Hepatitis B Virus Prevalence among Patients with Chronic Liver Hepatitis in Taif Region of Saudi Arabia: Serological and Molecular Methods

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Abstract: The prevalence of serological markers of HBV and most prevalent genotypes were determined in Taif region in the West area of the country among chronically HBV infected residents between 2001-2004. HBsAg, anti-HBc, HBeAg and anti-HBe were screened using an ELISA commercial reagent kit and existence of HBV-DNA was confirmed by Polymerase Chain Reaction (PCR). Higher prevalence of HbsAg (36.2%), anti-HBc (27.2%), anti-HBe (22.4%) and then HBeAg (19.4%) were detected in Saudi patients. Among non-Saudi carriers HBV markers were represented as 51.7, 43.5, 33.2 and 26.7%, for HbsAg, anti-HBc, anti-HBe and HBeAg, respectively. There was not a marked difference between Saudi and non-Saudi patients with regard to HBV marker distribution rates. Prevalence of HbsAg among non-Saudi nationalities showed a higher incidence among Filipinos (16/16; 100%), followed by Bangladeshis (19/21; 90.5%), Pakistanis (21/28; 75%), Egyptians (11/18; 61.1%) then Indians (21/35; 60%). In all groups prevalence of HbsAg was higher in males than females. HBV-DNA was detected only in 31.5% Saudi and 46.6% among non-Saudi patients who had a chronic hepatitis B infection by using PCR reaction. This indicating that level of HBV-DNA in serum of those patients was very low that couldn’t be detected by this method. The predominant genotypes in the selected patients in the Saudi population was B (34.8%), type C (4.4%) and type A (1.1%) and in non-Saudi patients was B (41.4%), type C (12.2%), type A (2.8%) and type E (2.2%). Type B as mainly expressed in patients with positive HbsAg and anti-HBc marker (77.2 and 72.7%, respectively), followed by patients showed positivity for HBeAg and anti-HBe (66.7 and 62.5%, respectively). These data clearly indicate that, detection of hepatitis B viral infection by a serological markers and PCR reaction may be helpful for identification of the phase of infection.

Key words: Chronic HBV infection, antibodies-HBcore, HbsAg, HBeAg, Hepatitis B type A, PCR-DNA, Enzymelinked immunosorbant assay, genotyping of HBV

INTRODUCTION

Hepatitis B virus (HBV) infection is one of the major public health problem in the world and may cause both acute and chronic infection in man (Maddrey, 2000), causes of most infectious disease mortality worldwide (Lee, 1997). The clinical spectrum of HBV infection ranges from sub-clinical to acute symptomatic hepatitis (acute phase) and from the inactive hepatitis B surface antigen (HBSAg) carrier state, chronic hepatitis of various degree of histological severity to cirrhosis and its complications during the chronic phase (Lok et al., 2001). Approximately 2 billion people have serologic evidence of past or present HBV infection (Alter, 2003). In addition to the deaths caused by acute infections (estimated at around 50,000 each year; Bonanni et al., 2003), HBV infection is the recognized cause of chronic infection affecting about 350 million people (Kane, 1995). Each year over 1 million people die annually from HBV-related chronic liver disease including cirrhosis and hepatocellular carcinoma HCC (Beasley, 1988). Hepatocellular carcinoma is one of the most common cancers worldwide and HBV is responsible for at least 75% of these cancers (Conjeevaram and Fong Lok, 2003).

The endemicity of HBV infection varies greatly world-wide and influenced primarily by the predominant age at which infection occur (Margolis et al., 1997; Yuen et al., 2009). Endemicity of infection is considered high in many parts of the world where 8% at least of the population is hepatitis B surface antigen positive (HBSAg carriers>8% in the general population) as South East Asia, most Africa and the equatorial area of South America. In

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area of the world with an intermediate pattern of HBV infection, such as pacific Islands and Arctic, the prevalence of HBsAg positivity ranges from 1 to 7%, while in most developed parts of the world, as Western countries, the prevalence of chronic HBV infection is <1% (Alter, 2003). Areas with a high endemicity of HBV infection have the highest rate of chronic HBV infection which is strongly associated with hepatocellular carcinoma (HCC) that leads to high death rates from this neoplasm (Alter, 2003). Approximately, 15-40% of patients with chronic hepatitis B progress to cirrhosis and end-stage liver disease (Maddrey, 2000; Yang et al., 2009). There is also data to suggest that survival among cirrhotic patients is lower among those who are HBeAg positive (Realdi et al., 1994).

Recent studies had shown that levels of HBV-DNA reflect the state of chronicity in HBsAg-positive patients and high level of HBV-DNA might lead to more severe liver damage (Lindh et al., 2000). Chronic hepatitis B infection consists of four phases. The first phase of chronic HBV infection is usually characterized by the presence of hepatitis B e antigen (HBeAg), HBsAg, low level of ALT (alanine aminotransferase) and high level of serum HBV-DNA (inactive HBsAg carrier state), the second phase is characterized by presence of HBeAg, HBsAg and high serum level of HBV-DNA and ALT (HBeAg-positive chronic hepatitis), non-replicative phase is the third phase of chronic HBV infection which characterized by clearance of HBeAg and HBsAg and sero-converted to anti-HBe and anti-HBs, respectively and undetected HBV-DNA level. The fourth phase of chronic hepatitis B infection (HBeAg, HBsAg negative and anti-HBs, anti-HBe positive) and this final phase (resolution phase of infection, Lok et al., 2001).

Persistence of HBsAg, HBeAg and HBV-DNA for more than 6 months implies progression to chronic HBV infection (Fong et al., 1994). Age at the time of infection is the best determinant of chronicity. Seroconversion of HBeAg in chronic hepatitis B to anti-HBe with marked reduction of HBV-DNA is associated with regression of inflammation (Whalley et al., 2001). The presence of HBeAg in serum correlates with higher tier of HBV and greater infectivity (Shikata et al., 1977). Appearance of anti-HBs is the best serological indicator of recovery from the infection. A persistence of high HBV-DNA and HBeAg positivity predict evolution of the infection (Whalley et al., 2001).

HBV now classified into seven major genotypes (A-G) based on an inter-group divergence in the complete nucleotide sequence (Kidd-Langgren et al., 2002; Chu and Lok, 2002). The geographical distribution of HBV genotypes is varied with genotype A being more common in Northwest Europe and North America; genotype B and C being more common in Asia; and genotype D most common in Southern Europe and India. The distribution of genotype E is restricted to Africa, while genotype F is found in central America. Genotype G has been identified in France and North America very recently (Stuyver et al., 1999; Morgan and Keeffe, 2009) and most recent reported about genotype H is less clear. Earlier studies (Kao et al., 2002a) indicated that HBV genotypes B and C are the most prevalent viral strains in Taiwan and genotype C is associated with the development of cirrhosis and HCC while genotype B may be associated with the development of HCC in young patients (Kao et al., 2002c). In addition, HBV genotype C infection have a lower rate of spontaneous HBeAg seroconversion and higher rates of cirrhosis compared to those with genotype B (Kao et al., 2002a). Among interferon alpha treated patients, genotypes A and B is associated with a lower frequency of core promoter mutation and reported to be associated with higher rates of antiviral response therapy compared to genotypes D and C (Zhang et al., 1996; Kao et al., 2000b).

The high prevalence of HBV infection in Kingdom of Saudi Arabia (KSA) is well reported. Since, 1990s approximately 8% of apparently health children are positive for HBsAg (HBV carriers), 60% have evidence of post exposure of HBV and 20% are positive for at least one marker of HBV (Faleh et al., 1992). The average prevalence rate of HBsAg in Saudi adults population is approximately 8% (Faleh et al., 1988; Tandon et al., 1995). However, several surveys have shown marked regional variations with prevalence of HBV in KSA (Ikram et al., 1988). The Jizan region of KSA was a focus of hyper-endemic HBV infection and its sequel of chronic liver disease and hepatocellular carcinoma. A comparative study of different regions of KSA reported a rate of HBsAg to be 32.2% in Jizan, compared to a rate of 4.7% in a similar population in Riyadh central region of KSA. Yet no survey was done for Taif Region in the West of KSA.

This study was designed to estimate approximately prevalence of HBV markers among the HBV chronically infected patients in Taif region in KSA by serological and molecular techniques and determine the most predominant genotypes of HBV among Saudi and non-Saudi patients during the period of study.

MATERIALS AND METHODS

Subjects: Serum samples were collected from 632 consecutive patients who admitted to gastroenterological clinics of different hospitals in Taif restrict area (King
Faisal Hospital (KFH), National Milliary Hospital (NMH),
King Mansour Hospital (KMH) from the period 1-2002 to
1425H (2001-2004) who were suffering from some
complications in gastrointestinal area for HBV detection.
Four hundred patients were eliminated from the test
because of positivity for antibodies to hepatitis C virus
(anti-HCV), hepatitis D virus (anti-HDV) or had any other
serological markers suggestive of autoimmune disease.
Two hundred and thirty two patients showed seropositivity for at least one marker of HBV (187 males and
45 females; aged from 25 to 70 years). Patients were
classified as a chronic HBV infection due to persistent
seropositivity for one marker of HBV for at least 6 months
divided mainly into two groups according to the status of
hepatitis B surface antigen (HBsAg). Group 1 comprised
166 patients who sera tested positive for HBsAg, among
them 64 were Saudi citizens and 102 were non Saudi.
Group 2 that showed a negative test for HBsAg and was
consisted of 66 patients (29 Saudi nationals and 37 non-
Saudi). Group 2 were subsequently divided into three
subgroups. Subgroup 2a consisted of 42 patients
(24 Saudi and 18 non-Saudi) that showed a positivity
towards hepatitis B e Ag (HBeAg), group 2b consisted of
7 patients (3 Saudi and 4 non Saudi) whose sera tested
negative for HBeAg and one positive for antibodies of
hepatitis core Ag (anti-HBc) and group 2c consisted of
17 patients (2 Saudi and 15 non-Saudi) were negative for
all above HBV markers and positive against antibodies to
hepatitis E Ag (anti-HBe). A questionnaire was completed
for each patient to obtain information on age, sex,
nationality and history of previous jaundice, blood
transfusion, or past surgical operation. These patients
received liver tests including serum Alanine
Aminotransferase (ALT) activity every 3 months. Serial
 sera samples taken from each patient were stored
at-20°C until used.

**Hepatitis virus markers:** Serological assays for hepatitis
markers were performed by using commercial available
enzymes immunoassay (EIA) reagent kits. For HBV
markers, serum HBsAg, anti-HBc, HBeAg and anti-HBe
were tested by Ausria-II/HbsAg, Ausah/anti-HBc and
Corab for remaining markers (Abbott Laboratories, North
Chicago, Illinois). Anti-HCV and anti-HDV were detected
by third generation EIA (Murex III) and anti-Delta (Abbott
Laboratories), respectively.

**Genotyping of HBV:** Serum HBV genotypes were
determined by using an HBV-Genotype EIA commercial
kit (Institute of Immunology, Tokyo, Japan) under
manufacturer’s instructions (Usuda et al., 1999).

**Molecular methods PCR amplification of serum DNA:**
One hundred microliter of serum was incubated at 65°C for
1 h. with proteinase K (100 μg mL⁻¹), 0.5% sodium
Dedosyle sulfate, 5 mM EDTA, 10 mM Tris-HCl, pH 8.0.
The solution was phenol/chloroform extracted and the
DNA was precipitated with ethanol in the presence of
salmon sperm as carrier (100 μg mL⁻¹). The precipitate
was dissolved in 50 μL of 10 mM Tris- HCl, pH 8.0 and 1 mM
EDTA. The entire core gene was amplified in 232 samples
by PCR reaction to confirm the viremia using
oligonucleotide primers specific for HBV core gene
sequence (1763F and 2032R) as described earlier
(Kaneko et al., 1989).

Analysis of amplified DNA. Amplified DNA reaction
mixture for each sample was fractionated by 3% agarose
gel electrophoresis and DNA was visualized by UV
fluorescence after staining with ethidium bromide. The
DNA was transferred to a hybridization nitrocellulose
membrane for Southern blot hybridization analysis using
the recombinant HBV DNA labeled to a high specific
activity as described before (Kaneko et al., 1989).

**Statistical analysis:** Collected data were analyzed
using Fisher’s exact test, Chi-square test with
Yates’ correction, univariant and multivariant analysis
by multiple logestic regression (Armitage, 1971) and
Student’s test where appropriate. Odds Ratios (OR)
and corresponding 95% confidence intervals (95% CI)
were used to estimate relative risk according to
(Castillo et al., 1979).

**RESULTS**

Of 232 chronic HBV infected patients, 198 (85.3%)
had elevated serum ALT levels (>40 U L⁻¹) among them
75 patients Saudi (32.3%) and 123 patients were non Saudi
(53.0%). The ratio of males to females in collective sample
was mostly about 4:1, respectively. The majority of
patients were non-Saudi 139 (59.9%) and they were
mainly from the Indian subcontinent and South East Asia
84 (60.4%), while Egyptians were 18 patients representing
12.9% and Filipino were 16 patients representing 11.5%.
other nationalities (21 patients) represented randomly
among different countries (15.0%) in this population
sample (Table 1).

Among all nationalities, 166 patients were found
positive for HBsAg giving an overall prevalence of
HBsAg of 71.6% that was highly indicated for chronic
hepatitis over other serological markers. The prevalence
of HBsAg among Saudi patients was 68.8% (64 patients
out of 93) with no significant differences compared to
non-Saudi patients (73.4%, 102 of 139). The remaining
patients (66 out of 232; 28.4%) who were HBsAg negative further tested for HBeAg. Overall prevalence of HBeAg was 18.1% (42 of 232), Saudi patients (24 of 64) represented 28.8% while non-Saudi (18 of 139) represented 13.0%. Twenty-four patients who represented negative for both HBsAg and HBeAg were tested for anti-HBe. Only 7 patients (3%) were positive for anti-HBe and distributed between Saudi (3) and non-Saudi (4) with no significant difference in prevalence (3.2 and 2.9%, respectively). Other 17 patients showed a positive test for anti-HBe with percentage of 7.3%. Two Saudi were positive for anti-HBe (2.2%), while 15 patients were positive among non-Saudi (10.8%). There was a significant higher in prevalence of anti-HBe positivity among non-Saudi (10.8%) compared to Saudi (2.2%; p<0.001). HBeAg prevalence in Saudi citizens was 2 fold higher compared with non-Saudis. The estimated risk of being HBeAg positive was 1.87% (OR = 1.15; 95% CI: 1.05-1.25), a relatively low figure compared to non-Saudis. Among non-Saudis HBsAg positivity was the best indicator for HBV infection and represented highest among Filipinos (100%) followed by Bangladeshis (90.5%), Pakistanis (75.0%), Egyptians had a very low prevalence rate (61.1%) of HBsAg positivity. On the other side, patients who were positivity for anti-HBe as only HBV marker was poorly expressed of HBV infection (2.9-9.5%; Table 2).

PCR amplifications were performed with primers specific for core region of HBV. Amplified product of 312pb was observed (Fig. 1; panel a). Amplified PCR products were ascertained by Southern blot hybridization with a cloned full length HBV probe obtained from King Abd Aziz University (Fig. 1; panel b). Among the sample population, 181 (78%) were positive for serum HBV DNA by PCR assay, 73 samples from Saudi (31.5%) and 108 from non-Saudis (46.6%). Only 58 out of 64 chronic Saudi patients who were found HBsAg positive were also found HBV DNA positive by the conventional serum DNA PCR (90.6%), while non Saudi showed 87 positive of HBV DNA out of 102 positive HBsAg (85.3%). Total samples of HBV-DNA detected and HbsAg positivity were 145 out of 166 (87.3%). Among the HBeAg positive carrier 12 and 10 were found positive for HBV DNA by serum DNA PCR out of 24 and 18, respectively with sensitivity of 50.0 and 55.6, respectively. HBV-DNA was less detected in this

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<th>Table 1: Distribution of patients according to nationality and gender in the Taif Area in Kingdom of Saudi Arabia</th>
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<td>Characteristics</td>
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<td>Nationalities (n = 232)</td>
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<td>Saudi</td>
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<td>Non-Saudi (50% U L^{-1})</td>
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<td>ALT (&gt;40 U L^{-1})</td>
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<td>Indian</td>
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<th>Table 2: Nationality specific prevalence of hepatitis B markers by serodiagnosis and PCR</th>
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<td>Nationalities</td>
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<td>Saudi +ve PCR</td>
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<td>Non-Saudi +ve PCR</td>
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n: No. of positive samples for any HBV markers
Table 3: Distribution of HBV genotypes in different nationalities in Taif region of chronic hepatitis B patients with +ve PCR samples

| Nationalities (n = 181) | Genotype A | Genotype B | Genotype C | Genotype E | Others*
|------------------------|------------|------------|------------|------------|--------
| No. | %  | No. | %  | No. | %  | No. | %  | No. | %  |
| Saudi (n = 73) | 2 | 1.1 | 63 | 34.8 | 8 | 4.4 | -- | -- | -- | 1 |
| Non-Saudi (n = 108) | 5 | 2.8 | 74 | 41.4 | 22 | 12.2 | 4 | 2.2 | 3 | 1.7 |
| Indian (n = 27) | -- | -- | 25 | 13.8 | 1 | 0.6 | 1 | 0.6 | -- | -- |
| Pakistani (n = 22) | 3 | 1.7 | 17 | 9.4 | 2 | 1.1 | -- | -- | -- | -- |
| Bangladeshi (n = 20) | 1 | 0.6 | 17 | 9.4 | 2 | 1.1 | -- | -- | -- | -- |
| Egyptian (n = 15) | 1 | 0.6 | 4 | 2.2 | 9 | 5.0 | 1 | 0.6 | -- | -- |
| Filipino (n = 11) | -- | -- | 2 | 1.1 | 8 | 4.4 | 1 | 0.6 | -- | -- |
| Others (n = 13) | -- | -- | 9 | 5.0 | -- | -- | 1 | 0.6 | 3 | 1.7 |

*Might be other genotypes or unidentified

Table 4: Distribution of different genotypes among HBV markers

| Features | Genotype A (n = 7) | Genotype B (n = 137) | Genotype C (n = 30) | Genotype E (n = 4) | Other genotype (n = 3)
|----------|-------------------|----------------------|---------------------|-------------------|---------------------
| +ve HBsAg marker (n = 145) | 5(3.4%) | 112(77.2%)* | 23(15.9%) | 2(1.4%) | 3(2.1%)
| +ve anti-HBe marker (n = 22) | 1(4.5%) | 16(72.7%)* | 5(22.7%) | none | none |
| +ve HBsAg marker (n = 6) | none | 4(66.7%)* | 1(16.7%) | 1(16.7%) | none |
| +ve anti-HBe marker (n = 8) | 1(12.5%) | 5(62.5%)* | 1(12.5%) | none | none |

*p<0.001

Positive samples for HBV-DNA by PCR assay (181) were selected for genotypes identification and was distributed among Saudi (73) and non-Saudi (108) as follows: A, 2 (1.1%) and 5 (2.8%), respectively; B, 63 (34.8%) and 74 (41.4%), respectively; C, 8 (4.4%) and 22 (12.2%) respectively; E, 0 (0.0%) and 4 (2.2%), respectively. Remaining 3 samples may be other subtype of HBV or mixed subtype. Accordingly, genotypes B was the predominant strains among these patients persistently infected with HBV 137 out of 181 (75.7%), followed by genotype C that was persist in this study in 30 (25.4%). Egyptians and Filipinos were the predominant countries for this genotype compared to other nationalities.

Genotype A was detected in two samples in Saudi nations and 3 samples from Pakistani. In general genotype B was more prevalent than genotype C in the different nationalities of HBV chronic infection. However the prevalence of genotype E was very rare (0.6%) in our sample collection and was detected in 4 different countries as one sample each (Table 3).

The distribution of HBV genotypes in different HBV markers of chronic hepatitis B patients was analyzed (Table 4). In general, genotype B was represented among all positive HBV markers groups with highly percentage in positive HBsAg marker (77.2%), anti-HBe marker (72.7%), followed by positive serum for HBcAg (66.7%) and positive anti-HBe marker (62.5%). On the other side, genotype C was mainly expressed in patients with positive anti-HBc marker (22.7%) and to lesser extent among patients positive for HBsAg, HBcAg and anti-HBe markers (15.9, 16.7 and 12.5%, respectively). Genotype E was more expressed among patients with positive HBcAg (16.7%) than other markers. However, genotype A was more prevalent among patients positive for anti-HBe marker (12.5%).

DISCUSSION

HBV infection is a global health problem and more than 350 million people of the world population are chronic carriers of the virus (Fong, 1994). Although, many studies have shown that clinical course of chronic hepatitis may be modified by several viral factors. The real significance of such factors with the course of chronic hepatitis remains unclear. One of reasons that make interpretation of the effect of such viral factors difficult is exclusion of HBV genotypes for analysis.

Prevalence of HBV markers: The majority of HBV positive number in our study were non-Saudi (93 Saudi and 139 non-Saudi out of 232; 60% versus 40% respectively; p<0.001), predominantly non-Saudi were from India subcontinent and Far East (87%), Egyptian HBV infected patients represented 13.0% from total non-Saudi HBV infected population, this is probably due to the mandatory screening of all expatriates prior to granting residency in KSA. In addition, the prevalence of HBsAg was significantly higher in non-Saudi compared with Saudi nationals (44.0% versus 27.7%; p<0.001).

This differences readily explained by the fact that these countries are hyper-endemic HBV foci (Gust, 1996; Elgouhari et al., 2009). This prevalence is higher than the rates reported before in KSA which proved that, the
overall prevalence of HBsAg is high as 16.7%, while the prevalence of HBV antibodies in the absence of HBsAg was 31.8% and the overall prevalence of anti-HBc was proven to be as high as 31.2% (El-Hazmi, 1989). However, the low prevalence of HBsAg negative but positive anti-HBe among Saudi (0.9%) that contradicts previously local studies, which indicating a higher prevalence up to 31.4% (El-Hazmi, 1989). This could be due to selection bias and small sample size. On the other side, high prevalence of patients with negative HBsAg but positive for anti-HBe among non-Saudi (6.5%) indicating that those patients were usually in recovery phase from past HBV infection. However, patients with HBeAg positive chronic hepatitis B, the incidence of cirrhosis ranges from 2-5 per 100 persons a year (Fattovich, 2003). The variability in the rate of progression to cirrhosis may be related to differences in the clinical and serological features of HBV infected patients (Van Thiel et al., 1999). Studies have shown that the risk of transmission of HBV infection has been as high as 78% when anti-HBe positive contact with HBV negative (El-Hazmi, 2004). This phenomena was explained by the existence of HBV in the liver tissues of healthy HBsAg negative and anti-HBe positive but not in their serum (Gust, 1996).

**Genotypes of HBV infection:** HBV genotypes related to severity of disease (Lindh et al., 2000; Elgouhari et al., 2009). Such facts demonstrate the importance of determining HBV genotypes for analysis of HBV related liver disease. A genetic classification of HBV isolates based on the comparison of complete genomes has been recently defined seven genotypes of HBV (A to G; Realdi et al., 1994). HBV genotypes have distinct geographical distribution (Kao et al., 2002). Generally, genotypes B and C are prevalent in Far-Asia (Chu and Lok, 2002) and in KSA as well. In HBeAg positivity patients with chronic HBV infection, present data consistently showed that genotype B (137/181; 75.7%) was the most predominant HBV followed by genotype C (30/181; 16.6%). Other genotypes accounted for only a minimal proportion (3.9% for genotype A, 2.2% for genotype E and 1.7% for other genotype or unidentifed three samples) with no significant difference between Saudi and non-Saudi. Out of 27 indians 25 patients were HBV genotype B representing 92.6%, while among Filipino chronic HBV infection patients showed only 2/11 for HBV genotype B (18.2%). On the other side, genotype C was mainly distributed among Filipinos (8/11; 72.7%) followed by Egyptians (9/15; 60%). Present results indicated that 63 out of 73 were genotype B (86.3%) of the Saudi HBV infected patients were infected with genotype B, confirming that genotype B in the most predominant HBV genotype in KSA followed by genotype C (8/73; 11%) that is consistency with previously published data (Kao et al., 2000b) that proved genotype B and C are the most prevalent HBV genotypes among Asian than other genotypes that were represented in minimal proportion (2/73; 2.7% for genotype A).

The clinical, virological and therapeutic implications of HBV genotypes in patients with chronic HBV infection have been partially clarified. Previous data (Kao et al., 2000b; Yeun et al., 2009) suggested that, HBV genotype C is associated with the severity of liver disease as development of cirrhosis and HCC as well as a lower response rate to interferon therapy compared to genotype B. Lindh et al. (2000) reported that, genotype C, compared to genotype B is associated with a higher frequency of HBeAg and HBV-DNA level, more pronounced liver inflammation and a lower frequency of pre-core mutations. The pathogenic link between genotype C and the progression of liver disease remains largely unknown. Koa et al. (2000c) implied that, genotype C seems to stay longer in the immune clearance phase of persistent HBV infection and shift to stages of severer liver inflammation and genotype B may be associated with a faster transition through the immunoreactive stage and evolve into the residual phase in which the serum HBV-DNA becomes barely detectable. This data is very consistence with our obtained data in Table 3.

**Detection of HBV-DNA in serum of patients with chronic hepatitis B:** The most sensitive method for detecting sera HBV DNA currently in use is PCR amplification of virus DNA (Kaneko et al., 1989) and its presence in chronic hepatitis patient serum indicates active virus replication. The presence of HBsAg and HBeAg in serum detected by EIA is used to identify the chronic earlier state of HBV infection. Sera analysis of such patients by PCR has demonstrated that most sera positive for HBsAg and HBeAg contain DNA. In present study the sera of 166 chronic hepatitis patients positive for HBsAg and sera of 42 were positive for HBeAg hepatitis patients were assayed for the presence of HBV DNA by PCR. DNA amplification (Table 2). Although, only 145/166 (87.3%) in HBsAg positive and 22/42 (52.4%) in HBeAg positive sera were tested positive for virus DNA by PCR present data was consistence with earlierly published (Gupta, 1992), whom reported that, HBV-DNA positivity is generally detected, but not all, in HBsAg and HBeAg positive sera. Low percentage of HBV-DNA detection specially among HBeAg might be related to (1) virus replication occurring in tissue but the titer of the virus in the serum is low and the amount of HBV-DNA present is below the detection
limits, (2) virus replication may be occurring with HBeAg expressed patients and integrated into host genome as in case of HBV-DNA containing hepatoma cell lines (Gupta, 1992). Overall, 80.3% (167/204) of all HBSAg and HBeAg positive chronic hepatitis patients tested possessed serum HBV-DNA that was detected by PCR technique.

Seroreversion from HBeAg to anti-HBe during the curse of acute hepatitis is usually accompanied by the resolution of clinic and biochemical evidence of liver disease and the loss of detectable serum and liver HBV-DNA (Kaneko et al., 1989; Yang et al., 2009). On the other hand, HBV-DNA is often detected in the liver of patients with chronic disease and rarely in their serum (Breehot et al., 1981), thus it is likely that circulating virus were present but below the detection limit of current technology. Therefore, the presence of anti-HBe in the serum of chronic hepatitis patients is not an accurate marker for cessation of virus replication in the host. In present study, the sera of 17 chronic hepatitis patients positive for anti-HBe were analyzed for the presence of HBV-DNA by PCR technique. Only 8/17 (47.1%) patients tested positive for serum HBV-DNA. This data is very consistent with previously reported. This result suggests that seroreversion from HBeAg to anti-HBe can be accompanied by a decrease of virus particle in serum but not a total disappeared.

With PCR nearly all (6/7) of the anti-HBe positive chronic hepatitis patients in present study group possessed serum HBV-DNA (85.7%). This finding supports the hypothesis that HBV-DNA continues to replicate in the liver of HBSAg negative patients after seeroreversion to anti HBe and that complete virions, continue to be released into the serum. Analysis of HBV-DNA in this group by using southern blot hybridization on PCR products indicated that, all positive PCR samples tested positive for virus HBV-DNA were also positive by PCR-SBH (100%). Therefore, PCR single band hybridization (PCR-SBH) analysis was able to detect HBV-DNA as more specific assay (because it uses hybridization of an amplified DNA fragment of specific size with a virus-specific radio-labeled probe, Fig. 1 panel b) in all sera that were positive for anti-HBe as proved before (Kaneko et al., 1989). Generally, in present study 181/232 (78%) of chronic hepatitis patients and positive for any HBV markers possess serum HBV-DNA that is detected by PCR analysis. This suggests that such patients continue to support active HBV replication. In addition, it is clear that serological analysis is not sufficient for predicting the course of chronic liver disease. Those data support the previous observations (Gupta, 1992) that HBV-DNA positivity is generally high in HBSAg- and anti-HBe positive sera (87.3 and 85.7%, respectively) compared with that in HBeAg and anti-HBe (52.4 and 47.1%, respectively) and seroreversion from HBeAg to anti-HBe may be accompanied by a decrease in, but not a total disappearance, circulating levels of virus particle from 52.4 to 47.1%, respectively.

In the natural course of chronic HBV infection, early seroreversion from HBeAg to anti-HBe (immune clearance phase) usually indicates a favorable outcome, because it is usually associated with the cessation of virus replication and non-progressive liver disease. In contrast, the late conversion of HBeAg may accelerate the progression of chronic hepatitis B and thus have a poor clinical outcome. Present data in Table 4 suggested that patients with genotype B tended to have a higher frequently of in HBeAg positivity than those with genotype C infection (66.7% versus 16.7%, p<0.01). In addition, when the seroreversion of HBeAg to anti-HBe marker of patients with chronic HBV infection, present data showed high significantly prevalence of genotype B in than those with genotype C among HBV carriers (62.5% versus 12.5%, p<0.01), in those with HBSAg (77.2% versus 15.9%, p<0.001), or in those positive for anti-HBe marker (72.7% versus 22.7%, p<0.001). These findings suggest that genotype C is less predominant among HBV carriers in different phases of viral seroreversion. Most recent studies, all from Asia, suggest that HBV genotype B is associated with early HBeAg seroconversion to anti-HBe than genotype C, thus most likely explaining the less progressive disease in patient with genotype B (Chu et al., 2001). Therefore, HBV genotype C is associated with a high risk of cirrhosis than genotype B (Chu et al., 2001).

Moreover, several studies suggested that HBV genotype may influence HBeAg seroconversion disease progression and even response to outer viral therapy (Scharazi and Rossau, 2000). Patients with genotype C infection have been found to have a lower rate of spontaneous HBeAg sera connection and hepatocarcinoma of cirrhosis compared to those with genotype B (Chu and Lok, 2002; Koa et al., 2002a). In addition, interferon γ (IFN) treated patient with genotype B have been response than genotype C (Zhang et al., 1996; Koa et al., 2002b). The availability of molecular diagnosis assay has improved our understanding of the clinical manifestation and facilitates the monitoring response to treatment (Lok et al., 2001; Elgouhari et al., 2009). Therefore, patients with positivity HBsAg and detectable HBV-DNA may be treated at that point. In present cases among 6 chronically HBV infected patients with positivity of HBeAg and detectable HBV-DNA level was examined, only one with genotype C considered difficult for treatment (16.7%). On the other side, 4
chronically infected HBV patients with positivity for HBeAg and HBV-DNA with genotype B was recommended for treatment as NIH determined.

In conclusion, serum HBV DNA in HBeAg positive patients presumably reflect high replication of hepatic HBV DNA might lead to more severe liver damage. However, it has not been examined in large scale study. Furthermore positivity of HBeAg and HBV DNA is different between HBV genotypes.

REFERENCES


