TURKISH REPUBLIC OF NORTH CYPRUS
NEAR EAST UNIVERSITY
HEALTH SCIENCES INSTITUTE

MUTATION DYNAMICS OF HBV STRAINS ISOLATED FROM CHRONIC TURKISH PATIENTS

ASHRAF ZAIED

Master Thesis

MEDICAL MICROBIOLOGY
AND CLINICAL MICROBIOLOGY DEPARTMENT

MENTOR
PROF. DR. MURAT SAYAN

2019-NICOSIA

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The director of the Health Science Institute

This study has been accepted by the thesis committee of medical microbiology and clinical microbiology programmer as a Master Thesis.

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Approval

According to the relevant articles of Near East University postgraduate study education and examination regulations, this thesis has been approved by the above mentioned members of the thesis committee and the decision by the board of directorate of the Health Science Institute.

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LIST of ABBREVIATIONS

AHB	Acute hepatitis B
ALK	Alkaline phosphatase
AMF	Lamivudin
CDC	Centre for Disease Control
cccDNA	Covalently closed circular deoxyribonucleic acid
CHB	Chronic Hepatitis B
DNA	Deoxyribonucleic acid
dsDNA	Double strand deoxyribonucleic acid
ETV	Telivudine
GOT	Glutamic Oxaloacetic Transaminase
GPT	Glutamat- pyruvic transaminase
HBV	Hepatitis B Virus
HBcIgG	Hepatitis B core IgG
HCC	Hepatocellular carcinoma
HbeAg	Hepatitis B envelop antigen
HBcIgG	Hepatitis B core IgG
HbeAg	Hepatitis B envelop antigen
HBM IgM	Hepatitis B middle IgM
HbsAg	Habitats B surface Antigen
HBx	Hepatitis B x Antigen
HBV cpz	Hepatitis chimpanzee strain
HBV org	Hepatitis orgultan strain
HBV gbn	Hepatitis Gibbon strain
INF	Interferon
Kd	Kilo Dalton

MHBs	Middle hepatitis B surface protein
NCBI	National Center Biotechnology Information
NUC	nucleoside analogues
NUCs	Nucleotid analog used
ORF	Open reading frame
PCR	Polymerace Chain Reaction
RNA	Ribonucleic acid
RFLP	Restriction fragment length polymorphism
RT	Reverse Transcriptase
TDF	Tinfovir
TSB	Total and direct bilirubin
WHO	World Health Organization
YMDD	tyrosine-D-methionine, D-aspartic acid, and aspartic acid protein

ÖZET

Kronik hepatiti B enkefsiyonu, Türkiye'de büyük önem arz etmektedir. Bunun sebebi ise %8'lik bir prevelansının olmasıdır. Bu çalışmanın amacı, tedavi alan hastalardaki S ve Pol geni mutasyonlarını belirlemektir. HBV ile enfekte olan Türk hastalarda genotipler (Restriction fragment lenght polymorphism) RFLP tekniği kullanılarak S geni bölgesinden tayin edildi. Toplamda 93 numune Ocak – Aralık 2018 tarihleri arasında Kocaeli Üniversite Hastanesinde toplandı. Tüm hastalar Türk vatandaşı idi. 93 hastanın 18'i HBeAg negatif Kronik Hepatit (HbsAg negatif) idi. 33 numune, Kocaeli hastanesi laboratuvarıında ELISA tekniği kullanılarak VİDAS sisteminde HbsAg için test edildi. Bu numuneler, 1-3 yıl arası tedavi alan hastalardan elde edildi. S geni mutasyonları 20 hastada (%21,5) gözlendi ve HBV genotipleri 73 hastada çalışıldı. Genotip D 72 hastada, genotip A ise 1 hastada saptandı. Genotip D için, 64 hastada subgenptip D1, 5 hastada D2 ve 1 hastada D3 olarak saptandı. Subgenotip A2 2 hastada gözlendi. S geni saptanan mutasyonlar W196L, EI64D, W196L, G130R, YI34N,WI96L,Q129H,Q129R(asittani)N131D (as1),W196L,196L,E164D,193M,T131 mutasyonları idi. Pol geni mutasyonları ise 25 hastada gözledi Q149K,L9II,Q215,M204I,V173L,L180M,S202G,M250V,N236D,L8OI,A194S,V214A, V173L,M204V,M250W,M250W,Q149K,L180L,M204V,M250T,M404I,,Q215H,L80V, Q215H,L80V,L91I,Q215S,L180M. Sonuç olarak, çalışamız indike ediyorki mutasyon oluşumunu önlemek amacı bölgesel genotip/subgenotip bakılmalıdır. Ayrıca ek olarak, mutasyon analizlerininde yapılası tedavi sürecinde önem arz etmektedir.

anahtar sözcükler ; HBV, direnç ,naif hastalar , antiviral ajanlar, mutasyon

ABSTRACT

Chronic hepatitis B virus infection is an important area of study in Turkey because of the intermediate prevalence (8 %) of persistent HBV infection. The purpose of this study is to demonstrate S and Pol gene mutations in chronic HBV for patients who undergo medical treatment. HBV genotypes were analysed in infected patients from the Turkish population, using restriction fragment length polymorphism (RFLP) of the S gene region.

A total of 93 patients and the research was carried out between January and December 2018 from different regions of Turkey Kocaeli University Hospital. All patients were native residents of Turkey and indicated positive for HBsAg. Of the 93 patients (n = 93), HBeAg-negative CHB (n = 18), and HBsAg-negative (n = 0). 33 Samples were collected and tested for the detection of serological markers using the ELISA technique. Positive specimens for HBsAg were confirmed by the VIDAS technique at the Kocaeli University laboratory Hospital. Patients had treatment between the range greater than one year to three years of nucleos(t)ide analogue (NUC). In addition, the total prevalence of typical surface gene mutation analysis, 20 (21.5%), the HBV genotypes were screened for 73 patients by PCR technology. Genotype D was found in 72 patients' samples and genotype A was only found in 1 patient sample. Also, 72 patients with subgenotype D and D1 were found in 64 patients (88.89%), with subgenotype D2 in 5 patients (6.94%). Nevertheless, subgenotype A2 was found in 2 patients (2.78%), subgenotype D3 in 1 (1.38%) and subgenotype D4, from the mutation analysis of the S gene, showed mutation with the following patterns:

W196L, EI64D, W196L, G130R, YI34N, WI96L, Q129H, Q129R (asittani) N131D (as1), W196L, 196L, E164D, 193M and T131, polymerase gene mutation only 25 (26.9%) showed mutation patients with the following patterns: Q149K, L9II, Q215, M204I, V173L, L180M, S202G, M250V, N236D, L8OI, A194S, V214A, V173L, M204V, M250W, M250W, Q149K, L180L, M204V, M250T, M404I, Q215H, L80V, Q215H, L80V, L91I, Q215S, L180M. Conclusively, the study recommends that

prevention policy to the HBV transmission needs to be mutation developed; ensuring that genotyping and subgenotype for a geographic region are carried out. In addition, HBV mutation dynamics should be monitored coupled with ensuring drug resistance analysis is performed on the viral rebound during the therapy.

Keywords: HBV, resistance, naïve patients, antiretroviral agents, mutations.

1.1. INTRODUCTION

Hepatitis B infection (HBV) is considered a serious health issue. World Health Organization (WHO) detected that around 2 billion individuals across the world are suffering from HBV. Moreover, there are approximately 350 million patients infected with HBV. (Rebecca T., 2011; Sorrell et al., 2009).The most common complication of HBV disease is requiring liver transplantation (Shepard et al.; 2006; Sorrell et al.; 2009).The nature of the biological disease of the hepatitis B virus-producing high levels of the virus with a few symptoms (Degertekin et al., 2009). However, the virus spreads and recurrent in liver cells of infected patients. To copy the DNA genome, the HBV virus uses reverse during the virus replication stage. This is as a result of Pol - and HBV deficiency to read counteractively, allowing gene mutations to occur. Therefore, a heterogeneous cell group of the hepatitis virus with a variable genome is obtained. Mutation viruses show dominant strains of the host's immune response and antiviral therapy, under selective pressure. Humans are the most susceptible to hepatitis B, due to HBV its high contiguous nature of about 50 times than HIV. Commonly, most affected cases of HBV happen thought child-care or at birth. Hepatitis B infection occurs by the means of contact with the infected serum, also by mother to her baby and sexual relation. The symptoms of this infection take a longer duration before its appearance. The spread of hepatitis B infection in the world varies from one part to another and is more prevalent in Asia and Africa (Mahoney et al., 1999).

Since 1998, the national hepatitis vaccination program has been implemented in Turkey. However; the incidence rate is still high with about 4.46 million Turks suffering from this infection (Mahoney et al., 1999). According to the Ministry of Health, 6,600 people died from liver diseases in 2006. WHO has classified Turkey as a country that has a moderate prevalence in terms of HBV infection, but some data illustrated the transmission of the virus is progressively increasing. However, there are certain modifications of nucleotide in the encoding and cryptographic zones of the virus. HBV DNA has qualities that are duplicate and cover within various open reading frames (ORFs) Figure 1.2 (Chisari et al., 1992; Liang et al., 2009; Günther et al., 2006). There

are four ORFs directing translation and transcription of all HBV proteins utilizing various starting codons (Fig 1.2). In addition, the polymerase protein (Pol gene), is the biggest HBV protein antigen; the S gene comprises of small, medium and large surface antigen proteins and X protein (X gene). As depicted in Figure 1.1, the overlay in ORFs does not seem to restrict the variety since the HBV qualities have variants (Liang *et al*., 2009). In the replication of HBV, the polymerization error rate is estimated to be one error per 107 bases (Coleman et al., 2006 –Harrison et al., 2006). Thus, during the only requirement for the production of mutations in HBV replication. Nevertheless, some alterations to HBsAg leads to specify HBV vaccination. Consequently, a new strain of the virus will multiply in the presence of anti-body vaccination.

In addition, antiviral therapy such as (famciclovir, lamivudine, lobocvir, and tenofovir) which include an inhibitor of nucleotides and polymerase activity also can minimize the hepatitis B virus to lowest levels (Andersson et al., 2009; Nguyen et al., 2009; Pawlotsky et al., 2008). However, these drugs also increase the formation of mutations to resist the hepatitis B virus.

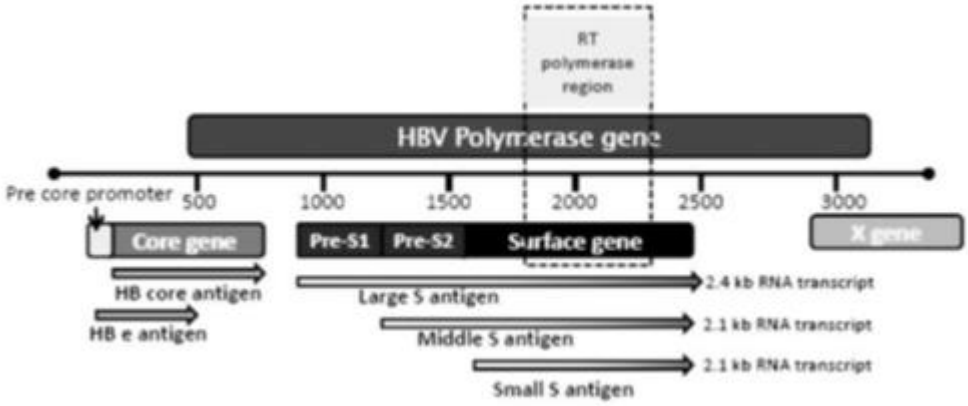

Fig 1.1 The HBV polymerase gene overlapping completely with the 3 surface (S) antigen proteins gene. Adapted from (Chisari et al., 1992; Liang et al., 2009; Günther et al., 2006; Harrison et al., 2006; Cao et al., 2009; Rebecca et al., 2011).

1.2 Significant of Study

This study will provide information on the nature of the HBV profile in chronically infected patients in Turkey as the main factor contributing to the resistance to antiretroviral treatment in the study area and the identification of a genetic mutation may occur. This, in turn, will help stakeholders with HBV-resistant data in Turkey.

1.3 Aim of Study

The aim of this study to define the occurrence of the hepatitis B virus in Turkey and the factors contributing to HBV infection in the region. And also to determine the variability in the genetics of the virus that helps it in escaping from antiretroviral therapy in chronic patients in Turkey.

1.4 Epidemiology

HBV is considered a health and epidemiological disease which leads to high rates of increased mortality worldwide, especially in developing countries (Alam et al., 2007). World Health Organization reported that 2 billion people worldwide are suffering from hepatitis B infection and 450 million people are infected in developing countries and pose a higher risk of HDV infection with HBV. According to international statistics, the WHO reported a rise in the number of deaths worldwide due to HBV complications. This issue is especially important in Asia, Africa, and the Western Pacific region, where HBV infection is spreading (from 5-20% to 80% of all infected people in the world) (Liberek et al., 2007). The prevalence of HBV infection in the world has been classified as high, medium and low epidemic as described in Table 1.2 (Hou et al., 2005; Thaiar et al., 2009).

Table 1.1 HBV infection endemic patterns. Adopted from (Alter, 2003; Thaiar et al., 2009).

Characteristic	Classification of infection		
	Low (%)	Intermediate (%)	High (%)
Prevalence of chronic infection	0.5—2	Uncommon (10-60)	≥ 8
prevalence of last infection	5-7	Uncommon (10-60)	70-95
Perinatal infection	Rare (<10)	Common (10-60)	Common (> 20)
Early childhood infection	Rare (< 10)	Common (10-60)	Very common (>60)
Teens / Adult infection	Common (70-90)	Common (20-50)	Uncommon (10-20)

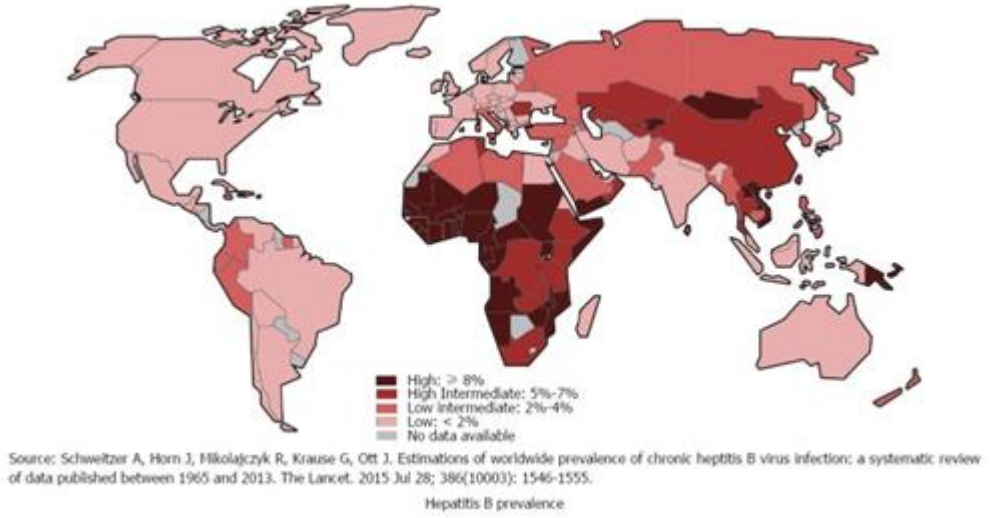

Figure 1.2 Universal distribution of hepatitis B. Adapted from (Sharma, Saini & Chwla, 2005)

1.5 Transmission of HBV infection in Turkey

According to WHO statistics, Turkey has an epidemic average (2-8%) of people infected with the hepatitis B virus. Since the starting of the comprehensive immunization program for all children and vulnerable groups in Turkey in 1998, a decrease in its prevalence has been observed. The spread of the disease among children in Turkey was also observed before the vaccination program. Prior to 1998, the overall prevalence rate was 5.90% among children aged 0-15 years. After the effect start of the vaccination program, this figure decreases to 2.84% for the same age group (Kanra et al., 2005; Hasan et al., 2018). The HBsAg positivity was found to be 2.3% with predominant D, (HDV) genotype in different parts of Turkey in 2012. Figure 1.3 shows the prevalence of HBV among different parts in turkey.

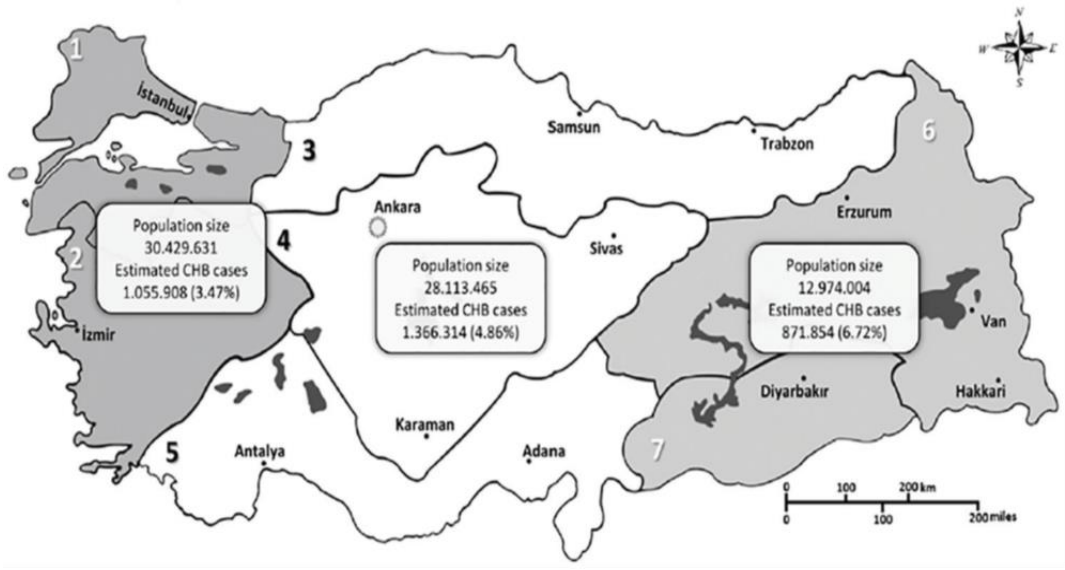

Fig 1.3 Distribution of HBV infection in different parts of Turkey. Adopted from (Hasan et al., 2018).

1.6. Methods of Transmission HB Virus

Hepatitis B virus is transmitted mainly through contact with the natural fluids of the infected person. Blood, semen, and saliva are the most important means of transmission of the infection (Robinson, 1995), as well as sweat, breast milk, urine, and stool (Prescott et al., 2005). Three methods have been identified: perinatal, sexual operation and injections for drug users / transdermal transmission. There is no evidence that infection is caused by air or feces. The virus cannot be transmitted via water, insects, contaminated food or other carriers (Hou et al., 2005).

1.7. Mother to Child Transmutation

Infants are exposed to the hepatitis B virus from their mothers during pregnancy. The postpartum period and breastfeeding seem to play an important role in determining the spread of the infection in endemic regions, especially China and South Africa (Hou et al., 2005). It might infect human ova and this could explain the high risk of vertical transmission in Asia. (Ye et al., 2006). There are three possible ways of transmitting the infection from the infected mother to the baby: transmission from the placenta to the uterus, transmission during childbirth; postnatal transmission during care or breastfeeding (Hou et al., 2005).

1.8. Sexual Rout Transmission

In all regions of the world, HBV is considered the main source of infection, especially in low-infection areas like North America. For many years, homosexuals, show more susceptibility to the infection by sex. In addition, 70% of the gays are infected proximately following to five years of sexual relation (Alter, 2003). There are other factors related to raising risk infection like the sexual partners' number and the sexual activity period, the duration of sexual activity, the history of disease transmitted by the sexual route and positive syphilis (Alter & Maast, 1994).

1.9. Parenteral / Percutaneous Transmission

The transfusion is considered injectable through injecting drug use, blood transfusion, dialysis, acupuncture, health care, ear piercing, and tattoos. Injecting drug use in the United States continues to be a very important means of transmission of the hepatitis B virus (15% of all patients) (Schiff, 2004). Nevertheless, Blood products and surgical instrument, which is contaminated with HBV are considered as a clear source of infection. Although, there is a control procedure in hospitals especially in dental clinics and dialysis units (Hou et al., 2005).

1.10. Risk factors of HBV Infection Transmission

Some people are more risk of infection than ordinary people: individuals exposed to current blood transfusion, dialysis patients, dentists, dentists, nurses, police, drug addiction and those are exposed to blood-stained or medical waste (Hou et al., 2005). Moreover, having sex with a person with severe or chronic illness also leads to high risk. In the United States, active gays make up 6%, while gay with multiple partners consists of 0.5% of all risk factors (CDC, 2008).

Table 1.2 HBV infection risk group (Thaiar, 2009).

No.	Risk Group
1	Health care workers.
2	Injection by glass syringe
3	Hemophiliac patients
4	Blood recipients
5	Health care workers
6	Parenteral drug administration

2.1. GENERAL INFORMATION

2.1.1. History and Definition of Hepatitis B

Lorman discovered hepatitis in 1885 when he found that about 15% of the 1,289 shipbuilding companies in Bremen had jaundice after being vaccinated with the human varicella vaccine. In addition, Bloomberg discovered Australia's antigen reaction with antibodies in 1963 in the serum of an Australian patient suffering from American hemophilia. The electron microscope also showed serotonin antigen with Dane molecules for hepatitis B (HBV) which is a superficial component pkg (Dane et al., 1970).

Hepatitis may be considered as inflammation of liver tissue. The causes in terms of etiologies including metabolism, such as alcohol toxicity, and other systemic viral infections that may influence the liver tissue such as hepatitis A, B, C, D, E, G viruses, cytomegalovirus, and hemorrhagic fever (Huda, 2013).

2.2. Stability of HBV

The hepatitis B virus stability is constantly fixed with HBs antigen exposure to pH 2.4 and heat 98 ° C for at least 6 hours but did not break down the antigen and also for 3 minutes HBsAg Exposure to 0.25% Sodium Hypochlorite (Hollinger and Liang, 2001). Infection after sterilization for 20 minutes at 121 ° C or one hour at 160 ° C dry heat dealing (Robinson, 1995). Hepatitis B virus can be prevented by exposure to hydrolyceride glutaraldehyde by 2% At room temperature for five minutes or 98% CI for two minutes, sporicidin (pH7.9 formaldehyde at 18.5 g /l (5% in formalin water), 70% isopropyl alcohol, 80% ethyl alcohol for 11 ° C for 2 minutes, Combined prop-propriolactone and ultraviolet radiation UV (Hollinger and Liang, 2001). However, the virus retains infection even when kept in temperature (30 - 32 ° C) for about six months also freezing at mines fifteen C for 15 years. In addition, HBV within the blood can stay on the drying surface for at least one week (Robinson, 1995; Thaiar et al., 2009).

2.2.1 Structure and Composition of HBV

The microscopic examination of the serum of hepatitis B patients shows three distinct antigenic particles: a 22nm non-spherical particle, a 42nm globule (DNA and DNA polymerase) called a less observed Dane particle, and tubular or filamentous particles that vary in length (Figure 1.1). The densest particles are spherical particles, which are made up exclusively of HBsAg as tubular or nematode shapes, which have the same diameter but maybe longer than that of the hemangiomas (Hollinger and Liang, 2001) 200 nm produced from the production of HBsAg.

The 22 nm particle is lacking in DNA and is therefore not contagious, but these particles have a high potential to stimulate immunity and induce an antimicrobial response (WHO, 2002). Hepatitis B virus (Figure 2.1) is 42nm, partially double the DNA virus (dsDNA), consisting of 27 nm nucleocapsid-core. HBcAg is surrounded by an external layer of lipid-protein (also called envelope) containing surface antigens (HBsAg) (Jawetz et al., 2007). The particle of 22 nm diameter lacks DNA, but these particles are highly immunogenic particles that stimulate the stimulation of antibodies to HBs (WHO, 2002). Hepatitis B first Alyalvsal literature review 7 surface antigen (HBsAg) (Ganem and Schneider, 2001). The 27 nm inner nucleocapsid core contains HBcAg a 3.2 kilometer double-byte circular DNA, DNA polymerase enzyme and protein kinase activity (Hollinger & Liang, 2001; Jawetz et al., 2007). The main structural protein of the nucleus is the basic 21kD phosphoprotein HBcAg (WHO, 2002) and nucleocapsid round with a diameter of 30-35 nm. The capsid consists of 180 capsomeres (Büchen-Osmond, 2003). It can be penetrated by stain and some appear dark in the center (Bruin et al., 1995; Thaiar, 2009).

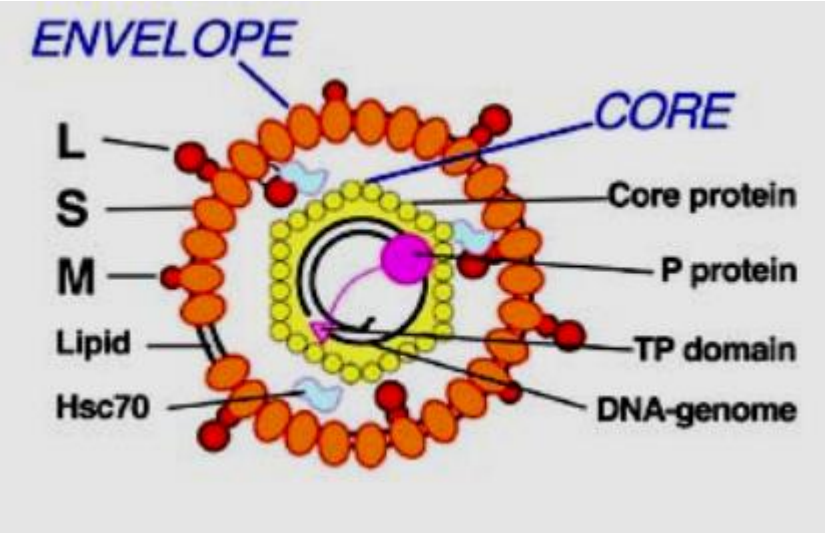

Figure 2.1 Hepatitis B whole virion. Adopted from (Scuderi, 2003; Thaiar, 2009)

2.2.2. Variability of HBV genome

HBV is one of the Hepadnaviridae family. The replication of slightly circular DNA in their double-stranded DNA genome (3.2 kb) in that stage include reverse transcription (Figure 2.2). The activity of RNA-oriented polymerase action (RT) is necessary to do reverse transcription that is encoded inside the virus. Another enzymatic action, the polymerase-bound DNA polymerase, is important to participate in the replication of the virus which is carried in the same dynamic active site via viral polymerization. HBV does not have a print-correction activity and does not have a 30-50 nuclease activity. The HBV polymerase-fault creates a genetic variation and was observed as a semi-viral type, i.e. closely related to a range of viral variants. This variation is considered for viral genotypes and variables with alien phenomena.

There is a typical genetic variation of HBV and variants of strange phenomena. These variants are the presence of antiviral drug-resistant gene mutations and escape from vaccine-bound datatypes (HBeAg). Hepatitis B virus (MHBs) is a large HBV surface that has recently been broken down by a protein that regulates TNF-related apoptosis, or mutation in regions regulated by the Genome (BCP) (Zhou et al., 1999).

There are many factors that affect the chances of the viral genome such as the infection method and the genetic host, including immune responses and antivirals drug, as well as the genomics and mutant dynamics that cause the reestablishments. ORFs overlapping open reading frames make a limitation in the evolution of the viral genome as many encode two viral proteins in nucleotides (Mizokami et al., 1997; Pavesi et al., 2015). The sequence indicates that between the genetic and assumed within the genotype is a recurrent event, while the recombination mechanism is still unknown (Hayer et al., 2013; Araujo, 2015).

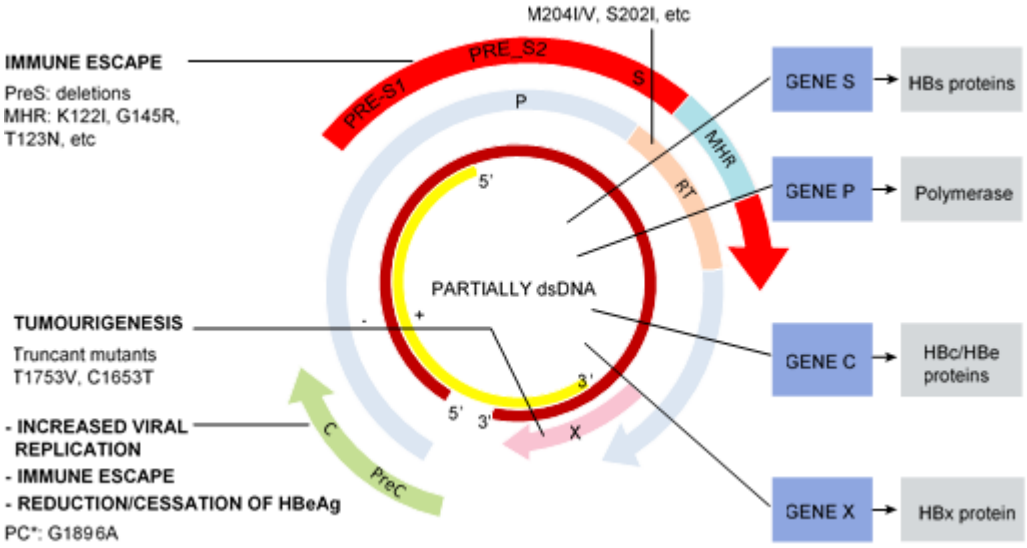

Figure 2.2 Map Hepatitis B genome with a summary of associated mutations and downstream effects. Adopted from (Neil et al., 2017).

2.2.3 Pathogenicity of HBV virus

According to the general classification of viruses, HBV as a hepatic virus is one of the family hepadnaviruses (Nassal, 1999; Howley, 2001). The HBV composed of an external envelope consisting of three surface antigens and nucleocapsid which have an important function in the diagnosis of the hepatitis B virus. Nucleocapsid contains a

nucleotide antigen of the HBcAg, a transcript of proteins of the cellular genome polynomial DNA.

The genome consists of partial pieces of ring DNA approximately 3200 pairs in the length also a sequence with gene regulation. Before surface 1 [before S1] / before surface 2 [before S2] / and surface genes [S] as a code for different HBsAg. Protein is encoded by the gene before the Ag (HBeAg), an indicator of the high replication of the virus (Howley, 2001). The principal nucleocapsid structural protein is the basic gene codes for HBcAg. Finally, the X gene codes for hepatitis B antigen (HBxAg) have strong coefficients of viral and cellular genes. The life cycle of the virus and the HBV infection remain un-established. Many genetic elements of cis and trans-act involved inaccurate control of the packaging of the gene, RNA expression, and then the replication of the virus should be known (Nassal, 1999; Howley, 2001; Thomas et al., 2007).

2.2.4 Genotype of HBV

HBV genotype is categorized as groups that have the same DNA series with over eight percent variations among the same strains (Okamoto et al., 1988). The HBV genotypes have been 8 types ranging from (A–H) (Arauz-Ruiz et al., 2002). Genomic nucleotide HBV level exchange is 105 to 104 per place per year. Alterations happen because of a long-term HBV adaptation of determined genetic in the certain host, recombination, and evolutionary drift (Gunther; 2006). Genotyping is a suitable agent used to investigate viral estimation, to know the common origin of infection, and to investigate viral evolution (Huda, 2013).

2.2.5 Sub genotypes of HBV

HBV subtypes depend on the "specific" antigen composition which occurs inside the amino acids 120-147 domain that contains a couple of loops and a projection build from the virus surface (Figure 2.3) (Bovier,1975). The HBsAg d and y subtypes were first discovered in 1971 after that discover w and r. All groups of the HBV subtype have

marked in 9 subtypes: ayw1, ayw2, ayw3, ayw4, ayr, adw2, adw4, adrqand dark (Liu et al., 2002), identified via amino acids arginine or lysine o in place of (122, 160) in HB surface antigen (Kao & Chen, 2005). Another subtype had been described as vectors of the amino acids (127, 144, 145, 158, 159, 177, and 178) (Kramvis et al., 2008). The Hepatitis B virus serum has been utilized for virological research studies and disease development. Ad subgenotype had a danger of lymphoid resistance higher reach 20 fold more than other strains (Zollner et al., 2004; Huda, 2013).

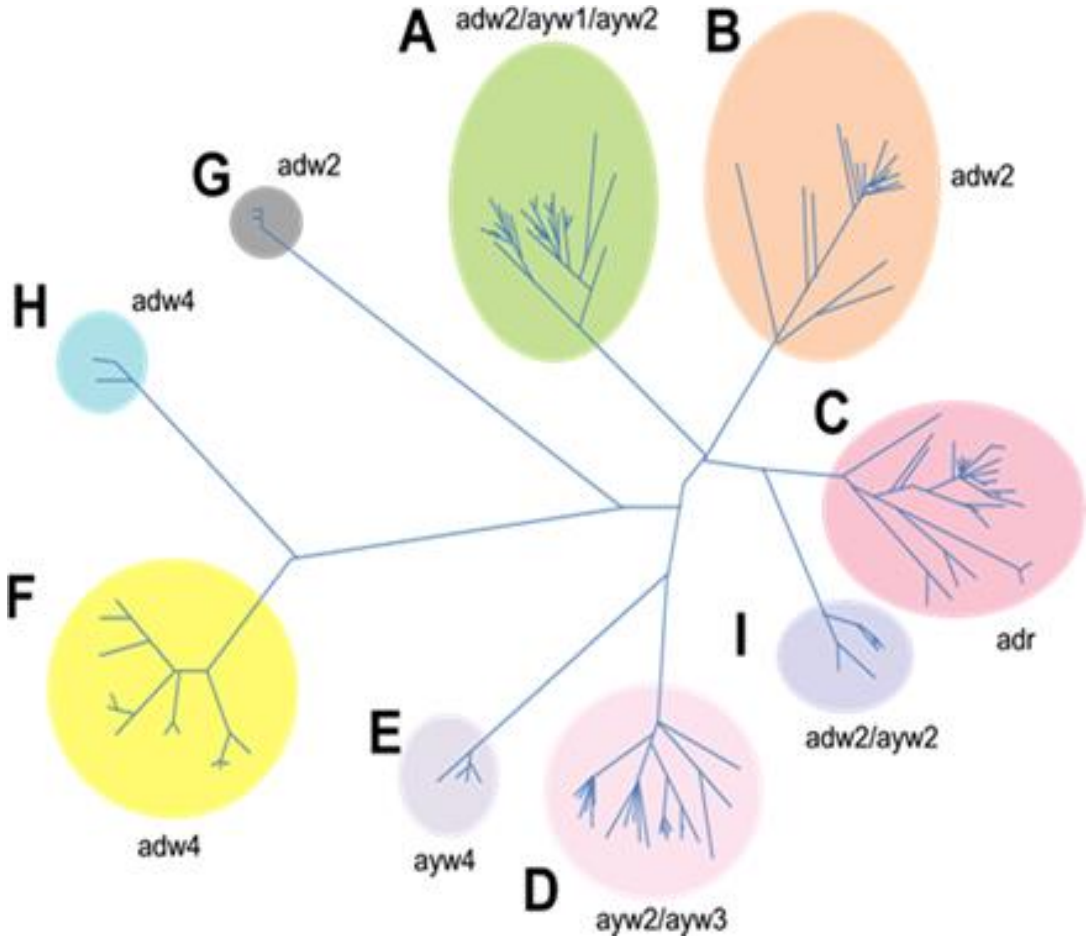

Figure 2.3 variability Hepatitis B virus genome: phylogenetic tree performed of the main nine genotypes (A–I). Adopted from (Neil et al., 2017).

2.2.6 Geographical Distribution HBV genotypes

Nine types of HBV genotype have a different geographical distribution (Figure 2.3), this distribution has connected to the mode of travel that leads to HBV genotype interaction. Also, the distribution informs us of how the disease evolution happened. If the patient in Northwestern Europe has over the last century, genotype A may be reflected in immigration to North America and South Africa. Genotype A, D, and C in Australia and New Zealand related to Southeast and the Pacific Island immigration (Bartholomeusz and Schaefer, 2004). In the UK, the most prevalent genotype is A and D (Dervisevic et al., 2007). However, the highest prevalence and more distributed is the genotype D distributed from India and Southern Europe to Africa see (Figure 5). Some factors influence the genotype prevailing change like in Japan homosexual people who have HIV harbor genotype C rather than (A or B) (Koibuchi et al., 2001). In Caucasians males, the genotype G is more pronounce and genotype E in West and South Africa (Bartholomeusz & Schaefer, 2004). Here we need repeated research and studies to discover the possible HBV genotypes change in all regions.

The genetic patterns of HBV are distributed according to the geographic distribution and have distinct genotypes. Like in Africa and Asia the genotype is A and Aa subtypes Respectively, and Ae subtype in Europe (Sugauchi et al., 2004). Genotype B has four subtypes (B1-B4). There is also a chemical structure for B, C, Ba and the (Bj) genotype in Japan breeds (Norder et al., 2004; Huy et al., 2004). Southeast Asia including Thailand and Vietnam have the C2 Ce genotype. Also the C2 (m) genotype is in the second part of East Asia in Korea, China and Japan (Norder et al., 2004). Australian strain has C Australia (C aus) Chimpanzee strain (HBV cpz), Orgutan strain (HBV org), and Gibbon strain (HBVgbn) (Bartholomeusz and Schaefer, 2004; Huda, 2013).

2.3. Clinical Picture

Hepatitis B virus infects the liver and causes several liver disorders, including chronic and acute hepatitis, cirrhosis, Hepatocellular carcinoma (HCC).

2.4. Acute HBV Infection

The incubation duration of acute hepatitis B (AHB) is between 45-120 days with an average of more than 60-90 days (Mahoney & Kane, 1999). Hepatitis B infection is common in about 90% of cases because the number of HBV infected cells is still small. Reviewing literature 17 is the time when the effective immune defense begins, so the infection is self-limited and remains symptom-free (Parslow et al., 2001). Most patients may recover after 4-8 weeks, without any indication. In some cases, there may be symptoms such as fatigue, abdominal discomfort, loss of appetite, vomiting, nausea, arterial pain and rash in many developed cases jaundice occurs, fever may be absent or moderate (Rosai, 2004; Thaiar, 2009).

2.5. HBV Chronic Infection

Chronic hepatitis B (CHB) is an infection for more than 6 months. Although the majority of adult patients heal completely from AHB infection in a large 5-10% proportion. The virus stays in the body but the ratio is much higher in children and 70-90% of infected Newborn in the first years of Infection become chronic carriers of HBV (Hollinger and Laing, 2001).

40-70% of hepatitis B virus infection before age 3 leads to CHB infection (Liberek et al., 2007). Related inflammatory liver disease, which happens is variable in terms of severity. Permanently more moderate than in AHB, however, continued to several years may lead to cirrhosis which related to increases in risk and development of the HCC (Hollinger and Liang, 2001). There are three stages of viral reproduction: High, low, and non-recurrent infection (Gitlin, 1997). The risk of death from liver cirrhosis

and HCC is 15-25% (Lin and Kirchner, 2004). CHB Infection is believed to be prevalent in Turkey as well as in many other developing countries (Thaiar, 2009).

2.6. Hepatocellular Carcinoma

Liver cancer is the most common type of human cancer. The number of annual infections is estimated at around 250,000 worldwide. Risk factors for viral hepatitis occur early or long after cirrhosis with maximum infection in the age group 30-50 years. The risk of liver cancer may be 200 times greater for other carriers such as HBsAg via similar controls (Liu et al., 2006; Huda, 2013).

2.7. Vaccines against Hepatitis B

HBsAg and spherical HBsAg particles were used in vaccine manufacture in the 1980s and pellets extracted from HBsAg plasma as HBV vaccine (Maupas et al., 1976).

Plasma clearance of chronic vectors has been eliminated to prevent possible risk of HBV infection and bacteria, HIV particle or any infection with another pathogenic organism.

The vaccine contains 22 nm of HBsAg molecules. The vaccines' second-generation composed of small surface proteins that are not associated with yeast glycosyl (S) obtained in yeast (McAleer et al., 1984). Vaccines are used in immunization programs in more than 150 countries around the world that have a strong immune effect. However, the third generation of the vaccine was recently developed, consisting of S protein (McAleer et al., 1984; Andre, 1990) As well as medium and large surface M proteins (L) (Hourvitz et al., 1996). Vaccines produced in China are considered to have an early and stronger immune response than that of S only (Raz et al., 1996) This is why proteins are called “unresponsive”, and instead a defensive immune response has been made (Rendi-Wagner et al., 200).

2.8. Treatment

Cirrhosis and hepatocellular carcinoma (HCC) are the main complications of HBV diseases. The Increased level of DNA HBV develops in cirrhosis and liver cancer.

Liver cancer occurs most often in those regions with a high incidence of hepatitis B virus. Alpha interferon therapy is the first treatment for chronic HBV infection.

About 30% of patients with chronic hepatitis B successfully use antiviral alpha-interferon therapy. In the past, lamivudine was commonly used as a nucleotide analog. (Takkenberg et al., 2010). Lamivudine inhibits the activity of pyrophosphoric in reverse transcription. HBV RNA is copied to the DNA prior to the secretion of viruses in the plasma. Due to resistance for lamivudine, the median age of the plasma molecules about a day and a half-day, the virus rate can be influenced quickly after the treatment. However, the drug cannot be eliminated with NUCs virus due to persistent HCV cccDNA (covalently closed circular DNA) in the nucleus of liver cells. After one year of treatment around 10 - 20% of cases lose HBeAg.

A group of patients with diphosphate resistant to lamivudine was developed owing to the occurrence of a mutation in the YMDD motif of HBV polymerization, and thus viral healing happiness. Due to the resistance lamivudine, new less vulnerable NUCs such as (Tenofovir, entecavir) (Table 3) (Schaefer et al., 2009). Endeavor and Tenofovir have significantly less dangerous to resist than NUCs used. So, recently antiviral HBV medications are utilized as the first choice of treatment.

Table 2.1. Confirmed nucleoside analogues (NUCs) for HBV treatment

Drug	Structure
Lamivudine	Cytidine analogue
Adefovir	Adenosine analogue
Enteoavir	Guanosine analogue
Telbivudine	Thymidine analogue
Tenofvir	Adenosin analogue

Due to the immunosuppressive of drugs, patients who are treated with chronic viral hepatitis after liver transplantation are more difficult. Therefore, these patients should use a combination of nucleoside analogues and immunoglobulin directed against HBsAg. New liver re-infection may occur due to re-spread of the virus molecules in the blood (Lai et al., 1998; Tillmann et al., 1999; Alexandra Schumann et al., 2014).

2.9. HBV Resistance

Five active drugs have approved the addition to INF to treat HBV and allow for numerous other methods to treat the infection. AMF, lamivudine (3TC), endeavor (ETV), telivudine (Tlb) and tinofovir (TDF) are either nucleotide isotopes or nucleotides which use 3TC and TDF in the treatment of HIV. The effectiveness of this cure depends on the similarity of the structure of HBV and HIV has a role in treatment. These viral changes of resistance are the main choice and challenges in the treatment of hepatitis B.

About 14-32% of patients treated with 3TC for one-year exhibit resistance variables, up to 80% after 4 years [Zoulim et al., 2009]. Response rates for treatment (20%) initially not respond, 29% resistance for ADF showed after 5 years of treatment.

Resistance rates for ETV are low in patients with naive treatment, 0.2% after 1 year, (1.2%) at 5 years. In contrast, 57% of patients treated with 3TC after 5 years (Zoulim et al., 2009; Tenney et al., 2009).

Until now, TDF therapy has been considered the only treatment that has not been identified as any resistance since adoption in 2008. Recently, this has been confirmed for 6 years of treatment (Kitrinos et al., 2014). It is announced that cases who are taking ADF neglect to react to TDF just like patients on other treatments. But, this low affectability cannot be clinically pertinent obstruction level (Van et al., 2010; Maria et al., 2014).

2.10. Mutations Allow Resistance to Antiviral Therapy

More recently, polymerization mutations were detected in cases treated with antiviral drugs. As a result, resistance to antiviral drugs was observed (Durantel, 2003). A nucleoside analog therapy group is a strong inhibitor for HBV replication that is clinically used as a treatment for chronic hepatitis B. Adyvir is a previously licensed hepatitis nucleotide for chronic hepatitis B (Marcellin et al., 2003; Hadziyannis et al., 2003). Presently, nucleotide isotopes include a clinical screening of entecavir, emriticetabine, clevudine, and telbivudine (Zoulim. 2004; Durantel, 2003). Most of these medicines have an important function of end-chain while synthesizing the budding DNA strand, thus finishing viral reproduction (Zoulim, 2004). Other nucleotides like adefovir and entecavir interfere with DNA to reduce the nucleic acid strand (Zoulim, 2004). Adefovir and to a lesser extent lamivudine as well targets additional primary DNA repair (Köck et al., 2003; Thomas et al., 2007).

2.11. Diagnosis Methods

Hepatitis B infection disease is diagnosed by clinical, serological, biochemical and histological analysis. Interpretation of these outcomes is important to analyze different clinical symptoms of hepatitis infection disease.

The first sign of diagnosis for the healing of infection is a decrease in viral load and then quickly constrain HBeAg and HBsAg in serum (Gerlich et al., 2009).

HBV chronic infection begins with a serotype same to acute infection(Fig.2.4). But chronic infection patients rarely show HBsAg and HBeAg.

HB surface antigen is helpful for diagnosing the change from acute to chronic disease. Also is important to diagnosis transition from acute to chronic infection. Chronic HBV infection after continues HBsAg more than six months can be confirmed. DNA HBV is an indication of HBV cloning in chronic and acute hepatitis infection.

quantities of HBV DNA estimated by quantitative chain polymerase chain reaction is indicative of infection. Anti- HBc is a decent marker for determining the rate of HBV in the number of individuals influenced. Until this time, since is not used as a part of the vaccine can only be detected after hepatitis B infection.

2.11.1. Clinical and Serological Course of HBV Infection

When AHB occurs, HBsAg appears 4-2 weeks before the onset of symptoms or jaundice, and the acute phase has reached its level and then gradually decreases within 4-6 months in a typical case (Keeffe et al; 2006). Synchronously and then decrease in HBV-DNA and HBeAg (Figure 2.4).

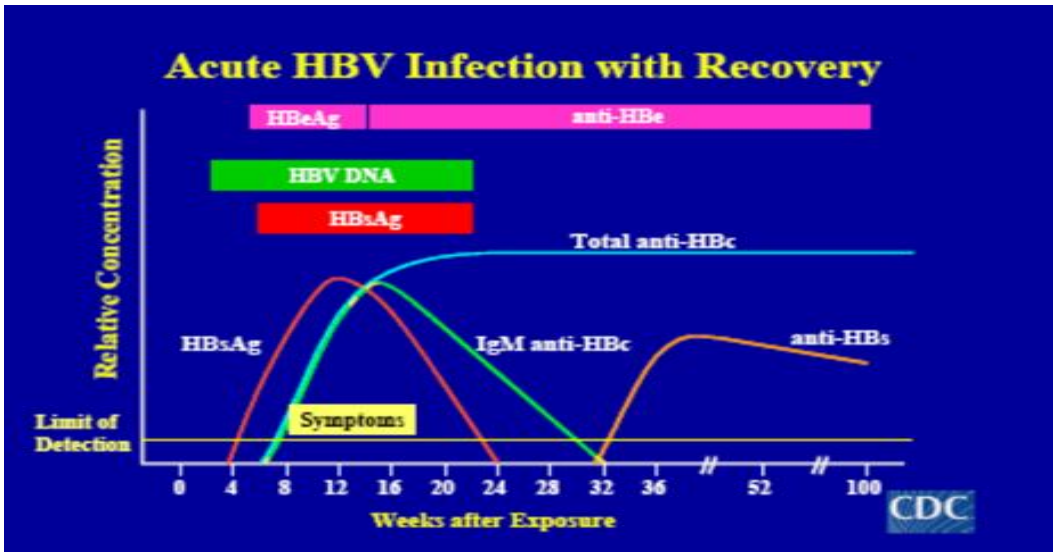

Fig.2.4 Clinical path and serological markers of acute HBV-infection. (Alter, 2003 - Thaiar K., 2009).

A decrease in anti-HBM IgM is observed regardless of where the disease resolves or becomes chronic may reappear during re-infection, whereas HBc IgG is still detectable for life in the chronic form (Alter, 2003).

HBV-DNA can be detected after 2-5 weeks of infection and up to 40 days before HBsAg (average 6 to 15 days) and rises slowly at relatively low levels during the

serum period, as detected during the CHB infection, while neutralizing the antibody (Anti-HBs) develops during healing It can be detected with anti-HBc or become undetectable in up to 20% of patients after several years (Figure 2.5) (Alter, 2003).

In the natural history of CHB infection, the loss of HBV-DNA traces the seropositivity of HBeAg to HBe (Ruiz-Moreno et al., 1999 - Thaiar K., 2009).

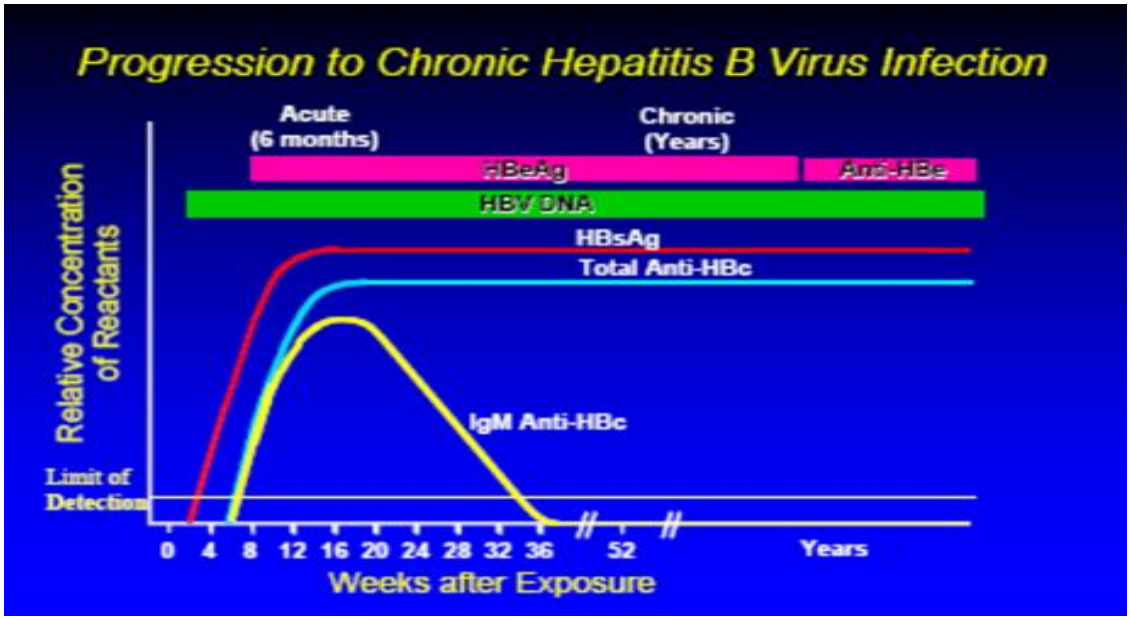

Fig. 2.5: A chronic serological and virological markers profile of HBV infection (Alter, 2003 - Thaiar K., 2009).

2.11.2. Biochemical Assays

The biochemical test of the liver function includes total bilirubin (TSB) and direct bilirubin (DSB), (GPT), (GOT), alkaline phosphatase (ALP), total protein, albumin, globulin, and coagulation factors test (Robinson, 1995 – Thaiar K., 2009).

2.11.3. Serological Diagnosis of HBV

One of the most useful assay to detect antibodies of virus HBV is ELIZA technology (Jawetz et al., Jawe 2007) The detection of serological indicators is based on the Ab&Ag interaction, so it is divided into two categories, the detection of Ags or the detection of antibodies Abs (Thaiar K., 2009).

2.11.4. Molecular Diagnosis

It is the measurement of the quantity of DNA-HBV in the infected serum and determines the level of virus reproduction. Recently, the HBV-DNA test was performed in the blood using non-amplified hybridization. These have a standard rate from (105 to 106copies / mL) and should not be used any more for patients with CHB infection as routine management. (Keeffe et al., 2006).

In addition, HBV-DNA tests also help to know information about virus replication, especially in patients Religion has signs that fall outside the normal pattern. The molecular test for HBV consists of two categories firstly, the quantitative method of HBV-DNA that measures the amount of DNA in the blood serum, reflecting the level of HBV replication, (viral load) in the liver. Secondly, specific parameters and determining the sequence of clinical significance or Pathophysiology in the HBV virus genome (Pallier et al., 2006 - Thaiar K., 2009).

2.11.5. Polymerase Chain Reaction Technique (PCR)

DNA virus of the hepatitis B virus can be detected by the interfering PCR technique that can be identified with a small rate of 102-103 repetitive mutations (Schutten and Niesters, 2001). At least 10 times more sensitive than a technique dot blot for HBV-DNA (Soni et al., 1994 – Thaiar K., 2009).

2.12. Viral Load

The researchers have developed tests that accurately detect and measure HBV DNA. These measures reveal the viral genome and measure the level of virus turnover in the infected person. HBV is often referred to as "viral load or load"; anticipating response to antiviral therapy based on viral load of treatment (Schiff, 2004). he Workshop of National health institute on the Hepatitis B Investigation Department considers that, if the patient has high load HBV DNA (ie 105 ccs/ ml or 20,000 IU / mL) the treatment here is important. Nevertheless, in some HBeAg negative patients with HBV-DNA stratified levels. This decline is less than (10 5copies / ml) (Chu et al., 2002).

According to expert tests, patients may develop the advanced liver disease may develop even if their HBV-DNA serum levels are consistently lower 20,000 IU / ml, therefore, the medical significance of the low level of HBV-DNA is uncertain and must be individualized. (Keeffe et al., 2006). PCR testing has now become more available for the initial diagnosis of patients and For both patients undergoing treatment and not undergoing. if diagnostic methods are not managed and regulated, making data comparison difficult.

In many laboratories, this variation is treated with the presence of HBV standard DNA (Saldanha et al., 2001). moreover, all results must be documented in IU / ml (approximately 5.6 g / mL) (Keeffe et al., 2006 - Thaiar K., 2009).

3.1. MATERIALS and METHODS

3.2. Patients and Sample Collection

This study was performed between the period of January and December 2018. It involves patients at the Hospital of Kocaeli University in Turkey. The study was specific to patients treated with the HBV antiviral group NUC, at the same time. All PCR results were positive and detected by real-time PCR technology. In general, 93 cases were used with 30 patients who were already undergoing NUC and, 64 cases were chronic hepatitis B patients. Serum blood samples were collected from patients who received NUC treatment according to viral penetration and/or biochemical examination. Separation of Blood samples was directly done by centrifuge, kept at -20 ^{o}C until testing.

Most serological tests included detecting antibodies to the HBsAg virus were regulated anti-HBs, a- antibody to hepatitis B, antiHBc core, HBeAg hepatitis envelop antigens were tested using antibody groups of particle enzymes (Roche Diagnostics, Germany - Axsym, Abbott Laboratories, IL, USA). However, all patients were HBsAg positive according to serum data.

3.3. DNA Extraction and HBV DNA Assay

Magnetic practice technology was used to extract DNA from serological samples by biorobot technology (NucliSENS-easy-MAG, bioMe' rieux, Boxtel). Examination of HBV DNA was done first by use PCR technology quantifies (Iontek Biotechnology Inc. Turkey; iCycler iQ5, BioRad Laboratories Inc., USA). As described by Karatayli et al., (2005).

3.4. HBV sequencing for the polymerase gene region

Used a pair of primary materials (eg: 50GTTGACAGACTTTCCAATCAAT-30) was designed for the polymerization region of the HBV.

The polymerase chain reactions for the polymerase fraction were: for 15 minutes at 95c, 45 cycles composed of 95 C for 45 seconds, and 72 C for 45 seconds, 56 C for 45

seconds. The initial pairs concentration was 0.3 mm. In HBV the volume of the amplicon was about 742, and include every NUC-resistant mutation. All PCR products were converted into the High Pure purification PCR kit product (Roche Diagnostics, Germany) by the use of the ABI PRISM 310 gene analyzer directly sterilized by using the dynamic ET Terminator Sequencing Kit (Amersham Pharmacia) Biotech Inc., Piscataway, USA). For the sequence of the session, after using the thermocouple protocol: 35 motorbikes consist of 95 8C20 seconds, 50 8C for 25 seconds, and finally 60 8C for2 min. The reverse primer was used as a sequence guide with a concentration of 0.5 mm electric images using Vector NTI v5.1 for assembling1 (Infor-MaxTM InvitrogenTM Life Science Software, Frederick, MD, USA).

3.4 HBV Genotype Determination

Determination of HBV genotypes was by the genotyping device of the National Center for Biotechnology Information (NCBI, US National Library of Medicine, Bethesda, MD, USA, http://www.ncbi.nih.gov/ projects/genotyping/form page.cgi), which distinguish the genotype based on the viral nucleotide sequences. The genotyping instrument works by using BLAST to compare a query sequence to a group of reference sequences for known genotypes.

3.5 HBV Genotype Determination and Mutation in the pol/surface Gene

The Arevir\Genafor –geno2pheno device for drug resistance device (Center of European Research, Germany; www.coreceptor.bioinf.mpi-inf.mpg.de).

The database is coreceptor.bioinf.mpi-inf.mpg.de) for HBV, which is designed for phenotyping of HBV and rapid virtual computer-assisted also accepts sequences of the genome (nucleic acid) as Input. Geno2pheno searches between input sequences and homology with others, this store immediately in its database, also relevant clinical data for HBV genotype/subgenotype stored with Drug resistance/S gene mutations. By direct sequencing data accumulated were analyzed by utilizing the geno2- pheno tool for drug resistance and S gene mutations or manually. The pol/S gene segments overlapped were

searched for 344/266 amino acid codons 266/344 in the pol \gene of reverse transcriptase (RT) domain.

3.6. Statistical Analysis

Descriptive analysis was performed on data collected and a table of percentage of variables has been presented. Using Pearson chi-square and Mann-Whitney test the data were analysed.

The *p*-value 0.05 considered significant for all the methods. All analyses were done using the Statistical Package for Social Science (SPSS) software (Version16.0 IBM Armonk, New York).

4.1 RESULT

Of 93 patients have CHB disease from Turkey recruited in this study, 64 (68.8%) were males and 29 (31.2%) were females with a mean age of 44.96 ± 13.98. The minimum age of the patients was 18 and the maximum age was 81 (Table 1). The city of Kocaeli was the highest HBV infection patients 51 (54.8%), followed by Istanbul with 18 (19.4%) and Bursa with 7 (7.5%). All other cities had about 2% or below as represented in Table 4.1.

Table 4.1 Characteristics of patients with chronic HBV infection in Turkey

Characteristic	Patient	
Patient, n	93	
Gender, n (%)	Male 64 (68.8%)	female 29(31.2%)
Age range	26 -70	
HBsAg positive, n (%)	93 (100%)	
HBeAg positive, n (%)	18 (19.4%)	
HBV DNA load, median IU/ml	10330.00 (1.2+E2 – 4.2+E8)	

All patients were native residents of Turkey and indicated positive for HBsAg. Of the 93 patients, 75 (80.6%) were negative and 18 (19.4%) were positive for HBeAg. Using Pearson chi-square, the results indicated that there was no significant relationship between gender and HBeAg status, X2 (1, N = 93) = 0.835, p >0 .05. This means that the male and female were equally affected by the HBV. The Mann-Whitney test of the relationship between the ages of the patients with HBeAg positive and negative status showed that there was no statistically meaningful relationship between the groups (P>0.05). The mean rank of the age of HBeAg positive patients was 40.08 and that of the age of HBeAg Negative patients was 48.66.

From the total only twenty cases unsuccessful determine Genotype. Thus, the HBV genotypes were screened for 73 patients. Genotype D was recognized in 72 patients' samples and genotype A was determined in 1 patient's sample as shown in Figure 4.2. Of the 72 patients with subgenotype D, subgenotype D1 was found in 64

(88.89%), with subgenotype D2 in 5 (6.94%), subgenotype A2 in 2 (2.78%), subgenotype D3 in 1 (1.38%) and subgenotype D4 in 1 (1.38%) as shown in Figure 4.3.

Table 4.2 Mutation features of chronic hepatitis B infected patients in Turkey.

Patient	Gender	Age	Treatment Status	Polymerase gene mutation pattern	Surface gene mutation pattern
1	Female	55	3 years LAM	US	US
3	Male	35	5 years TDF	US	US
4	Male	46	1-year LAM, after 2 years ETV, and lastly 3 year TDF	US	US
5	Male	29	3 year TDF	US	US
6	Male	32	2 year ETV	MND	MND
8	Female	34	1.5 year LAM	Q149K, Q215S	MND
9	Female	55	ND	L91I, Q215S	MND
10	Male	67	ND	M204I	W196L
15	Female	55	3 year LAM	US	US
17	Male	43	ND	Q149K	MND
19	Male	44	ND	L91I	MND
21	Male	62	ND	V173L	E164D
23	Male	29	1 year ETV, 2 month TDF	US	US
24	Male	53	ND	L180M, M204I	W196L
27	Male	36	ND	L91I, M204I,	G130R, Y134N, W196L
29	Male	34	ND	MND	Q129H
31	Male	36	1-year classical IFN, 2-year LAM, 1-year ADV, and lastly 2 years ETV	MND	MND
33	Male	52	ND	S202G,M250V, M204I, N236D	Q129R , N131D , W196L,
35	Male	27	ND	L80I, M204I	W196L
36	Male	46	ND	A194S, V214A	MND
38	Male	39	ND	V173L, L180M, M204V	E164D, S193L, I195M
41	Male	26	9 year ETV	V173L, M204I,	T131I, E164D, W196L
42	Female	52	ND	M250W	MND
44	Female	67	LAM, LDT, ADV, ETV and TDF	US	US
45	Male	70	2-year LAM, 7-year ADV, 2 year TDF	MND	MND
46	Male	70	ND	M250W	MND
48	Female	47	ND	MND	S193L
49	Male	34	4 year LDT	US	US
51	Female	56	ND	Q149K, L180M, M204V,	I195M
52	Male	42	5-year IFN, 5-year LAM, 3-year ADV, 6 year TDF, 2 years ETV respectively	V173L, L180M, M204V	E164D, I195M
53	Female	52	5 year ETV	US	US
54	Male	38	1 year TDF + ETV	US	US
55	Male	36	ND	M250T	MND
56	Male	56	1year IFN, 4 year LAM, 9 year TDF	US	US

57	Male	58	1 year TDF	US	US
58	Male	28	1 year ETV	MND	MND
59	Male	27	7 year ETV	MND	MND
61	Male	57	2 year ETV	MND	MND
63	Female	38	ND	MND	Q129H
65	Male	60	2 year ETV	L91I, M404I, Q149K	T126S, W196S
69	Male	27	ND	MND	I110L, S143L
70	Male	51	1 year TDF	MND	MND
71	Female	65	ND	Q215H	MND
73	Male	35	ND	MND	T118K, T126I, Q129H, P142S
74	Male	78	3 year TDF	MND	D144E
75	Male	49	1 year TDF	MND	MND
76	Male	31	ND	L80V, M204I	W196L
77	Male	49	3 year ETV, 1 year TDF	MND	MND
78	Male	54	ND	Q215H	MND
80	Female	57	ND	Q149K, M250V	MND
81	Female	71	1 year ETV, 1 year TDF	MND	MND
86	Male	81	10-year LAM, 9 year TDF	MND	MND
87	Female	57	ND	L80V, L91I, L180M, M204V, Q215S	I195M
88	Male	51	ND	L180M, M204V	I195M
90	Male	33	5 year TDF	MND	MND
92	Female	45	3 year ETV	US	US

Note: ND, No data; MND: Mutation not detected; US: Unsuccessfully Sequencing; LAM: lamivudine; ADV: adefovir; LdT: telbivudine; TDF: tenofovir; ETV: entecavir

A large percentage of the patient's treatment information 62 (66.7%) could not be found (Figure 4, Table 2). Of the other patients, 13 (14.0%) The largest group used for treatment was between 1 to 3 years (> 1 year ≤ 3 years), 6 (6.5%) had treatment between the largest range than 3 years to 5 years (> 3 years ≤ 5 years), 5 (5.4%) had treatment for more than seven years (> 7 years), 4 (4.3%) had treatment between the range greater than five years to seven years (> 5 years ≤ 7 years) and 3(3.2%) had treatment between the range of one year and below (≤ 1 year) as represented in Figure 4.4.

From the mutation analysis of the polymerase gene in the 93 patients with CHB in Turkey, only 25 (26.9%) showed mutation, 48 (51.6%) indicated no mutation and 20 (21.5%) were unsuccessfully sequenced as shown in Figure 4.5 and Table 2. For the surface gene mutation analysis, 20 (21.5%) showed mutation, 53 (57.0%) indicated no

mutation and 20 (21.5%) were unsuccessfully sequenced as shown in Figure 4.6 and (Table 2).

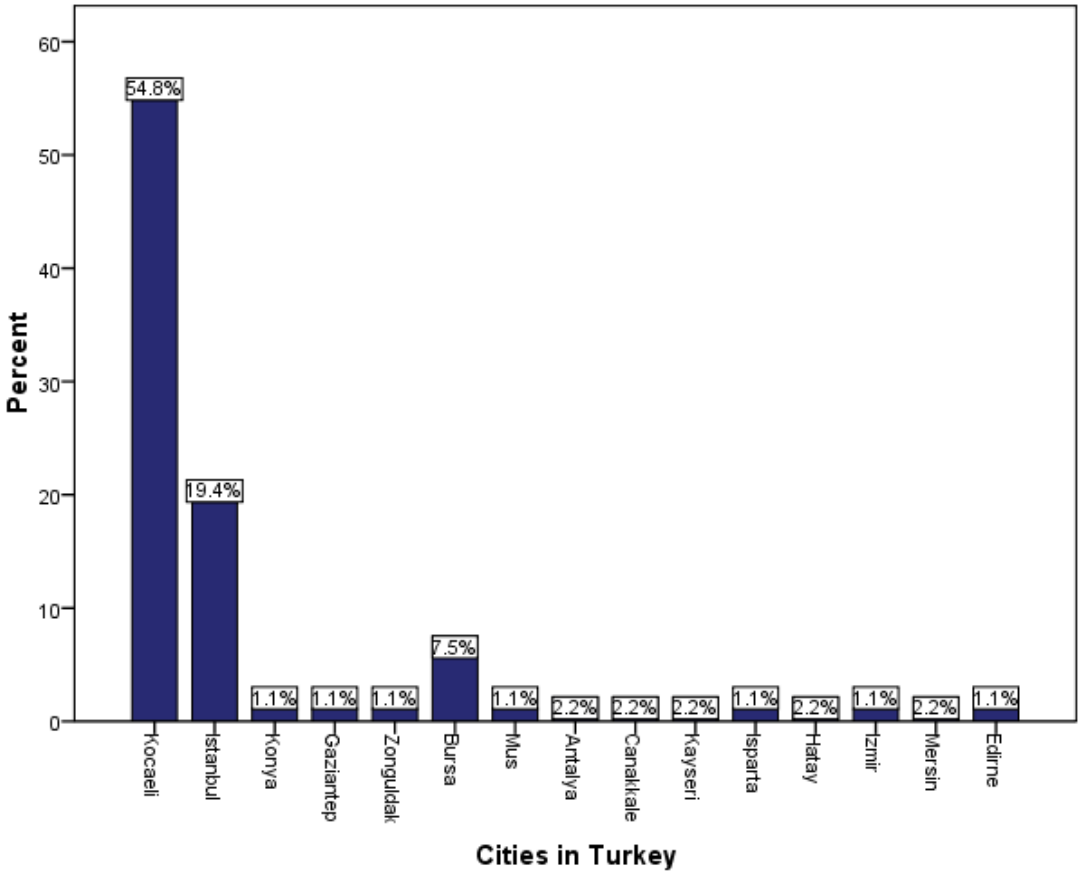

Figure 4.1 Distribution of hepatitis B virus in the cities of Turkey.

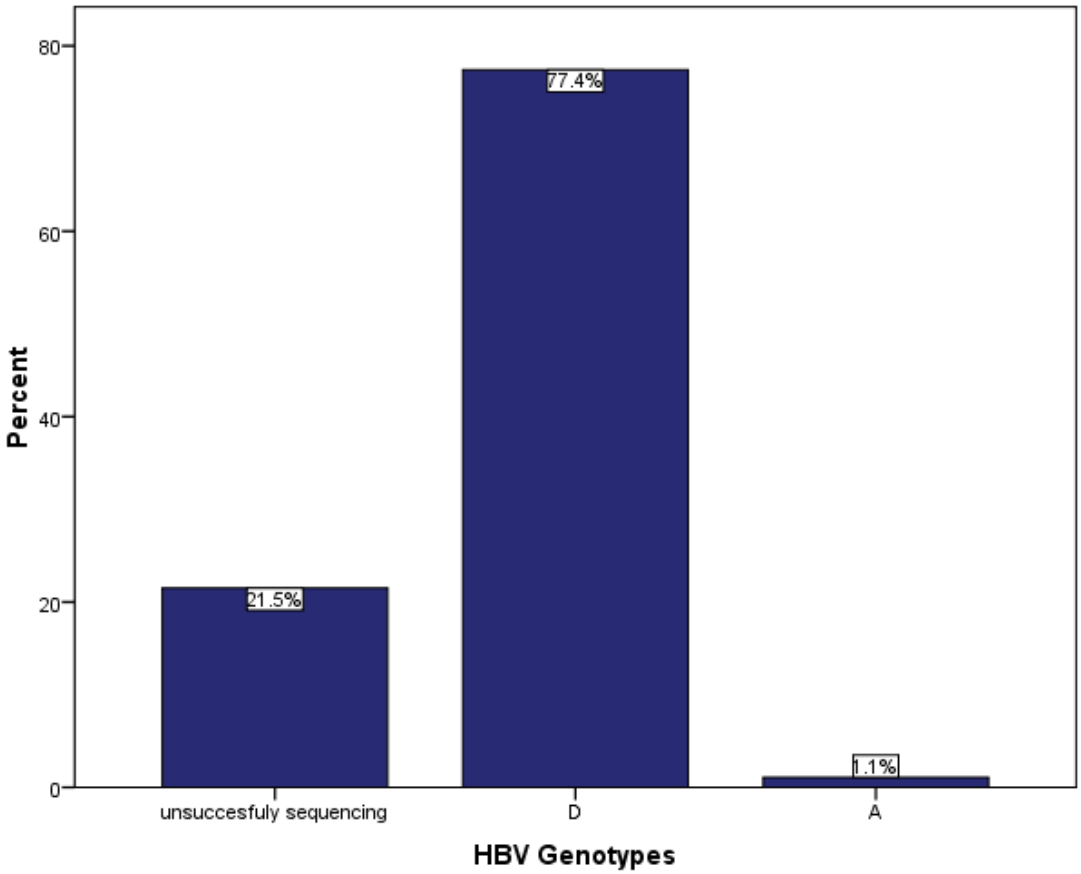

Figure 4.2 Hepatitis B virus genotype results in infected patients in Turkey.

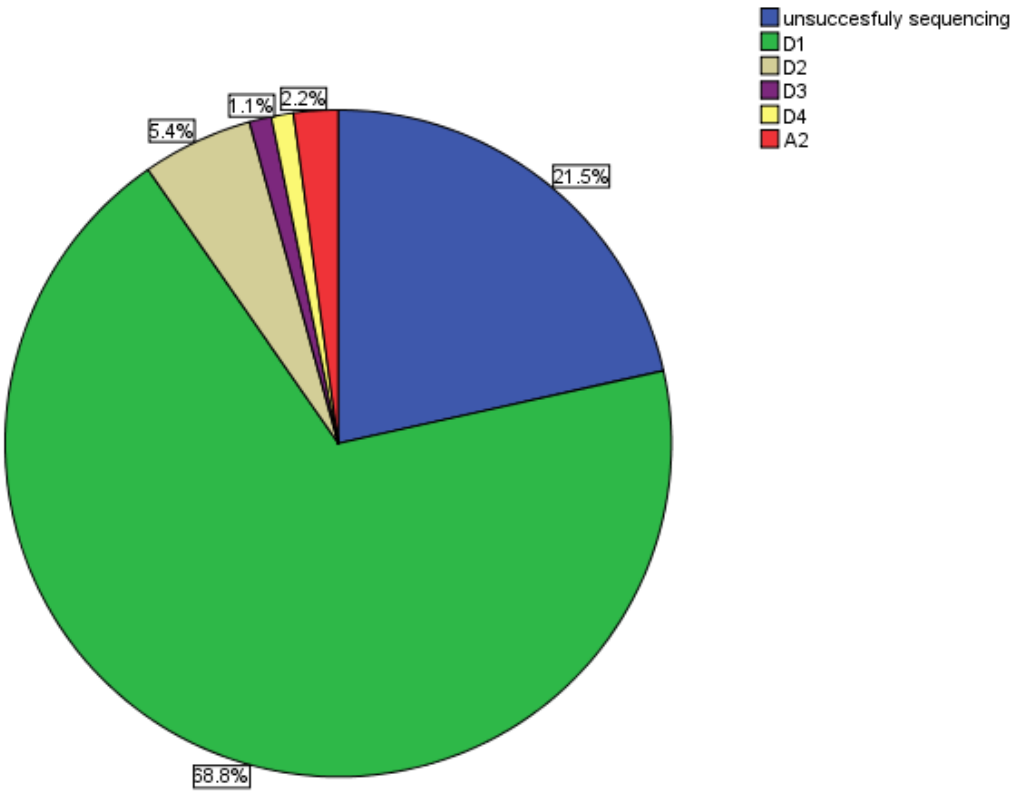

Figure 4.3 Hepatitis B virus subgenotype results in infected patients in Turkey.

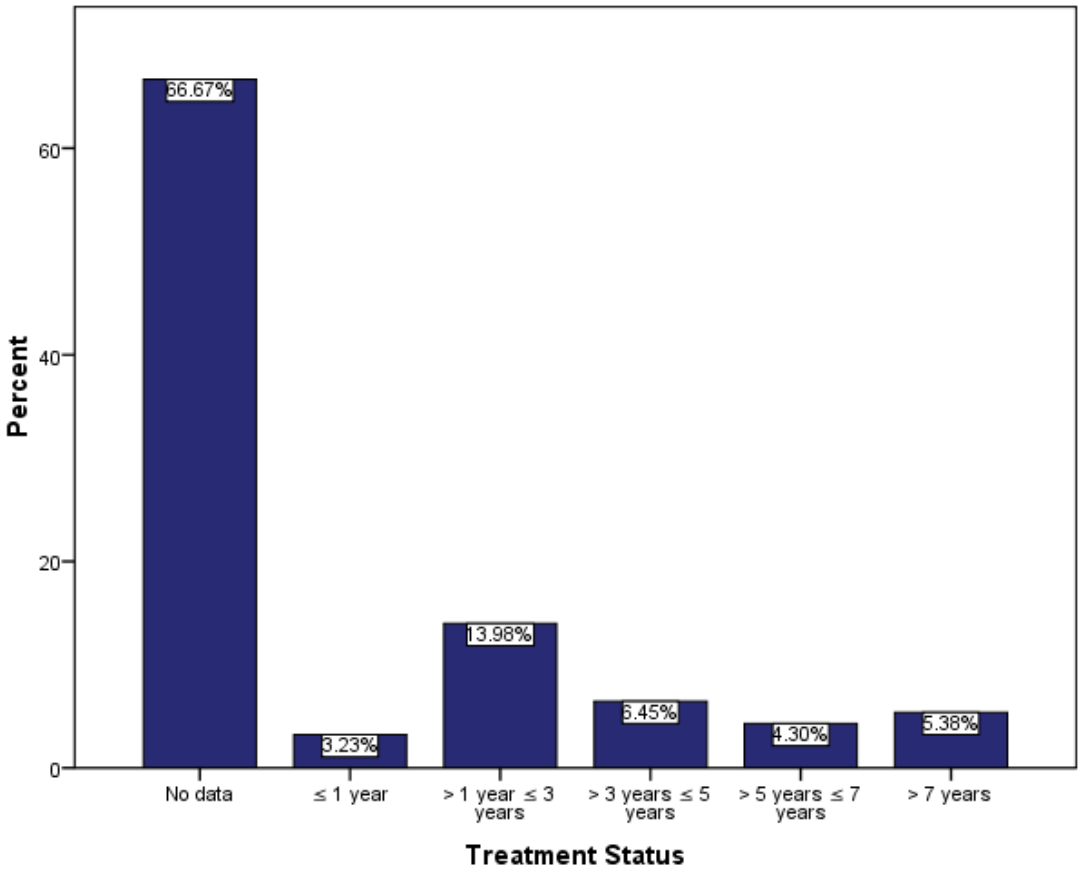

Figure 4.4 Treatment status of chronic hepatitis B patients in Turkey.

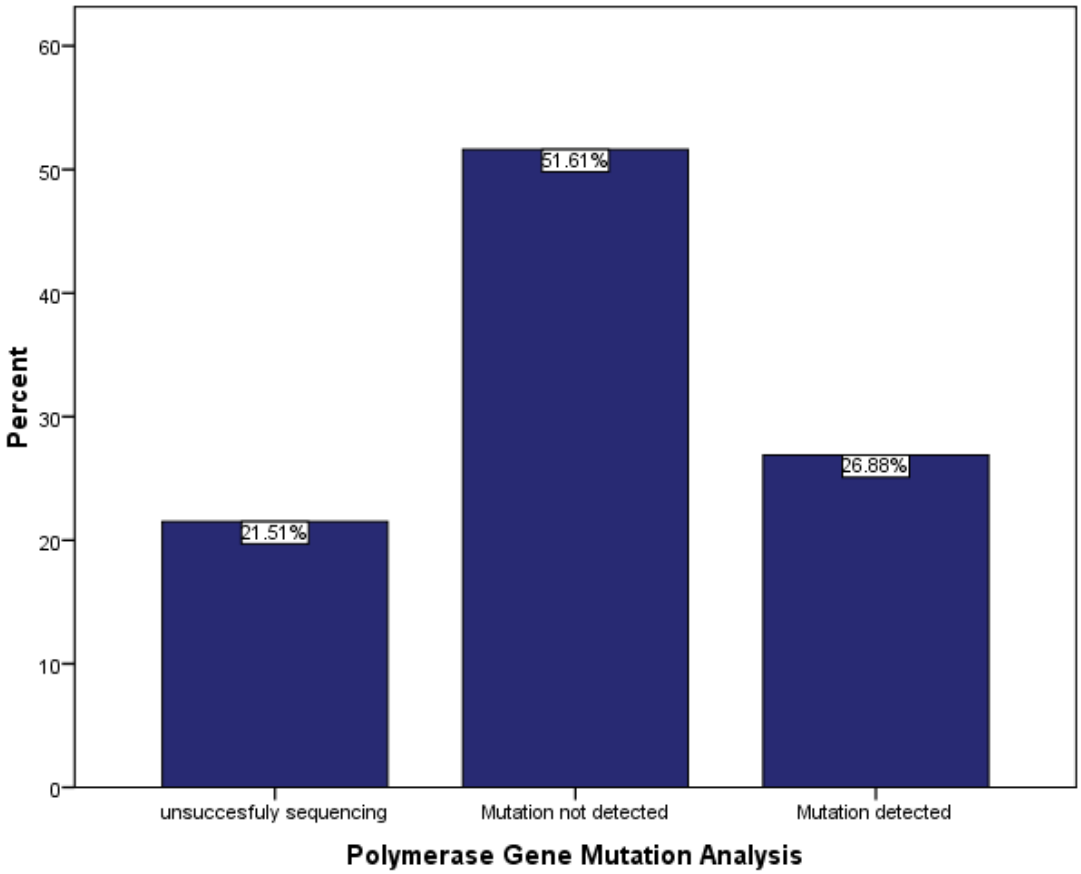

Figure 4.5 Polymerase gene mutation analysis in chronic hepatitis B patients in Turkey.

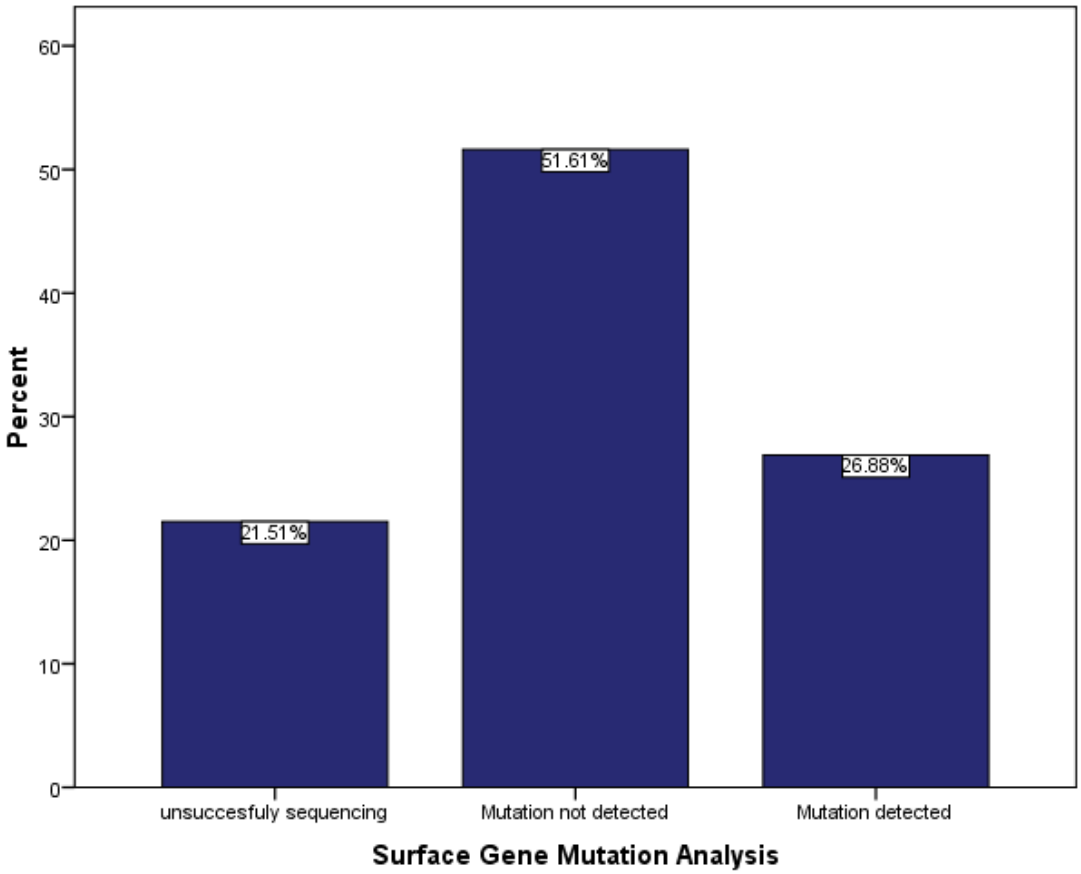

Figure 4.6 Surface gene mutation analysis in chronic hepatitis B patients in Turkey

5.1. DISCUSSION

Turkey is one of the WHO European Region (51 countries) who by 2000 has implemented a universal HBV immunization program (Van Damme, 2001). The expectation was that the immunization program would aid the protection of people from HBV infection. About 20 years have passed now; it seems more need to be done to completely eradicate HBV.

A number of HBV transmission routes have been shown in Turkey, the most common being blood contact, followed by heterosexual (Leblebicioglu, et al., 2004). Perhaps, the sexual transmission may have been underreported due to social issues.

In this study, all patients indicated positive for HBsAg (Table 1). This implies that all these persons can pass on the virus to others through blood contact.

In Turkey, the prevalence of HBV varies across different geographical regions. The results in this study indicate that Kocaeli has the highest HBV infection 51 (54.8%), followed by Istanbul 18 (19.4%) and Bursa 7 (7.5%) with other cities following with about 2% or below (Figure 4.1). In a study in 2004, 6% of infection was found in western parts of Turkey with the eastern and southeastern part of Turkey has the highest prevalence of about 12.5–14.3% (Leblebicioglu, et al., 2004).

Of the 93 patients, 75 (80.6%) were negative and 18 (19.4%) were positive for HBeAg. In contrast with the findings in this study, a study in Germany indicated more HBeAg positive (64) than and HBeAg negative (32) after analyzing the sera of 96 patients with chronic hepatitis B (Erhardt et al., 2000). HBeAg is a marker of active viral proliferation in the liver cell and infectivity (You, et al., 2004). Using Pearson chi-square, there was no significant relationship between gender and HBeAg positive status, $X2\ (1, N = 93) = 0.835$, $p > 0\ .05$. This means that both the male and female were equally affected by the HBV. However, a previous study reported that male gender was an important risk factor for HBV exposure both in the rural and urban areas of Turkey (Mehmet et al., 2005). The results in this study did not corroborate that.

There was no statistically meaningful relationship between the ages of the patients with HBeAg positive and negative status (P>0.05). This means age does not confer susceptibility to HBV. In contrast with this study, Mehmet and colleagues reported that the exposure to HBV in the south-eastern part of Turkey increases with age having the age group greater than 64 susceptible to HBV in both rural and urban areas (Mehmet et al., 2005). In yet another study in Taiwan, the prevalence of HBeAg in chronically HBV infected persons decreased with the increase in age (You, et al., 2004). From these results, it suggests that the role of age in the HBV infection is still contentious.

Genotyping in HBV infected patients may serve as viral genetic markers to assist in predicting the disease progression as well as aid therapy development (Lin, and Kao, 2015). In this study, genotype D and A were recognized in 72 and 1 patient respectively, indicating that the D genotype is prevalent in Turkey than other HBV genotypes. This result is in agreement with what has been reported earlier that Genotype D is predominant in the Mediterranean region (Kao, 2002). Specifically, many studies have indicated that genotype D represent almost all the HBV patient population in Turkey (Bozdayi et al., 2004; Bozdayi et al., 2005; Sayan et al., 2009a; Sayan et al., 2010). At least 10 HBV subgenotypes (A to J) have been identified worldwide (Lin, and Kao, 2015).

The prevalence of certain genotypes play an important role in heterogeneous clinical results often find in HBV chronic patients (Bozdayi et al., 2004). HBV genotypes D patients have been noted to have a lower rate of seroconversion of HBeAg, higher frequency of pre-S mutations and core promoter, risk of chronically infected by HBV increased of development hepatocellular and cirrhosis, lower response to interferon-based therapy (Kao, 2002; Lin, and Kao, 2015).

Although 18 (19.4%) out of the 93 patients' sample indicated positive for HBeAg in this research, a correlation between HBeAg and the genotypes was inconclusive because a screen of genotypes was unsuccessful in 20 patients (Figure 4.2). In their study of the epidemiology and genotype distribution of acute HBV infection in

Turkey, Lin and Kao (2015) used RFLP assay to classify HBV genotypes. However, genotype was not detected via PCR in 18 samples. Thus, nested PCR which is 1000-fold more sensitive was used. In this study, the 20 unsuccessful cases were not able to be further determined by a more sensitive method because of constraints. However, there is a link between HBeAg and HBV genotypes has been shown by many previous studies In a study from Switzerland genotype A was found to be predominant in chronic HBV patients. While genotype D in acute HBV patients was more common. indicating such genotype D has been decreased among chronic HBV patients (Mayerat et al. 1999). Yet genotype D seemed to be more linked to the most severe disease in the liver and possibly hepatocellular carcinoma among young patients in India. (Thakur et al 2002).

Finding out genetic mutation in HBV patients from surface genes and polymerase have immense diagnostic relevance and health benefits (Sayan et al., 2009b). From the mutation analysis of the polymerase gene in this study, nineteen (19) polymerase gene mutation was detected in the 25 (26.9%) patients: A194S, L180M, L80V, L91I, L80I, M204V, M250V, M250V, M204I, M404I, M250T, M250W, N236D, Q215S, Q149K, Q215H ,S202G, V173L, V214A (Table 2 and Figure 4.5). The mutation was not detected in 48 (51.6%) patients and sequencing was unsuccessful in 20 (21.5%) patients' samples (Figure 4.5). For surface gene mutation, eighteen (18) were detected from 20 (21.5%) patients: D144E , E164D, G130R, I195M, I110L, N131D, P142S, Q129H, Q129R, S193L, S143L, T131I, T118K, T126I, T126S, W196L, W196S, Y134N (Table 2 and Figure 4.6). From the remaining patients, 53 (57.0%) indicated no mutation and 20 (21.5%) were unsuccessfully sequenced as shown in (Figure 4.6). The relationship between HBV infection and genotype or between treatment status and mutation could not be ascertained in this study due to either incomplete sequencing or the absence of complete data about patients' treatment status.

5.2. Conclusion

In conclusion, this study showed that the percentage of the surface gene, polymers gene mutation detected is (22%) and (25%), respectively. While, the

percentage of not detected mutation on both of them (55%)and (53%), with most of the HBV genotype and Sub genotype as D and D1 respectively. However, the study is restricted by the small sample size and some of the data could not be established. The study recommends that prevention policy to the HBV transmission needs to be mutation developed; ensuring that genotyping and subgenotype for a geographic region are carried out. In addition, HBV mutation dynamics should be monitored coupled with ensuring drug resistance analysis is performed on the viral rebound during the therapy.

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