WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 5.045
Volume 4, Issue 4, 1599-1610. Research Article ISSN 2277– 7105

HEPATITIS B VIRUS SUBCLINICAL CASE INFECTION IN BASRA PATIENTS DISCOVERED BY PRE-OPERATION OF VIRAL SCREENING INVESTIGATIONS

Awatif H. Issa (Prof.Dr.)*[1], Noor Abd. Hamdan (MSc)[1] and Hazim T. Thwiny (Ph.D)[2]

[1]College of Science -Department of Biology University of Basrah, Iraq
[2]College of Veterinary Medicine-Microbiology faculty University of Basrah, Iraq.

Article Received on
03 Feb 2015,

Revised on 28 Feb 2015,
Accepted on 25 March 2015

***Correspondence for Author**
Prof. Dr. Awatif H. Issa
College of Science-Department of Biology University of Basrah, Iraq.

ABSTRACT

Hepatitis B is an inflammation of liver that is caused by hepatitis B virus (HBV), HBV infections can be asymptomatic, or can present as acute or chronic hepatitis that can then lead to primary heptacellular carcinoma or liver cirrhosis. This disease considered as the major disease of humaneness and a serious global public health issue. HBsAg, HBsAb, HBeAg, HBeAb and HBcAb are biomarkers used to detect the presence and the stage of the infection. The Iraqi ministry of health applied pre-operation viral screening investigation, according to statistics in the largest hospitals in Basra province: Al faihaa general hospital, Alsadir teaching hospital, Ibin Ghazwan hospital, Basra general hospital, Public health laboratory. For the period from 2012 till 2014. Two thousand twenty eight infections with HBV were 617, 697 and 714 patients, respectively. The current study including 169 cooperated cases from them (91) males and (78) females. By ratio (1.14 : 1). The mean age of patients for males (39.9) and for females (39.7) range from 5-75 years as follows < 20 (11.83%), 21-30 (18.93%), 31-40 (25.44%), 41-50 (15.38%), 51-60 (15.38%) and > 61 (13.01%). The clinical state, viral load and their distribution according their different residential area in Basra province were performed. 169 patients were divided into 3 studied groups including: Actually infected, active acute and chronic infections with rates (87.83%) (10.81%) (8.11%), respectively. Viral load have been classified according to the level of viral load with the maximum number of cases have got a viral load below 50,000 IU/ml (about 37.8% of the cases). Where the infections distributed by districts and center city, founded that the highest prevalence in Al-Zubair district (13.01%) followed by Abu Al-

kaseeb (10.65%) and Shatt al-Arab district (5.91%). As for the city center, the infection was scattered in all the province, but the highest prevalence was in Al-Basra Alkadema (24.26%), although there are difference in the infection rates, but the statical analysis showed that there is no great significant difference. We concluded that HBV infection remains a dangerous health problem because it's prevalence is still significant among patients in all age groups and in all residential area in Basra province, all viral markers is very important for the disease diagnosis, rt-PCR a precise tool for diagnosis of HBV infection in Iraq cause it is a very sensitive scientific technique that gave the exactly number of copies/ml in a closed system.

KEYWORDS: diagnosis, Hepatitis B virus (HBV), Hepatitis, real time PCR .hepatitis B surface antigen, Basra.

INTRODUCTION

Hepatitis B virus (HBV) infection become a significant public health problem in a recent years, according to the world health organization (2013) two billion people worldwide have been infected with HBV at some point in their life and approximately (350) million individuals suffer from chronic HBV infection, over (600,000) deaths each year, mainly from cirrhosis or liver cancer (Horvat, 2011). Without intervention (15%) to (40%) of chronic HBV infected individuals will develop cirrhosis, heptacellular carcinoma (HCC) or in other cases may be need to liver transplantation (Sheppard *et al*., 2006; Sorrel *et al*., 2009). Blumberg and colleagues, 1965 was first identified HBV infection when they found hepatitis B infection surface Antigen (HBsAg). HBV is a member of the orthohepadnavirus genus in the hepadnaviridae family, it is highly species specific (Schaefer *et al*.,1998). All member of this family are small, hepatotropic DNA viruses which display a similar virion morphology and genome organization, and replicate via reverse transcription of an RNA intermediate. HBV is a partially double – stand circular DNA virus that infects the human and some animals such as chimpanzees and birds (Salim and Abdullah, 2014). According to CDC (2013) seventy percent of adult will develop symptoms from the infection, and these symptoms characterized by fever, fatigue, loss of appetite, nausea, vomiting, abdominal pain, dark urine, joint pain and jaundice. In highly endemic area, HBV is usually transmit by many ways such as blood, semen, vaginal fluid and other body fluids, there are two ways of transmission, horizontally such as a sexual contact with an infected person and vertical transmission from the mother to her offspring (Lin and Kirchner, 2011). An accurate investigate of the infection is essential for disease diagnosis management and prevention in

the community. HBV vaccination become available in the mid of 1980s, infant vaccination was introduced in 1992(Liang *et al*., 2009). The introduction of HBV vaccine play role in reduce the rate of the new infections by effectively preventing the transmission of the virus, but despite the availability of an prophylactic of HBV vaccine , the completely control of hepatitis B virus infection still very difficult issue work thus ,a further extensive efforts are require to monitor the emergence of vaccination and therapy resistance HBV variants and to prevent their spread in the general population (Baumert *et al*., 2011).

The genes of HBV comprise genetics codes that great numerous protein product included hepatitis (B) surface antigen (HBs Ag), hepatitis B core antigen (HBc Ag), hepatitis B envelope antigen (HBeAg) and DNA polymerase (Valente *et al*., 2010), these four proteins are of pivotal significance as they are measured in the blood tests and help in the diagnosis of the virus (Uttreshwar *et al*., 2008). Hepatitis B patients also contain circulating antibodies against Hepatitis B s antigen ,Hepatitis B e antigen and Hepatitis B c antigen called Hepatitis B s antibody (HBsAb), Hepatitis B e antibody (HBeAb) and Hepatitis B c antibody (HBcAb) hepatitis B core antigen doesn't found in blood stream it's found only in hepatocyte (Salim and Abdullah, 2014). The hepatitis B evolution leads to occurrence of various genotype sub genotype, mutants, recombination and even quasispecies (Hunt *et al*.,2007). Genotype have been identified by linked to clinical outcomes, drug responses, and mutation (Huang *et al*., 2006).

HBV consists of eight members (genotypes A-H) which differ genetically by greater than 8 % (Khouri *et al.*, 2008), this diversity is driven by viral replication and the survival of various that exhibit an advantage These variant in HBV have been able to evade specific human immunity due to natural selection of mutants during viral replication (Horvat, 2011). Outcome of acute hepatitis B virus infection range from symptomatic acute hepatitis (30%) and asymptomatic subclinical infection (70%) to fulminant hepatic failure (0.1-0.5%) (Hammadi *et al*., 2012). Acute Hepatitis B virus infection is occurs within the first six months after someone is exposed to the Hepatitis B virus and it does not always lead to chronic HBV infection ,a proportion of patients infected with hepatitis B virus (5-10% among adults) develop to chronicity, defined as persistence of infection for more than six months (Aggarwal,2004). During HBeAg seroconversion HBV mutations gradually occur with the progression of chronic infection, a significant effects of viral mutations on hepatocellular

carcinoma and cirrhosis were selectively obvious in those with HLA-DP polymorphisms promoting HBV persistence (Zhang *et al*., 2013).

MATERIAL AND METHODS

One hundred sixty nine patients who have been identified to have positive HBsAg serological test were been selected to be the raw material of this study. All subjects have been stratified against age, sex, geographical distribution and viral load. The subjects have been obtained from the largest hospitals in Basra province: Al faihaa general hospital, Alsadir teaching hospital, Ibin Ghazwan hospital, Basra general hospital, Public health laboratory. For the period from 2012 till 2014.

DNA Extraction

The DNA-sorb-B extraction Kit (Sacace, England) was used for DNA extraction from serum samples according to the manual. The extracted DNA was used for amplification in the LiPA procedures. LiPA analysis was performed within approximately 5 days following DNA extraction. If DNA extracts were not used immediately, they were stored at -20°C.

Virologic Testing

Genotypic testing was performed in only those with a detectable HBV DNA (qualitative) in serum. HBV genotyping was determined from serum samples by performing nested PCR-mediated amplification of the target sequence and hybridization with sequence-specific oligonucleotides.

Preparation of standard HBV DNA

HBV DNA X region (1414-1744 nt) was amplified (forward primer, 5'-ACGTCCTTTGTYTACGTCCCGT-3', nucleotides 1414 to 1435; reverse primer, 5'-CCCAACTCCTCCCAGTCYTT AA-3', nucleotides 1744 to 1723) and cloned into pMD18-T vector. After quantification, recombinant HBV plasmid was serially diluted from 1011 copies per mL to 102 copies per mL. Ten microliters of each diluted recombinant HBV plasmid was used as HBV standard PCR template.

Real-time PCR reaction parameters

Amplification reaction mixture(50 µL) contained 10 µL of DNA, 5 µL of 10 × PCR buffer, 200 µmol/L dATP, 200 µmol/L dCTP, 200 µmol/L dGTP, 500 µmol/L dUTP, 1.5 mmol/L MgCl2, 400 nmol/L forward primer, 400 nmol/L reverse primer, 400 nmol/L TaqMan-MGB

probe, 2 U of hot start DNA polymerase (Takara), and 0.5U of uracil DNA glycosylase (UNG). Thermal cycling conditions were as follows: initial activation of UNG at 50 for 2 min followed by inactivation of UNG at 95 for10 min. Subsequently, 60 cycles of amplification were performed at 95 for 20 s, at 55 for 30 s and at 72 for 30 s.

RESULTS AND DISCUSSION

According to statistics in the largest Basra hospitals: Al faihaa general hospital, Alsadir teaching hospital, Ibin Ghazwan hospital, Basra general hospital, Puplic health laboratory there are Two thousand twenty eight infections with hepatitis B virus in Basra province For the period from 2012 till 2014. As shown in (table 1).

Table 1: Annual statistics in the largest Basra hospitals from (2012-2014).

Years	total No.	infections	%
2012	34,976	617	1.76%
2013	37,931	697	1.83%
2014	38,382	714	2.16%

The results indicated that the rates of infections were 1.76%, 1.83% and 2.16% infections, respectively. Recently, HBV infection has truly become a problem which we still face. In Basra city, the number of patients has become noticeably medium, the infections with hepatitis B virus increased from one year to another. The Iraqi ministry of health applied pre-operation viral screening investigation to detected virus before doing operations, worth noting is that all cases were detected accidently either during pre-operation test, blood donation, marriage investigation or during pregnancy test, that noticeably at the largest Basra hospitals, note that disclosure of the preparation of this infections is not real cause when it was investigate families of these patients, founded that many individual were infected, during investigating the family of one patient we noticed that eight members within his family were infected with HBV and are not registered within this statistical.

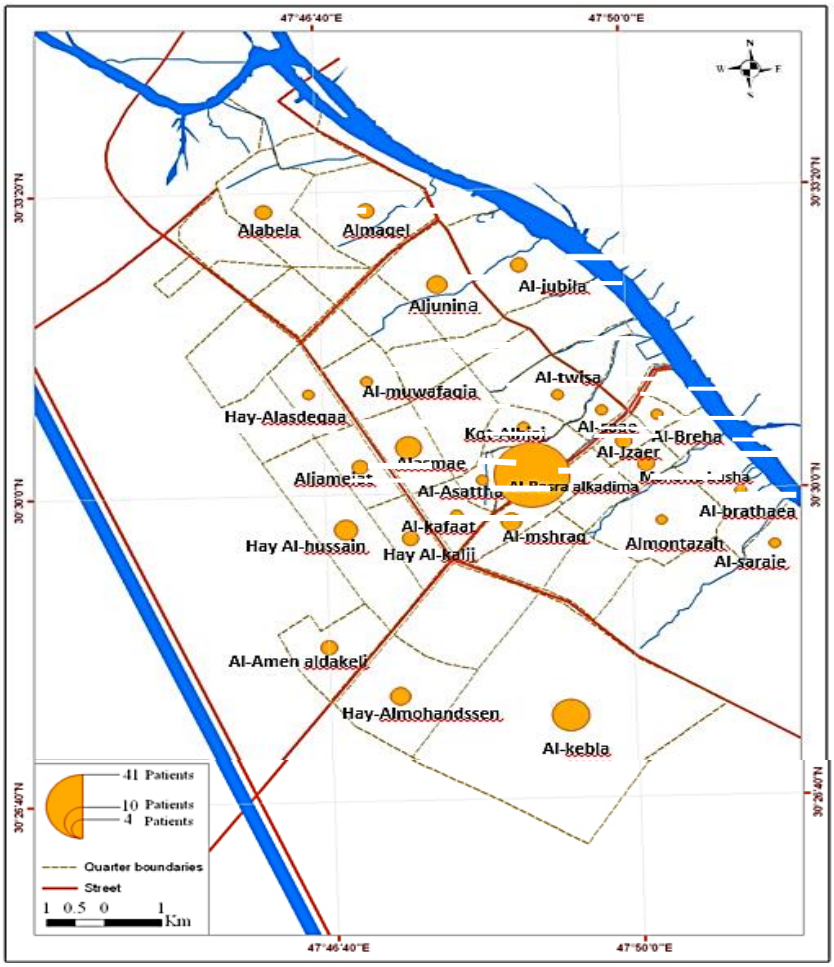
Table 2: Distribution of patients according to age and gender.

Age (years)	Male	female	total NO.	Percent
>20	12	8	20	11.83%
21-30	15	17	32	18.93%
31-40	26	17	43	25.44%
41-50	13	13	26	15.38%
51-60	14	12	26	15.38%
> 61	11	11	22	13.01%
Total	91	78	169	100.00%

Risk factor for HBV infections may vary considerably depending on age, gender, epidemiological data, culture characteristics of different population (Fang *et al* .,2009). The current study included 169 cooperated patients from a different residential area for the both sex and in all age groups, that male were 91 patients (53.25%) while female 78 (46.15%). In table (2) which shows the relation between age and sex which showed that the highest attendance of the hospital within the male groups were in those of less than 20 years of age and represent 20 patient (11.83%) out of 91 patient (53.25%) while those in age higher than 61 years of age which represent only 11 patient (13.01%).In female group it is seen that female within the age group less than 20 years of age were the lowest among the group which 8 patient (10.12%) out of 78 patients. the highest attendance were 17 patients (25.44%) at the age 31-40 years of age out of 78 patients.

HBV virus spreads through contacts with body fluids of an infected person and sexual contacts, carrier of hepatitis B can spread the virus among different contacts (Poukarium *et al*., 2010). Vertical transmission of HBV from mother to her offspring always result in chronic HBV infection (Allain *et al*., 2004). An infection during puberty result in livelong protective immunity.

The present study documents high prevalence of HBV infection in all residential area in Basra city as show in Figure (1) and (2), this epidemiological data was make Basra in a risk region for HBV infection, the infection distributed according to city center and districts in Basra province, founded that the highest prevalence in Al-Zubair district (13.01%) followed by Abu Al-kaseeb (10.65%) and Shatt al-Arab district (5.91%). As for the city center, the infection was scattered in all the province, but the highest prevalence was in Al-Basra Alkadema (24.26%), although there are difference in the infection rates, but the statical analysis showed that there is no great significant difference.



Figure(1) distribution of HBV patients in Basra province according to city center.

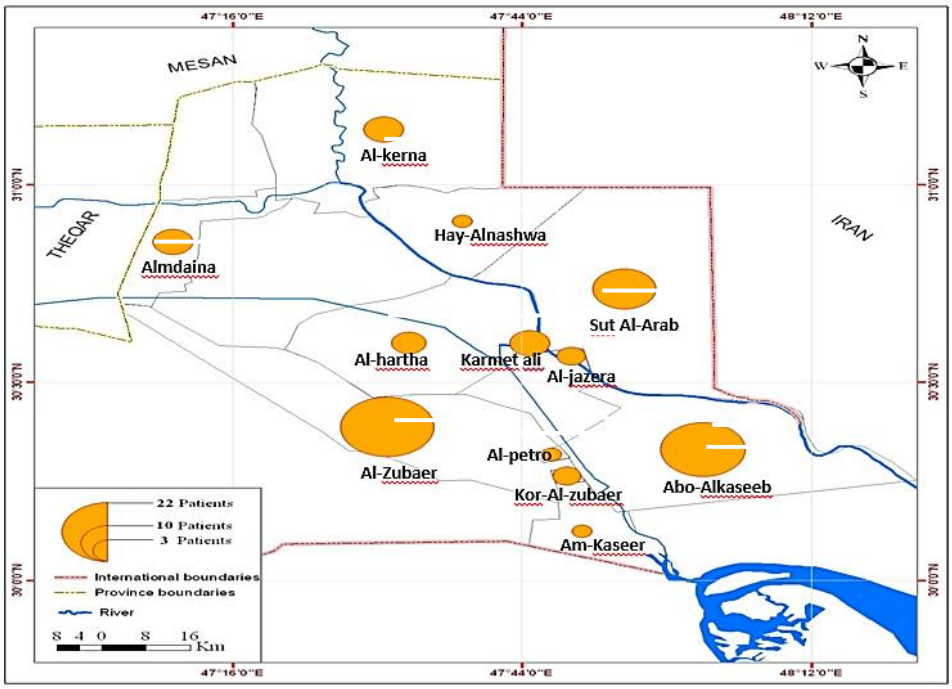


Figure (2): distribution of HBV patients in Basra province according to districts

74 subclinical case of hepatitis B virus infection were followed-up divided into three groups according there serological markers and there viral copies/ml. As show in table(3).

Table (3): Distribution of HBV markers with gender, subclinical cases and viral load.

Subclinical cases	%	Gender		Markers					mean of copies/ml
		Male	Female	HBs Ag	HBs Ab	HBe Ag	HBe Ab	Anti-THBc	
Actually infected	87.83	29	27	+	-	-	+	+	181672.6
Chronic	8.11	4	2	+	-	-	-	+	137012.5
Active acute	10.81	5	4	-	-	+	-	+	155101.2
				+	-	+	-	+	

*Actually infected carrier (87.83%):positive of HBsAg ,HBeAg and Total anti-HBc.

**Chronic (8.11%):positive of HBsAg and Total HBc.

***Ative acute (10.81%):positive HBeAg ,Total anti-HBc and with or without HBsAg.

A key serological marker for detection and diagnosis of hepatitis B virus infection is Hepatitis B surface antigen (HBsAg) , is an important viral envelope protein which appear shortly after infection , it is detectable within 3 to 4 weeks of the infection and may be up to 5 months in an acute infection (Perrillo *et al*.,2009).

The results indicated that the majority of patients were actually infected group (87.83%) followed by active acute (10.81%) and chronic infection (8.11%). An actually infected forms the largest group in chronic HBV infected, approximately 300 million people are actually infected (Lin *et al*.,2004). Positive HBsAg, HBeAg and total anti-HBc is an indicator for actually infected .HBeAg is produce during active viral replication and may be act as immunogen or a tolerogen, leading to appearance of infection (Hammadi *et al*.,2012). Anti HBc are evident in an infection but not with vaccinated patients and can therefore use as a differential tool for the presence of anti-HBs is due to an infection or vaccination.

During the used of the wide range of hepatitis B virus biomarkers as shown in table(3), Actually all viral markers were essential in the diagnosis of the disease and in giving an obvious indicated of the cases tested. In approximately 50% of patients with the self limited HBV infection range of up to several months between the disappearance and the detectable of anti-HBs, during this time only the total anti-HBc is detectable; this period is referred to as

window phase. So using HBsAg marker only in diagnosis of infection in the medical situation is not enough to detecting all HBV infections (AlHmudi, 2012).

The annual rate of HBsAg clearance has been estimated to be less than 2% in western patients and even lower (0.1-0.8%) in Asia patients (Chwal, 2005) .The present study showed unusual markers of patients with rate (4.05%) in which antigen and their antibody presence in the same patient in the same time. Furthermore, unusual markers were markers that existed in patients in a way that is not interpretable or identified, such as in usual markers in which the cases can be readily classified as acute or chronic. Repeating testing of the same sample or possibly of an additional sample is advisable when tests yield discordant or unusual results (Hollinger & Liang, 2001). These markers were present in the immunocompromised patients.

Viral load determinations assist with diagnosis, treatment decisions, and response to antiviral therapy. HBV DNA is possibly detected 21 days earlier, where as HBsAg becomes detectable 6-10 weeks after exposure to the virus (Servoss and Friedman, 2004). The serum level of HBV DNA largely depended on viral genotype, and the quantity of HBeAg in serum which determines the progression of liver cirrhosis to carcinoma. HBV viral load determination can be advantageous in distinguish between active and inactive infection, in assess response to therapy, detect the development of antiviral resistance, mediate atypical serology profiles and to measure infectivity, as with vertical transmission (Gish and Locarnini, 2006). Although the world health organization has established an international compare serial results on the same patient using only the same assay. Viral load has been be obtained for all cooperate participants and their results have been classified according to the level of viral load as shown in table (4) with the maximum number of cases have got a viral load below 50,000 IU/ml (about 37.8% of the cases).

Table 4: Distribution of cases according to their viral load

NO.of patients (%)	category of IU/ml
37 (22.6%)	≥ 10000
62(37.8%)	≥ 50000
45(27.4%)	≥ 100000
10(6.1%)	≥ 150000
10(6.1%)	< 150000

Currently, more than 10 different methods have been developed for HBV genotyping with variable specificity, sensitivity and turnaround time. Structural and functional difference

between genotype linked to severity,clinical outcomes, and response to treatment of HBV infection and possibly against the virus (Chu and Lok, 2002). According to intergroup divergence of 8% or more in the complete nucleotide sequence, HBV can classified into 8 genotype (Mehmet *et al*., 2007). This virological diversity of HBV has been associated with difference in clinical and virological characteristics, indicated that they play a role in the virus host relationship (Araujo, 2011). In the resent study have been detected genotype D for 24 patients and has been founded two infected patients with mixed genotype D and E. Genotype may be result from natural evolutionary drift of the virus genome, from recombination of the specific host population (Jayalakshmi *etal*., 2013).

RECOMMENDATION

Study recommends intentional screening of all residents of Basra province to investigate hepatitis B virus infections to identify patients and dealing with them and vaccinated close contacts to reduce the spread of HBV infection.

REFERENCES

1. Aggarwal R. Preventing and treating hepatitis B infection. British Medical Journal, 2004; 329: 1080-1086.
2. Al Hmudi H.(2011). Some immunogenetic aspects for diagnostic of HBV in Basra province- Iraq. PhD thesis. college of science. University of Basra. Basra. Iraq.
3. Allain JP. Occult hepatitis B virus infection. Transfus Clin. Biol, 2004; 11: 18-25.
4. Araujo NM. Hepatitis B virus infection from an evolutionary point of view: How viral, host, and environmental factors shape genotypes and subgenotypes. Infect Genet Evol, 2011; 11(6): 1199-207.
5. Baumert TF and Blum HE. Genetic variants of hepatitis B virus and their clinical relevance .Minerva Gastroenterol Dietol. 2011; 51: 95-108.
6. Blumberg BS, Alter HJ and Visnich S. A "new" antigen in leukemia sera. JAMA, 1965; 191: 541–546.
7. CDC. Recommendations for identification and public health management of persons with chronic hepatitis B virus infection.USA, 2013; 57(8): 9-11.
8. Chu CJ and Lok ASF. Clinical significance of hepatitis B virus genotypes. Hepatology, 2002; 35(5): 1274-6.
9. Chwla Y. Hepatitis B Virus: Inactive carriers. Virol. J, 2005; 28: 82.

10. El Khouri M and dos Santos VA. Hepatitis B: epidemiological mutation. Rev Hosp Clin Fac Med Sao Paulo, 2008; 59: 216-224.
11. Fang Y, Shang QL, Liu JY and Li D. Prevalence of occult hepatitis B virus infection among hepatopathy patients and healthy people in china. J. Infect, 2009; 58: 383-8.
12. Gish RG and Locarnini S A. Chronic hepatitis B: current testing strategies. Clin. Gastroenterol. Hepatol. 2006; 4(6): 666–676.
13. Hamadi A, Al-Hmoudi H and Issa A. Determination of clinical state of hepatitis B virus infection of patients in Basra governorate. Proceeding of the Eleventh Veterinary Scientific Conference. 2012; 72 – 75.
14. Hollinger FB and Liang TJ. Hepatitis B Virus. J .Virol, 2001; 4: 2971-3036.
15. Horvat R. Diagnostic and clinical relevance of HBV mutations. Lab Med, 2011; 42(8): 488-496.
16. Huang C, Lin S, Ho Y, Chen F and Yang C. The immune response induced by hepatitis B virus principal antigen .Medical laboratory and Biotechnology. 2006; 402: 97-106.
17. Hunt C M, McGill JM. and Allen MI. Clinical relevance of hepatitis B viral mutations. Hepatol, 2007; 31: 1037–1044.
18. IJpelaar H, Zhang C, Yin J, ZhangY, Deng Y, Ji X, Du Y, Pu R, Han Y, Zhao J, Han X, Zhang H and Cao G. HLA- DP polymorphisms affect the outcomes of chronic hepatitis B virus infection possibly through interacting with viral mutations. J. Virol, 2013; 87(22): 12176-13. immunological, and serological considerations emphasizing
19. Jayalakshmi M, Kalyanaraman N and Pitchappan R. Hepatitis B virus genetic diversity: disease pathogenesis. In: German Rosas-Acosta Eds. Immunology and Microbiology "Viral Replication". InTech. 2013; 69-82.
20. Liang TJ. Hepatitis B: The virus and disease. Hepatology, 2009; 49(5): 13–21.
21. Lin HH and Wang LY. Vaccination for hepatitis B. Tzu. Chi. Med. J, 2005; 17(5): 303 – 309.
22. Ozaslan M, Ozaslan E, Barsgan A and Koruk M. Mutations in the S gene region of hepatitis B virus genotype D in Turkish patients. J Genet, 2007; 86(3): 195-201.
23. Perrillo R, Richman D & Sherman K. Pocket guide to hepatitis B. University of Wisconsin Board of Regents and MDG Development Group, 2009; 1-63.
24. Pourkarim MR, Lemey A, Maes P and Ranst V. Novel hepatitis virus sub genotype A6 in African-Belgian patient. J. Clin. Virol, 2010; 47: 93-96.
25. Salim R and Abdullah B. The prevalence of hepatitis B virus in high risk groups in Nineveh governorate/Iraq. J. Baghdad for science, 2014; 11(2): 888-893.

26. Schäefer S, Tolle T, Lottmann S and Gerlich WH. Animal models and experimental systems in hepatitis B virus research. In: Koshy RC and Caselmann WH Eds. Hepatitis B Virus: Molecular Mechanisms in Disease and Novel Strategies for Therapy. Imperial College Press, London, UK, 1998; 51–74.
27. Servoss JC and Friedman LS. Serologic and molecular diagnosis of hepatitis B virus. Clin. Liver Dis, 2004; 8(2): 267–281.
28. Shepard CW, Simard EP and Finelli L. Hepatitis B virus infection: Epidemiology and vaccination. Epidemiol Rev, 2006; 28: 112–125.
29. Sorrell MF, Belongia EA, Costa J. National Institutes of Health Consensus Development Conference Statement: Management of hepatitis B. Ann Intern Med, 2009; 150: 104–110.
30. Uttreshwar GS and Ghatol AA. Hepatitis B diagnosis using logical inference and self-organizing map. J Computer Sci, 2008; 4(12): 1042-1050.
31. Valente F, vieria do Lago B. Epidemiology and molecular characterization of hepatitis B virus in Luanda, angola. Mem. Inst. Oswaldo cruz, Rio de Joneiro, 2010; 105(8): 970-977.
32. WHO.(2013). Hepatitis B. www.who.int/eht/.