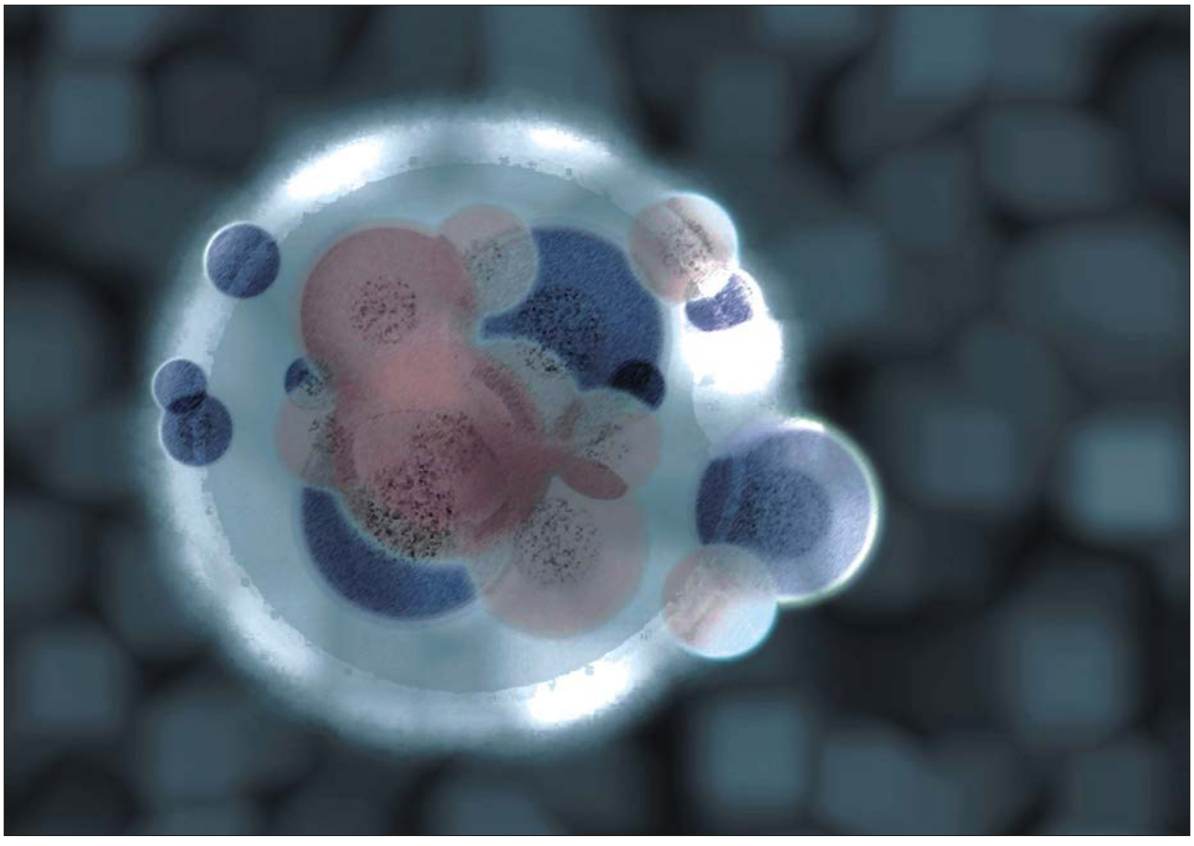

Journal of Biological Sciences

ISSN 1727-3048

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Journal of Biological Sciences 9 (6): 524-532, 2009
ISSN 1727-3048

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 predominant 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 Filipino (16/16; 100%), followed by Bangladeshi (19/21; 90.5%), Pakistani (21/28; 75%), Egyptians (11/18; 61.1%) then Indian (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 neoplasma (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 titer 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-Lunggren *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*., 1966; 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 squeal 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 Millatiry Hospital (NMH); King Mansour Hospital (KMH) from the period 1422 to 1425H (2001-2004G) 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 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 serum 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, Ausab/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 earlierly (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 extract 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 25.8% while non-Saudi (18 of 139) represented 13.0%. Twenty four patients who represented negative for both HBsAg and HBeAg were tested for anti-HBc. Only 7 patients (3%) were positive for anti-HBc 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 = 1.15-1.21; CI = 95) times higher for a Saudi compared to non-Saudi. Among non-Saudis HBsAg positivity was the best indication 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 Saudis (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

Table 1: Distribution of patients according to nationality and gender in the Taif Area in Kingdom of Saudi Arabia

	Males		Females		Total	
Characteristics	No.	%	No.	%	No.	%
Nationalities (n = 232)	72	31.0	21	9.10	93	40.1
Saudi ALT (>40 U L^{-1})	60	25.9	15	6.50	75	32.3
Non-Saudi	115	49.6	24	10.30	139	59.9
ALT (>40U L^{-1})	103	44.4	20	8.60	123	53.0
Indian	27	11.6	8	0.03	35	15.1
Pakistani	21	9.1	7	0.03	28	12.1
Bangladeshi	17	7.3	4	0.02	21	9.1
Egyptian	16	6.9	2	0.01	18	7.8
Filipino	13	5.6	3	0.01	16	6.9
Others	21	9.1	--	--	21	9.1

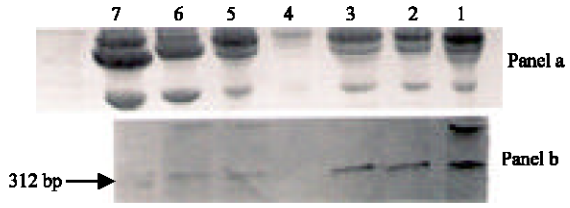

Fig. 1: HBV-DNA in the serum of patients positive for anti-HBc. The serum of patients was analyzed for HBV-DNA by PCR-EB panel a and PCR-SBH, panel b. Positive PCR products were represented in 312-bp HBV-DNA. Autoradiography demonstrated the presence of HBV-DNA in the serum samples of patients in lane 1, 2, 3, 5, 6 and 7

Table 2: Nationality specific prevalence of hepatitis B markers by serodiagnosis and PCR

		-ve sample of HbsAg									
		+ve HbsAg		+ve HbeAg		+ve Anti-HBc		+ve anti-HBe		PCR	
Nationalities	Sample tested (n = 232)	No.	%	No.	%	No.	%	No.	%	No.	%
Saudi +ve PCR	93	64	68.8	24	25.8	3	3.2	2	2.2		
		58	25.0	12	5.2	3	1.3	--	--	73	31.5
Non-Saudi	139	102	73.4	18	13.0	4	2.9	15	10.8		
+ve PCR		87	37.5	10	4.3	3	1.3	8	3.4	108	46.6
Indian	35	21	60.0	6	17.1	7	20.0	1	2.9	27	11.6
Pakistani	28	21	75.0	5	17.9	--	--	2	7.1	22	9.5
Bangladeshi	21	19	90.5	1	4.8	1	4.8	--	--	20	8.6
Egyptian	18	11	61.1	4	22.2	2	11.1	1	5.6	15	6.5
Filipino	16	16	100.0	--	--	--	--	--	--	11	4.7
Others	21	12	57.1	4	19.0	3	14.3	2	9.5	13	5.6

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

	Genotype A		Genotype B		Genotype C		Genotype E		Others*	
Nationalities (n = 181)	No	%	No	%	No	%	No	%	No	%
Saudi (n = 73)	2	1.1	63	34.8	8	4.4	--	--	--	--
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-HBc marker (n = 22)	1(4.5%)	16(72.7%)*	5(22.7%)	none	none
+ve HBeAg 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%)	1(12.5%)	none

*p<0.001

group 22 of 42; 52.4%). The remaining HBV markers (anti-HBc and anti-HBe) showed sensitivity for viral detection by PCR representing 100 and 0.0% for Saudi and 75.0 and 44.4 for non Saudi respectively and total HBV-DNA samples detected in anti-HBc subgroup were 6 of 7 (85.7%), while in anti-HBe subgroup were 8 of 17 (471%; Table 2).

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 persisted 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-HBc marker (72.7%), followed by positive serum for HBeAg (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, HBeAg and anti-HBe markers (15.9, 16.7 and 12.5%, respectively). Genotype E was more expressed among patients with positive HBeAg (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 51.2% (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-HBc 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-HBc 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 unidentified 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 Fillipino (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 consistence 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 replications. 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.

Seroconversion 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 (Brechot *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 seroconversion 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-HBc 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 seroconversion to anti HBc 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-HBc 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-HBc positive sera (87.3 and 85.7%, respectively) compared with that in HBeAg and anti-HBe (52.4 and 47.1%, respectively) and seroconversion 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 seroconversion 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 seroconversion 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% versus15.9%, $p<0.001$), or in those positive for anti-HBc marker (72.7% versus 22.7%, $p<0.001$). These findings suggests that genotype C is less predominant among HBV carriers in different phases of viral seroconversion. 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). Therefor, 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 (Schinazi and Rossau, 2000). Patients with genotype C infection have been found to have a lower rate of spontaneous HBeAg sera connection and hepatocarcenoma 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 facilities the monitoring response to treatment (Lok *et al*., 2001; Elgouhari *et al*., 2009). Therefore, patients with positivity HBeAg 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.

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