CHRONIC CARRIERS OF HEPATITIS B VIRUS IN BANGLADESH: A COMPARATIVE ANALYSIS OF HBV-DNA, HBeAg/ANTI-HBe, AND LIVER FUNCTION TESTS

KN Hasan[1], MAK Rumi[1], MA Hasanat[2], MG Azam[1], S Ahmed[1], MA Salam[1], LN Islam[3] and MS Hassan[1]

[1]Department of Immunology, Bangladesh Institute of Research and Rehabilitation in Diabetes, Endocrine and Metabolic Disorders (BIRDEM); [2]Department of Medicine, Bangabandhu Sheikh Mujib Medical University (BSMMU), Bangladesh; [3]Department of Biochemistry, University of Dhaka, Bangladesh

Abstract. Serological markers of hepatitis B virus (HBV), liver function tests and quantitative estimation of HBV-DNA are important in the assessment of the state of infection and prognosis following treatment for hepatitis B**.** This study aimed to determine whether low-cost assays, *eg* hepatitis B e antigen (HBeAg) and liver function tests, could be used for the assessment of infectivity as an alternative to HBV-DNA estimation. We tested 125 hepatitis B carriers for HBeAg, antibody to HBeAg (anti-HBe), and serum HBV-DNA; we also carried out a range of standard liver function tests. Seventy-three subjects were positive and 52 were negative for HBeAg. Of the HBeAg positive cases, 3 were also positive for anti-HBe; of the HBeAg negative cases, 5 were also negative for anti-HBe. Of these 8 cases, 7 had no detectable HBV-DNA. Most of the HBeAg positive but anti-HBe negative subjects were positive for HBV-DNA (74.3%; 52/70) whereas most of the HBeAg negative and anti-HBe positive subjects (93.6%; 44/47) were also negative for HBV-DNA. Of 56 HBV-DNA positive individuals, alanine transaminase (ALT) was found to be raised in 69.6% (p=0.066) and aspartate transaminase (AST) was raised in 66.1% (p=0.011), while 67.9% had normal alkaline phosphatase (ALP) (p=0.054). HBeAg (p=0.018) and raised ALT (p=0.008) were found to be independent predictors for HBV-DNA positivity among HBV carriers. This study suggests that HBeAg positive and anti-HBe negative hepatitis B carriers with raised ALT and AST are likely to be positive for HBV-DNA; the combination of routine serology and biochemical tests may be considered as an alternative to HBV-DNA in evaluating the state of chronic HBV infection. However, HBV-DNA should be specifically assessed if discordance is observed between seromarkers and transaminases.

INTRODUCTION

Hepatitis B virus is a recognized cause of chronic hepatitis, cirrhosis and hepatocellular carcinoma (Lee, 1997). In Bangladesh, 36% of hepatocellular carcinoma is associated with hepatitis B infection (Khan and Ahmed, 1996). Unlike healthy carriers, patients with evidence of chronic active hepatitis require treatment because their condition may lead to cirrhosis and hepatocellular carcinoma (Zoulim *et al*, 1992). The disease status of the patient has to be evaluated before initiation of antiviral therapy and during follow-up. (Zuckerman and Thomas, 1998).

Hepatitis B surface antigen (HBsAg), e antigen (HBeAg), and their respective antibodies (anti-HBs and anti-HBe) have been used as serological markers of the status of HBV infection (Pao *et al*, 1991). Serum HBeAg is thought to be associated with active HBV replication and the synthesis of complete virions and has been used to identify highly infectious hepatitis carriers (Negro *et al*, 1984; Govindarajan *et al*, 1988; Scott *et al*, 1990a; Pao *et al*, 1991; Maruyama *et al*, 1993).

Correspondence: Dr MS Hassan, Department of Immunology, BIRDEM Hospital, 122 Kazi Nazrul Islam Avenue, Dhaka-1000, Bangladesh.
Tel: ++880-2-9661551-59/Ext 2262; Fax: ++880-2-8613004
E-mail:mshassan@dab-bd.org; nadim124@yahoo.com

Seroconversion generally indicates the cessation of viral replication and therefore lower infectivity (Lieberman *et al*, 1983; Harrison *et al*, 1985; Scoot *et al*, 1990b). Other markers of replication include DNA polymerase activity and the hepatitis B core antigen (HBcAg) in hepatocytes (Ditzhuijsen *et al*, 1985). Quantitative determination of serum HBV-DNA has been considered to be an accurate and reliable marker of HBV replication (Tur-kaspa *et al*, 1984; Bonino *et al*, 1986).

Although liver histology remains the mainstay of the investigation of hepatitis activity, it is an invasive procedure that is not without its complications (Sherlock and Dooly, 1997). Alternative liver function tests, *eg* liver transaminases, prothrombin time and serum albumin/globulin estimation, may be used to assess indirectly the inflammatory activity and cirrhotic process in the liver. HBV-DNA estimation is expensive and many laboratories in developing countries lack the logistical support required for the test. The objective of this study was to determine whether low cost serological and biochemical tests could provide an alternative to expensive HBV-DNA analysis for assessing the disease status and the indications for treatment in cases of chronic hepatitis B infection.

MATERIALS AND METHODS

Study subjects

One hundred and twenty-five chronic hepatitis B patients [age (mean ± SD): 29±14 yrs; m/f: 111/14] who were referred to the Department of Immunology of the Bangladesh Institute of Research and Rehabilitation in Diabetes, Endocrine and Metabolic Disorders, Dhaka, were studied. Patients with a past history of taking immunosuppressive or immunomodulatory drugs were excluded from the study. All samples were tested for hepatitis C, hepatitis D, and human immunodeficiency virus: none was positive. Serological markers of hepatitis B and liver function tests were also done. All subjects consented to participate in the study (protocol approved by the Ethical Review Committee).

Sample collection

Five ml of venous blood were collected from each of the subjects. Serum was separated and preserved at -80ºC for assay of serological markers and HBV-DNA; biochemical markers were assayed on the day of collection.

Analytical methods

HBV-DNA was estimated with an Enhanced Microplate Hybridization Assay (EMHA) kit (Enzo Diagnostic Inc, NY, USA), a non-radioactive colorimetric hybridization procedure with a micro-well format. Serum samples (125 μl) were used for the assay. The kit also contained five titration-standard sera of different HBV-DNA concentrations (from 156 pg/ml to 2,500 pg/ml) and a HBV-DNA negative serum. Titration standards were hepatitis B recombinant plasmid DNA. Standard curves generated by assaying the titration standards were used to estimate the amounts of DNA in the serum samples; the lowest detection limit for HBV-DNA was 156 pg/ml.

Serum transaminases, alkaline phosphatase, total protein, albumin and globulin were assayed by a chemistry analyser (RA-50; Bayer, Dublin, Ireland) using commercial reagents (Randox Laboratories Ltd, Antrim, UK). Prothrombin time was estimated by an automated analyser (Coag-a-mate II; Organon Teknika, Boxtel, The Netherlands) using a commercial thromboplastin reagent (Pacific Hemostasis, NC, USA).

Assays of the serological markers for hepatitis B (HBsAg, HBeAg, anti-HBe and anti-HBc IgM), hepatitis C (anti-HCV), hepatitis D (anti-HDV) (Diasorin, Saluggia, Italy) and anti-HIV (1+2+0) (Uniform II, Organon Teknika, Boxtel, The Netherlands) were performed by commercial enzyme immunoassay kits.

Statistical analysis

Data are expressed as frequencies, per-

Table 1
Association of HBe antigen/antibody with HBV-DNA level among HBV carriers (n=125).

HBV DNA (pg/ml)	HBeAg			
	Positive		Negative	
	Anti-HBe +	Anti-HBe -	Anti-HBe +	Anti-HBe -
Positive	0	52 (74.3)	3 (6.4)	1 (20.0)
Negative	3 (100)	18 (25.7)	44 (93.6)	4 (80.0)
Total	3	70	47	5
χ^2	7.74		1.18	
p	0.021		0.341	
<156	3 (100)	18 (25.7)	44 (93.6)	4 (80.0)
156-500	0	13 (18.6)	2 (4.3)	0
501-1,000	0	7 (10.0)	0	0
1,000-2,500	0	4 (5.7)	0	1 (20.0)
>2,500	0	28 (40.0)	1 (2.1)	0

(Percentages are of their respective column totals)
HBV-DNA : Hepatitis B virus DNA; HBsAg : Hepatitis B surface antigen; HBeAg : Hepatitis B e antigen; Anti-HBe : Antibody to hepatitis B e antigen.

centages and means ± SD, unless stated otherwise. Association between groups was determined by the chi-square test. The predictive capability of various biochemical and related parameters composed with HBV-DNA status was checked by logistic regression analysis. P values ≤ 0.05 were considered as significant.

RESULTS

Association of HBV-DNA with HBeAg/Anti-HBe status

Of the 125 carriers, 3 were positive for both HBeAg and anti-HBe; 70 were positive for HBeAg but negative for anti-HBe; 47 were negative for HBeAg but positive for anti-HBe and 5 negative for both markers. Most of the HBeAg positive but anti-HBe negative subjects were positive for HBV-DNA (74.3%, 52/70) whereas most of the HBeAg negative and anti-HBe positive subjects (93.6%, 44/47) were negative for HBV-DNA. However, 25.7% (18/70) of HBeAg positive and anti-HBe negative patients had no detectable HBV-DNA; in contrast, 6.4% (3/47) of HBeAg negative and anti-HBe positive subjects were found to be positive for HBV-DNA. Seven of 8 patients (3 HBeAg positive and anti-HBe positive and 5 HBeAg negative and anti-HBe negative) had no detectable HBV-DNA.

Of the 52 HBV-DNA positive and HBeAg positive subjects, 13 had 156-500 pg/ml, 7 had 501-1,000 pg/ml, 4 had 1,001-2,500 pg/ml, and 28 had more than 2,500 pg/ml of HBV-DNA. Of the three HBeAg negative and HBV-DNA positive patients, 2 had 156-500 pg/ml and 1 had more than 2,500 pg/ml of HBV-DNA. Of the 5 HBeAg negative and anti-HBe negative patients, 1 had 1,000-2,500 pg/ml of HBV-DNA (Table 1).

Association of HBeAg/anti-HBe with biochemical markers

Of the HBeAg positive subjects, 65.8% had raised alanine transaminase; 60.3% showed raised aspartate transaminase; 67.1% had an altered albumin-globulin ratio; of the HBeAg negative patients, these markers were abnormal in 51.9%, 42.3%, and 69.2% of cases respectively. The anti-HBe negative subjects showed a similar patient of markers; a good

Table 2
Association of seromarkers with ALT, AST, alkaline phosphatase, PT and A:G ratio in HBV carriers (n = 125).

Biochemical markers	HBeAg		p-value	Anti-HBe		p-value
	Positive (N=73)	Negative (N=52)		Positive (N=50)	Negative (N=75)	
ALT						
Raised	48 (65.8)	27 (51.9)	0.140	25 (50.0)	50 (66.7)	0.093
Normal	25 (34.2)	25 (48.1)		25 (50.0)	25 (33.3)	
AST						
Raised	44 (60.3)	22 (42.3)	0.069	20 (40.0)	46 (61.3)	0.028
Normal	29 (39.7)	30 (57.7)		30 (60.0)	29 (38.7)	
Alkaline phosphatase						
Raised	21 (28.8)	08 (15.4)	0.090	09 (18.0)	20 (26.7)	0.288
Normal	52 (71.2)	44 (84.6)		41 (82.0)	55 (73.3)	
PT (n = 115)						
Raised	02 (2.9)	02 (4.3)	1.000	02 (4.4)	02 (2.9)	0.644
Normal	66 (97.1)	45 (95.7)		43 (95.6)	68 (97.1)	
A:G ratio						
Altered	49 (67.1)	36 (69.2)	0.848	35 (70.0)	50 (66.7)	0.845
Normal	24 (32.9)	16 (30.8)		15 (30.0)	25 (33.3)	

(Percentages are of their respective column totals)
HBsAg: Hepatitis B surface antigen; ALT : Alanine transaminase; AST : Aspartate transaminase; ALP : Alkaline phosphatase; PT : Prothrombin time.

Table 3
Association of HBV-DNA with ALT, AST, alkaline phosphatase, PT and A:G ratio in HBV carriers (n = 125).

Biochemical markers	HBV-DNA		χ^2	p-value
	Positive (N = 56)	Negative (N = 69)		
ALT				
Raised	39 (69.6)	36 (52.2)	3.931	0.066
Normal	17 (30.4)	33 (47.8)		
AST				
Raised	37 (66.1)	29 (42.0)	7.17	0.011
Normal	19 (33.9)	40 (58.0)		
Alkaline phosphatase				
Raised	18 (32.1)	11 (15.9)	4.554	0.054
Normal	38 (67.9)	58 (84.1)		
PT (n = 115)				
Raised	1 (1.9)	3 (4.8)	0.684	0.625
Normal	51 (98.1)	60 (95.2)		
A:G ratio				
Altered	39 (69.6)	46 (66.7)	0.126	0.847
Normal	17 (30.4)	23 (33.3)		

(Percentages are of their respective column totals)
HBV-DNA : Hepatitis B virus DNA; HBsAg: Hepatitis B surface antigen; ALT : Alanine transaminase; AST : Aspartate transaminase; ALP : Alkaline phosphatase; PT : Prothrombin time; A:G ratio : Albumin : Globulin ratio.

number of anti-HBe positive subjects also showed raised transaminses and altered albumin-globulin ratios. Table 2 shows the association of serological and biochemical markers.

Association of HBV-DNA with biochemical markers

Of the HBV-DNA positive patients, alanine transaminase was raised in 69.6%; aspartate transaminase in 66.1% (p=0.066 and 0.011 respectively). Although positive for HBV-DNA, 30.4% had a normal level of alanine transaminase and 33.9% had a normal level of aspartate transaminase (Table 3). Although 84.1% of the subjects having no detectable HBV-DNA had a normal level of alkaline phosphatase, 67.9% of HBV-DNA positive subjects also had a normal level of alkaline phosphatase (p = 0.054). All but one of the HBV-DNA positive patients, as well as 95% of HBV-DNA negative patients were found to have a normal prothrombin time (p = 0.625). Albumin-globulin ratios were altered in 69.6% of HBV-DNA positive and in 66.7% of HBV-DNA negative subjects (p = 0.847).

Table 4
Logistic regression analysis of HBV-DNA against various serological and biochemical markers.

Variables	Regression coefficient	p-value
Age	0.125	0.6062
Sex	-0.3990	0.6512
HBeAg	3.4737	0.0180
Anti-HBe	-2.0915	0.1326
ALT	0.0271	0.0088
AST	-0.0157	0.1822
ALP	0.0030	0.1293
A/G ratio	0.0939	0.5330
PT	-0.2747	0.4555
Constant	-0.5860	0.9036

HBeAg: Hepatitis B e antigen; Anti-HBe : Antibody to hepatitis B e antigen; ALT : Alanine transaminase; AST : Aspartate transaminase; ALP : Alkaline phosphatase; PT : Prothrombin time A:G ratio : Albumin : Globulin ratio

Regression analysis

Logistic regression of age, sex, serological and biochemical markers over the prediction of HBV-DNA status revealed that HBeAg (p = 0.018) and raised alanine transaminase (p = 0.008) were independent predictors for HBV-DNA status (Table 4).

Association of HBV-DNA with combined HBeAg and serum transaminases

None of the patients found to be HBeAg negative and to have normal serum transaminases (n = 22), had detectable HBV-DNA. Of 17 HBeAg positive patients with normal transaminases, 12 (70.6%) were positive for HBV-

Table 5
Association of HBV-DNA with combined HBeAg and serum transaminases.

Status of HBeAg and transaminases	HBV-DNA	
	Negative (N = 69)	Positive (N = 56)
HBeAg positive, ALT/AST/both ALT and AST raised (N = 56)	16 (28.6)	40 (71.4)
HBeAg negative, ALT and AST normal (N = 22)	22 (100.0)	0
HBeAg positive, ALT and AST normal (N = 17)	5 (29.4)	12 (70.6)
HBeAg negative, ALT/AST/both ALT and AST raised (N = 30)	26 (86.7)	4 (13.3)

(Percentages are of their respective row totals) $\chi^2 = 50.496$; p = 0.000
HBV-DNA : Hepatitis B virus DNA; HBeAg : Hepatitis B e antigen; ALT : Alanine transaminase; AST : Aspartate transaminase

DNA. On the other hand, 71.4% (40/56) of the subjects positive for HBeAg and with raised transaminases (alanine and aspartate; one or both) as well as 13.3% (4/30) of the subjects negative for HBeAg but with raised transaminases, were positive for HBV-DNA ($p = 0.000$) (Table 5).

DISCUSSION

Assessment of viral replication is indicated in hepatitis B patients requiring antiviral therapy. This study of 125 chronic hepatitis B carriers revealed a significant association between circulating HBV-DNA and HBeAg/anti-HBe status. Similar findings were made in several other studies (Tur-kaspa *et al*, 1984; Ditzhuijsen *et al*, 1985; Alberti *et al*, 1986; Govindarajan *et al*, 1986; 1988; Scott *et al*, 1990; Scoot *et al*, 1990; Stroffolini *et al*, 1992; Maruyama *et al*, 1993).

It has been reported that aspartate transaminase is one of the best predictors of the hepatitis activity index (histological hepatitis activity) and chronic active hepatitis (Borg *et al*, 1998). We found a significant association of positive HBV-DNA with raised aspartate transaminase. However, a good association was also observed between positive HBV-DNA and raised alanine transaminase.

Discordance between HBV-DNA with HBeAg/anti-HBe status was also found in this study. Three HBeAg negative and Anti-HBe positive patients were found to have a detectable level of HBV-DNA. This pattern has been reported in many other studies (Ditzhuijsen *et al*, 1985; Alberti *et al*, 1986; Krogsgaard *et al*, 1986; Govindarajan *et al*, 1988; Scott *et al*, 1990; Scoot *et al*, 1990). The presence of circulating HBV-DNA in HBeAg negative patients may be due to mutation in the precore region of HBV.

There were 18 HBeAg positive sera without detectable HBV-DNA. It was found observed that 28.6% of subjects positive for HBeAg and with raised transaminases (one or both) were negative for HBV-DNA: similar discordance was found in a study comprising 899 hepatitis B carriers from four ethnic groups (Caucasian, Australian Aboriginal, Melanesian, and Asian) (Scott *et al*, 1990). This discordance was unusual in Caucasians but frequently occurred in the other three ethnic groups (11-27%), most commonly among the Asians (Scott *et al*, 1990). In the present study, discordance of this type was 25.7% and 28.6% when HBV-DNA status was compared with combined HBeAg and serum transaminases respectively. It has been reported that HBeAg may be synthesised and secreted in the absence of an intact virus assembly and that this phenomenon may be related to the integration of viral DNA into the host genome with loss of the replication stage (Scoot *et al*, 1990; Ou *et al*, 1997). We cannot say with certainly that this was the case in the discordant type in our study, because confirmation by detection of precore sequence integrated in liver tissue which was not undertaken. Alternatively, the discordance may have been due to HBV-DNA clearance prior to HBeAg clearance, as reported in a follow-up study (Moestrup *et al*, 1985). The sensitivity of the kit used for HBV-DNA detection in our study may also have been partly responsible for the discordance.

This study suggests that raised serum transaminases and HBeAg positivity represent inflammatory activity of the liver and HBV replication; these markers may therefore be used in the diagnosis of chronic HBV infection. Quantitative HBV-DNA detection does not give any additional information about patients with chronic HBV infection who are being considered for antiviral therapy; moreover, HBV-DNA is expensive and needs an advanced laboratory with skilled personnel. Combined serological and biochemical testing costs ~US$ 2.00 per patients whereas HBV-DNA assay costs ~US$ 16.00 per patient and may be yet more expensive if more sensitive kits are used, *eg* bDNA hybridization assay (Quantiplex, Chiron Corporation, Emeryville, CA, USA), Amplicor HBV monitor assay (Roche Diagnostics GmbH, Germany). However, the HBV-DNA level can give additional informa-

tion about chronic hepatitis B patients in cases in which discordance of HBV-DNA with serological and biochemical markers is suspected.

HBV-DNA status was strongly correlated with HBeAg/anti-HBe and aspartate transaminase. Logistic regression analysis revealed that HBeAg and alanine transaminase were independent predictors for HBV-DNA positivity. It was also found that HBeAg positive patients with normal serum transaminases and HBeAg negative patients with raised serum transaminases may be positive for HBV-DNA. The discordance of HBV-DNA with serological and biochemical markers may be suspected by looking for disparity between HBeAg and serum transaminases.

In conclusion, the present study indicates that HBeAg positive and anti-HBe negative chronic carriers who have raised levels of liver transaminases are likely to be positive for HBV-DNA and are therefore candidates for antiviral therapy. However, HBV-DNA may require independent determination in chronic carriers who show discordance between HBeAg and serum transaminases.

ACKNOWLEDGEMENTS

This study was supported in part by Diamed (local representative of Diasorin, Italy) and Bio-trade International (local representative of Randox Laboratories Ltd, UK).

REFERENCES

Alberti A, Pontisso P, Fattovich G, *et al*. Changes in serum hepatitis B virus (HBV) DNA positivity in chronic HBV infection: results of a long-term follow-up study of 138 patients. *J Infect Dis* 1986; 154: 562-9.

Bonino F, Rosina F, Rizzetto M, *et al*. Chronic hepatitis in HBsAg carriers with serum HBV-DNA and Anti-HBe. *Gastroenterology* 1986; 90: 1268-73.

Borg F, Kate FJW, Cuypers HTM, *et al.* Relation between laboratory test results and histological hepatitis activity in individuals positive for hepatitis B surface antigen and antibodies to hepatitis B e antigen. *Lancet* 1998; 351: 1914-8.

Ditzhuijsen TJM, Selten GCM, Van Loon AM, Wolters G, Matthyssen L, Yap SH. Detection of hepatitis B virus DNA in serum and relation with the IgM class Anti-HBc titres in hepatitis B virus infection. *J Med Virol* 1985; 15: 49-56.

Govindarajan S, DeCock KM, Valinluck B, Ashcavi M. Serum hepatitis B virus DNA in acute hepatitis B. *Am J Clin Pathol* 1986; 86: 352-4.

Govindarajan S, Fong TL, Valinluck B, Edwards V, Redeker AG. Markers of viral replication in patients with chronic hepatitis B virus infection. *Am J Clin Pathol* 1988; 89: 233-7.

Harrison TJ, Bal V, Wheeler EG, Meacock TJ, Harrison JF, Zuckerman AJ. Hepatitis B virus DNA and e antigen in serum from blood donors in the United Kingdom positive for hepatitis B surface antigen. *Br Med J* 1985; 290: 663-4.

Khan M, Ahmed N. Seroepidemiology of HBV and HCV in Bangladesh. *Intern Hepatol Commun* 1996; 5: 27-9.

Krogsgaard K, Wantzin P, Aldershvile J, Kryger P, Andersson P, Nielsen JO. Hepatitis B virus DNA in hepatitis B surface antigen-positive blood donors: relation to the hepatitis B e system and outcome in recipients. *J Infect Dis* 1986; 153: 298-303.

Lee W M. Hepatitis B virus infection. *N Engl J Med* 1997; 337: 1733-45.

Lieberman HM, Labrecque DR, Kew MC, Hadziyannis SJ, Shafritz DA. Detection of Hepatitis B virus DNA directly in human serum by a simplified molecular hybridisation test: comparison to HBeAg/Anti-HBe status in HBsAg carriers. *Hepatology* 1983; 3: 285-91.

Maruyama T, Iino S, Koike K, Yasuda K, Milich DR. Serology of acute exacerbation in chronic hepatitis B virus infection. *Gastroenterology* 1993; 105: 1141-51.

Moestrup T, Hansson BG, Widell A, Blomberg J, Nordenfelt E. Hepatitis B virus –DNA in the serum of patients followed-up longitudinally with acute and chronic hepatitis B. *J Med Virol* 1985; 17: 337-44.

Negro F, Chiaberge E, Oliviero S, *et al*. Hepatitis B virus DNA (HBV-DNA) in anti-HBe positive sera. *Liver* 1984; 4: 177-83.

Ou JH. Molecular biology of hepatitis B virus e antigen. *J Gastroenterol Hepatol* 1997; 12 (suppl):

S 178-87.

Pao CC, Yao DS, Lin CY *et al*. Serum hepatitis B virus DNA in hepatitis B virus seropositive and seronegative patients with normal liver function. *Am J Clin Pathol* 1991; 95: 591-6.

Scoot JS, Pan P, Pace RA, Sloots TP, Cooksley WG. The absence of hepatitis B virus DNA in hepatitis B e antigen positive sera from chronic hepatitis B surface antigen carriers in China. *J Med Virol* 1990a; 30: 103-6.

Scott JS, Pace RA, Sheridan JW, Cooksley WG. Discordance of hepatitis B e antigen and hepatitis B viral deoxyribonucleic Acid. *J Med Virol* 1990b; 32: 225-31.

Sherlock S, Dooly J. Disease of the liver and biliary system, 10th ed. London: Blackwell Science, 1997.

Stroffolini T, Sagnelli E, Rapicetta M, *et al*. Hepatitis B virus DNA in chronic HBsAg carriers: correlation with HBeAg/Anti-HBe status, Anti-HD and liver histology. *Hepato-Gastroenterol* 1992; 39: 62-5.

Tur-kaspa R, Keshet E, Eliakim M, Shouval D. Detection and characterisation of hepatitis B virus DNA in serum of HBe antigen-negative HBsAg carriers. *J Med Virol* 1984; 14: 17-26.

Zoulim F, Mimms L, Floreani M, *et al*. New assays for quantitative determination of viral markers in management of chronic hepatitis B virus infection. *J Clin Microbiol* 1992; 30: 1111-9.

Zuckerman AJ, Thomas HC. Viral hepatitis, 2nd ed. London: Churchill Livingstone, 1998.