical operation, and 40 (45.46%) no history of surgical operation. In this study, the proportion of patients with hepatitis B blood transfusion 49 (56%), which is less than the proportion of non-



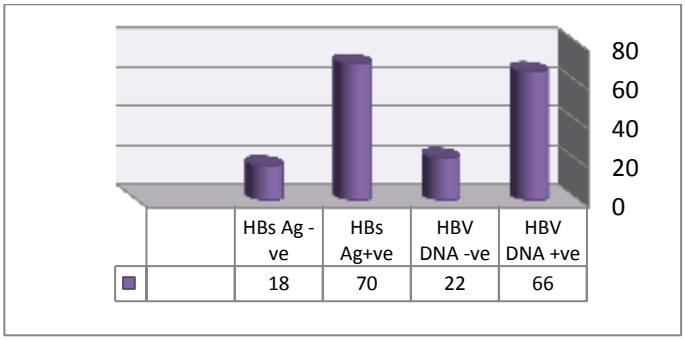
Format(1) Seroprevalence of *HBV* markers and molecular technique result among population in Al-Diwinyia-Iraq



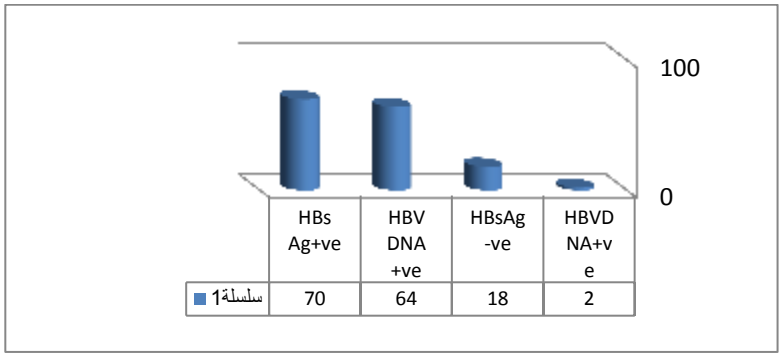
Format(2) Seroprevalence of *HBV* markers among in Al-Diwanyah of Iraq according to the sex.



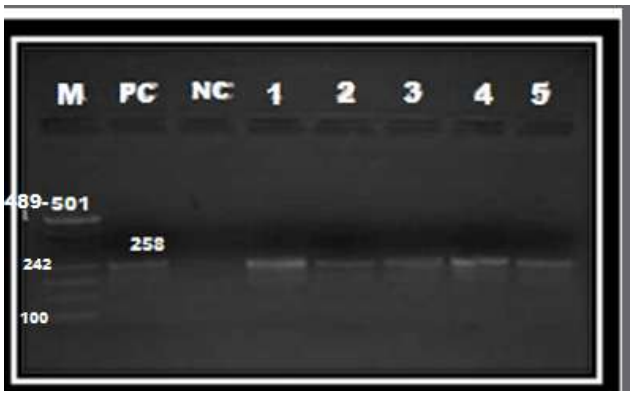
Format(3) detection of *HBV* stage among in Al-Diwanyah of Iraq



Format(4) Seroprevalence of HBsAg and HBV DNA markers among in Al-Diwanyah of Iraq



Format(5)Seroprevalence of HBsAg(-ve) and HBVDNA(+ve) markers among in Al-Diwanyah of Iraq .



Format (5): HBV DNA in patient's serum by PCR. HBV DNA bands obtained by PCR, on 3% agarose gel at 100 v. for 45min. M: Molecular weight of marker (501 bp), PC:Positive Control , NC:Negative Control. 1- 5: DNA bands Positive (258bp).

Table (1): Seroprevalence of HBV marker and HBV DNA mong normal population according to the seven age groups

Age group	HBsAg		Anti-HBs		Anti-HBc IgM		Anti-HBc total		HBeAg		Ani-HBe		HBV DNA	
	No	%	No	%	No	%	No	%	No	%	No	%	No	%
15-24	13	14.77	3	3.40	8	9.09	4	4.54	7	7.95	8	9.09	12	13.63
25-34	18	20.45	2	2.27	7	7.95	6	6.81	5	5.68	11	12.5	13	14.77
35-44	18	20.45	2	2.27	9	10.22	4	4.54	9	10.22	12	13.63	16	18.18
45-54	8	9.09	4	4.54	7	7.95	6	6.81	4	4.54	7	7.95	10	11.36
55-64	5	5.68	0	0	4	4.54	4	4.54	1	1.13	3	3.40	7	7.95
65-74	5	5.68	3	3.40	3	3.40	1	1.13	1	1.13	4	4.54	4	4.54
75-84	3	3.41	2	2.27	1	1.13	2	2.27	9	10.22	4	4.45	4	4.54
total	70	79.54	16	18.18	39	44.31	27	30.68	36	40.90	49	55.68	66	75

Table (2): Seroprevalence of HBsAg marker in normal population

No.of individuals tested	HBsAg Positive	
	No	%
23123	**88**	**0.38**

Table (3)Marker *HBs* Ag Positive among married, surgical and blood transfusion

HBsAg Positive	
Married	68 (77.27%)
No married	20(22.73%)
History of surgical	48 (54.54%)
No history of surgical	40(45.46%)
blood transfusion	56.82%)
non- blood transfusion	43.18%)

Table(4)Primary and secondary tests to diagnose/monitor hepatitis B virus (HBV)infection

Marker	Incubation period	Acute HBV	Recovery of HBV	Chronic HBV	Carrier HBV	Window period	False Positive	Vaccination
HBsAg	±	+	–	+	+	-	-	–
Anti-HBs	–	–	+	–	-	-	-	+
Anti-HBc-Total	–	±	+	+	+	±	+	–
Anti-HBc-IgM	–	+	–	±	-	+	-	–
HBeAg	±	+	–	±	-	-	-	–
Anti-HBe	–	–	±	±	+	±	-	–
HBV-DNA	±	+	±	+	-	+	-	–

Discussion

The meaning of the present study may emerge from the fact that *HBV* infections represent a worldwide epidemic. Iraq was ranked in the intermediate region of endemicity similar to other East Mediterranean countries(14). these results showed important correlation (P > 0.05). As the prevalence of HBsAg among adult age groups may go back to blood, blood products transfusion or other horizontal risk factors such as sexual transmission alike to many developing countries (15). The high occurrence of hepatitis

B was observed for the middle age groups from 15 years to 44 years, while the lowest incidence was the age groups greater than 50 years. The present study consistent with results of post studies that showed the rate of hepatitis B higher in age 25-50 years, and less *hepatitis B* in age better than50 years (16). The highest rate of anti-HBc Igm was observed in the age group (35-44 years) was 10.22% and 1.13% were found in the age group of 75-84 years, this results similar with other results were reported, since,(17).Other researchers were reported (3.4%) in Iraq (18). (5.1%) reported by Zahn, et al in Iran(19). The anti-HBc total was 6.81% found in tow age groups 25-34 and 45-54 years old and the lowest ratio was in the age group 65-74 years. Which if present denote preceding or current infection with the virus(Sadik A.S. *et al*) (20). For HBe Ag, the highest rate in two age groups 35-44 and 75-84 years was 10.22% and the lowest was in the age groups 55-64 years . He stressed , *HBV* reactivation in HBs Ag-positive patients is a common complication, occurring in 21–53% of *HBV* carriers who receive cytotoxic agents or immunosuppressant's, and may yield to acute hepatitis and even hepatic failure (21). The results showed a positive sera HBV DNA for 66 (74%) patients from 88 Positive sera HBs Ag. The highest *HBV* DNA percentage in the age group 35-44 years was 18.18% and the lowest in the age groups 65-74 and 75-84 years were 4.54% for both of them. HBV viral load measuring is a very important instrument for monitor HBV infected patients (22), indicate that the PCR technique is more sensitive and dependable than the ELISA technique. The hepatitis B surface antigen (HBs Ag) is most often used to screen for the presence of this infection. It is the first detectable viral antigen to show during infection. But near the beginning in an infection, this antigen may not be present and it may be untraceable later in the infection as it is being cleared by the host (23). The obtain results confirm the great importance of the PCR system in correctness and reliability of detection and diagnosis of hepatitis viral infection during the cancelation of the antigen ‘‘window period” of hepatitis B infection(24). He sex differences among population could be explained on the foundation that males may have a better chance to come in contact with danger factors of *HBV* than females, or alcohol intake being common in males that may develop the liver damage caused by HBV infection(25). Married patients 68 (77.27%) showed high significance than single patients 20(22.73%) in acquiring the infection. In low prevalence areas sexual transmission is the major rout of transmission. about 40% of new HBV infections in the United States is considered to be transmitted via heterosexual contact, and 25% happen in men who have sex with men(26),(27). Position in many countries of the world, particularly in underdeveloped countries. Most common source of spread of these infections is through the use of unsterilized syringes or instruments especially dental instruments or

unimpeded blood transfusion (28),(29). Agreed the lack of interaction of hepatitis B sero positivity with other risk factors that are better common in males, particularly alcohol consumption and smoking, the multivariable adjusted population-attributable risks for hepatitis B (66.7%) and smoking (25.1%) accounted for the majority cases, and we found small indication of a combined contribution (30). Some surveys of voluntary blood donors have shown marked local variations in the prevalence of (*HBV*)(31). Blood transfusion continues to cause hepatitis B in countries where giver blood is not screened for HBsAg. Transmission is more likely with blood from paid donors than from volunteer blood(32). In the U.S.A and other developed countries, transfusion acquired hepatitis B is rare because of the testing and removal of HBV contaminated donor blood(33).Atotal of 88 serum samples from apparently healthy population , who were Positive to HBsAg .marker underwent for additional investigations of HBs)As shown in (format 4). 70 (79.54% Ag positive,18 (20.46 %) of HBs Ag negative, it was detected 16 (18.18 %) with anti-HBs positivity, this result displayed a previous HBV infection and immunity to hepatitis B, whereas 66 (75%) were positive to HBV DNA and 22(25%) ware negative, those possibilities contain HBV, infection in remote past; "low-level" HBV carrier; "window" between vanishing of HBsAg and appearance of anti-HBs(34).Also detection of HBV DNA among 2 (12%). HBs Ag negative was investigated sample frome 15 sample of HBsAg negative of this group was positive for HBV DNA (format 5), which signified for attendance of the virus, despite the non attendance of HBs Ag and still capable of transmitting infection. This result is in accord with that was reported by Lebanese researchers

in 2007, whom found that 5.4 % of healthy blood donors with core antibody have detectable viral load(35). In this study, hepatitis was diagnosed with the following tests HBs Ag, anti-HBs, anti-HBc IgM, anti-HBc total using the ELISA test. HBV DNA was also tested using PCR. To determine the period of the disease, the HBe Ag, anti-HBe and ALT test was added and the stages of the disease were determined in comparison with Table(4). Acute HBV infection generally presents after an incubation period of six weeks to several months with an onset of nonspecific symptoms that may include fever, malaise, anorexia and nausea, followed by the onset of jaundice, dark urine and pale stools. Approximately 25% to 40% of infected adults will be symptomatic, and most will show elevations in ALT; however, infants, toddler and immune suppressed persons may not manifest signs (eg, jaundice) or symptoms of infection(36). If HBs Ab positive, the patient is careful immune to HBV (either because of resolved infection or as the result of past vaccination). Very rarely (less than 1%) can chronic carriers be positive for HBsAg and antibody to hepatitis B surface protein (anti-

HBs) at the similar time . In such cases, the patient is considered infectious(37). Incubation period of the hepatitis B virus is 6-24 weeks. The virus can be detected within 1-2 months following infection(38). Representative the start of recovery. Antibody to HBsAg arises late during infection, typically during recovery or period of recovery after clearance of HBs Ag. Anti-HBs persists after recovery, being the antibody linked with immunity against HBV. However, between 10% and 15% of patients who recover from hepatitis B do not develop detectable anti-HBs and have anti-HBc only as a marker of previous infection. For this cause, anti-HBc testing is the most reliable means of assessing previous infection with HBV, whereas anti-HBs testing is used to review immunity and response to HBV vaccine(39). With time, however, the disease activity can resolve either with persistence of high levels of HBeAg and HBV DNA (the "immune tolerance stage") or with loss of HBe Ag and fall of HBV DNA to little or undetectable level ("inactive carrier state"). Other patients continue to have chronic hepatitis B, although some mislay HBe Ag and develop anti-HBe (HBeAg-negative chronic hepatitis B)(40). Window period – length of time between infection and laboratory finding of infection(41). Persons positive only for anti-HBc are likely not infectious ,bar under unusual circumstances of large direct percutaneous exposure of susceptible recipients (e.g., blood transfusion, organ transplant)(42).

Referance:

1. Locarnini, Stephen, and Fabien Zoulim. "Molecular genetics of *HBV* infection." *Antivir Ther* 15.Suppl 3 (2010): 3-14.
2. Heise, Tilman, et al. "The *hepatitis B virus* PRE contains a splicing regulatory element." *Nucleic acids research* 34.1 (2006): 353-363.
3. Oakes, Kathryn. "Hepatitis B: prevalence and pathophysiology." *Nursing times* 110.7 (2014): 12-16.
4. Pan, Calvin Q., and Jin X. Zhang. "Natural history and clinical consequences of hepatitis B virus infection." International journal of medical sciences 2.1 (2005): 36-40.
5. Kao, Jia-Horng. "Molecular epidemiology of hepatitis B virus." The Korean journal of internal medicine 26.3 (2011): 255.
6. Horvat, Rebecca T. "Diagnostic and clinical relevance of *HBV* mutations." *Laboratory Medicine* 42.8 (2011): 488-496.
7. Baumert, Thomas F., Robert Thimme, and Fritz von Weizsäcker. "Pathogenesis of hepatitis B virus infection." World journal of gastroenterology*: WJG* 13.1 (2007): 82.
8. Liang, Tao, En-Qiang Chen, and Hong Tang. "Hepatitis B virus gene mutations and hepatocarcinogenesis." Asian Pacific journal of cancer prevention 14.8 (2013): 4509-4513.
9. Lin, Chih-Lin, and Jia-Horng Kao. "Hepatitis B virus genotypes and variants." Cold Spring Harbor perspectives in medicine 5.5 (2015): a021436.
10. Mora, Monica Viviana Alvarado, et al. "Molecular epidemiology and genetic diversity of hepatitis B virus genotype E in an isolated Afro-Colombian community." *Journal of General Virology*91.2 (2010): 501-508.
11. Raimondo, Giovanni, et al. "Statements from the Taormina expert meeting on occult hepatitis B virus infection." Journal of hepatology 49.4 (2008): 652-657.
12. Koike, Katsuro, et al. "Hepatitis B virus DNA is frequently found in liver biopsy samples from hepatitis C virus-infected chronic hepatitis patients." *Journal of medical virology* 54.4 (1998): 249-255.
13. Chemin, Isabelle, et al. "High incidence of hepatitis B infections among chronic hepatitis cases of unknown aetiology." *Journal of Hepatology* 34.3 (2001): 447-454.
14. Ott J, Stevens G, Groeger J, Wiersma S. Global epidemiology of hepatitis B virus infection: new estimates

of age-specific HBsAg seroprevalence and endemicity. Vaccine. 2012;30(12):2212-9.
15. Chowdhury A. Epidemiology of hepatitis B virus infection in India. Hepatitis B Annual. 2004;1(1):17.
16. Fung J, Cheung C, Chan SC, Yuen MF, Chok KS, Sharr W, et al. Entecavir monotherapy is effective in suppressing hepatitis B virus after liver transplantation. Gastroenterology. 2011;141(4):1212-9.
17. Olotu AA, Oyelese AO, Salawu L, Audu RA, Okwuraiwe AP, Aboderin AO. Occult Hepatitis B virus infection in previously screened, blood donors in Ile-Ife, Nigeria: implications for blood transfusion and stem cell transplantation. Virology journal. 2016;13(1):76.
18. Ibtihal HM. Detection of Occult hepatitis B virus infection by polymerase chain reaction in Diyala province 2012 [M.Sc. thesis. Collage of Education for Pure Science
19. Zahn A, Li C, Danso K, Candotti D, Owusu-Ofori S, Temple J, et al. Molecular characterization of occult hepatitis B virus in genotype E-infected subjects. Journal of General Virology. 2008;89(2):409-18.
20. Sadik A, Alzailayee A, Foda M. Serological and molecular detection of hepatitis B viral antibodies and its surface antigen in blood samples of different age stages at Taif Governorate, KSA. Int J Curr Microbiol App Sci. 2014;3(3):758-73.
21. Lan J-L, Chen Y-M, Hsieh T-Y, Chen Y-H, Hsieh C-W, Chen D-Y, et al. Kinetics of viral loads and risk of hepatitis B virus reactivation in hepatitis B core antibody-positive rheumatoid arthritis patients undergoing anti-tumour necrosis factor alpha therapy. Annals of the rheumatic diseases. 2011;70(10):1719-25.
22. Sitnik R, Paes Â, Mangueira CP, Pinho JRR. A real-time quantitative assay for hepatitis B DNA virus (HBV) developed to detect all HBV genotypes. Revista do Instituto de Medicina Tropical de Sao Paulo. 2010;52(3):119-24.
23. Ganem D, Prince AM. Hepatitis B virus infection—natural history and clinical consequences. New England Journal of Medicine. 2004;350(11):1118-29.
24. Kurdi M, Abughararah M, Mulike M, Yamani O, Bugdady M, Noor M. Molecular detection of hepatitis B virus (HBV) among voluntary ELISA positive blood donors in Almadinah Almunawwarah. Journal of Taibah University Medical Sciences. 2014;9(2):166-70.
25. Villa E, Barchi T, Grisendi A, Bellentani S, Rubbiani L, Ferretti I, et al. Susceptibility of chronic symptomless HBsAg carriers to ethanol-induced hepatic damage. The Lancet. 1982;320(8310):1243-4.
26. Lavanchy D. Hepatitis B virus epidemiology, disease burden, treatment, and current and emerging prevention and control measures. Journal of viral hepatitis. 2004;11(2):97-107.
27. Bertoletti A, Gehring AJ. The immune response during hepatitis B virus infection. Journal of General Virology. 2006;87(6):1439-49.
28. Nangrejo KM, Qureshi MA, Sahto AA, Siddiqui SJ. Prevalence of Hepatitis B and C in the patients undergoing cataract surgery at eye camps. Pak J Ophthalmol. 2011;27(1):27-9.
29. Khan H, Hayat Z, Rehman R, Zarif M. Comparative analysis of risk factors and complications of hepatitis B and C infections at Khyber Teaching Hospital, Peshawar. 2007.
30. Jang MK, Lee JY, Lee JH, Kim YB, Kim HY, Lee MS, et al. Seroepidemiology of HBV infection in South Korea, 1995 through 1999. The Korean journal of internal medicine. 2001;16(3):153.
31. Arya SC, Ashraf SJ, Parande CM, El-Sayed M, Sahay R, Ageel AR, et al. Hepatitis B virus in Gizan, Saudi Arabia. Journal of medical virology. 1985;17(3):267-74.
32. Afrin N. Survey on Hepatitis B Knowledge and Awareness among the university students of Bangladesh: East West University; 2017.
33. Park KW, Chandhok D. Transfusion-associated complications. International anesthesiology clinics. 2004;42(3):11-26.
34. Jawetz M, and Adelberg Hepatitis viruses. . In: ed t, editor. Medical Microbiology 2007. p. 466-85.
35. El-Zaatari M, Kazma H, Naboulsi-Majzoub M, Haidar M, Ramlawi F, Mahfoud Z, et al. Hepatitis B virus DNA in serum of 'anti-HBc only'-positive healthy Lebanese blood donors: significance and possible implications. Journal of Hospital Infection. 2007;66(3):278-82.
36. Will H, Reiser W, Weimer T, Pfaff E, Büscher M, Sprengel R, et al. Replication strategy of human hepatitis B virus. Journal of Virology. 1987;61(3):904-11.
37. Dufour DR, Lott JA, Nolte FS, Gretch DR, Koff RS, Seeff LB. Diagnosis and monitoring of hepatic injury. I. Performance characteristics of laboratory tests. Clinical chemistry. 2000;46(12):2027-49.
38. Organization WH. Guidelines for the Prevention Care and Treatment of Persons with Chronic Hepatitis B Infection: Mar-15: World Health Organization; 2015.
39. Sato A, Kato Y, Nakata K, Nakao K, Daikoku M, Ishii N, et al. Relationship between sustained elevation of serum alanine aminotransferase and progression from cirrhosis to hepatocellular carcinoma: comparison in patients with hepatitis B virus-and hepatitis C virus-associated cirrhosis. Journal of gastroenterology and hepatology. 1996;11(10):944-8.
40. Perrillo R, Schiff E, Yoshida E, Statler A, Hirsch K, Wright T, et al. Adefovir dipivoxil for the treatment of lamivudine-resistant hepatitis B mutants. Hepatology. 2000;32(1):129-34.
41. Heckman N. Immunoglobulin Therapy and Primary Immunodeficient Patients' Health-Related Quality of Life and Well-Being. 2018.
42. Brownson RC, Colditz GA, Proctor EK. Dissemination and implementation research in health: translating science to practice: Oxford University Press; 2017.