Hepatitis B Viral Factors in HBeAg-Negative Carriers with Persistently Normal Serum Alanine Aminotransferase Levels

Chih-Lin Lin,^{1*} Li-Ying Liao,^{1*} Chun-Jen Liu,² Ming-Whei Yu,³ Pei-Jer Chen,^{2,4} Ming-Yang Lai,^{2,4}
Ding-Shinn Chen,² and Jia-Horng Kao^{2,4,5,6*}

Chronic hepatitis B patients with high-normal serum ALT (levels of 0.5-1 × upper limit of normal) are still at risk of liver disease progression. We thus investigated the correlation between serum ALT level and hepatitis B viral factors in HBeAg-negative carriers with persistently normal serum ALT level (PNALT). Baseline clinical and virological features of 414 HBeAg-negative carriers, including 176 (42.5%) with low-normal ALT (levels of less than 0.5 × upper limit of normal) and 238 (57.5%) with high-normal ALT, were compared. Compared with HBV carriers with low-normal ALT, those with high-normal ALT were older (41 vs. 37 years, $P < 0.001$) and had a greater frequency of serum HBV DNA level $>10^4$ copies/ml (63.4% vs. 47.5%, $P < 0.001$) as well as a higher prevalence of basal core promoter T1762/A1764 mutant (36.5% vs. 24.2%, $P = 0.01$). Multivariate analysis showed that factors associated with a high-normal serum ALT level included male sex [odds ratio (OR), 1.82; 95% confidence interval (CI), 1.10-3.01, $P = 0.019$], increasing age (OR, <30 years: 1, reference; 30-39 years: 2.43, 95% CI, 1.18-5.03, $P = 0.016$; 40-49 years: 4.22, 95% CI, 1.99-8.93, $P < 0.001$; ≥ 50 years: 4.06, 95% CI, 1.69-9.78, $P = 0.002$) and serum HBV DNA level $>10^4$ copies/ml (OR, 1.83; 95% CI, 1.07-3.13, $P = 0.027$). **Conclusion: HBeAg-negative patients with persistently normal ALT are not a homogenous group, and those with high-normal ALT share some of the characteristics that have been associated with adverse long-term outcomes. (HEPATOLOGY 2007;45:1193-1198.)**

The natural history of chronic HBV infection could be generally divided into three chronological stages.¹ During the immune tolerance stage, serum HBV DNA levels are high and hepatitis B e antigen (HBeAg) is present. In the immune clearance stage, most

carriers eventually seroconvert from HBeAg to anti-HBe. After HBeAg seroconversion, patients were in the integration or residual stage, serum level of HBV DNA decreased, and ALT became normal. Accordingly, HBeAg-negative carriers were usually considered to have low-replicative HBV infection, and normal or nearly normal serum ALT levels. However, progressive liver disease still develops in HBeAg-negative carriers because of bouts of hepatitis flare. Thus, the clinical spectrum of HBeAg-negative chronic HBV infection may range from inactive carrier to aggressive chronic hepatitis with or without cirrhosis.² The differential diagnosis of HBeAg-negative chronic hepatitis from an inactive carrier mainly depends on sequential determinations of serum ALT.³ However, slightly increased serum ALT level, although within the normal range, has been reported to be significantly associated with risk of liver-related mortality in the general population.⁴ Furthermore, recent large-scale cohort studies showed that chronic hepatitis B patients with a normal serum ALT level, irrespective of HBeAg status, were also at a risk for the development of cirrhosis and HCC.^{5,6} Therefore, chronic hepatitis B patients with high-normal serum ALT levels (0.5-1 × upper limit of normal) may be

Abbreviations: CI, confidence interval; HBeAg, hepatitis B e antigen; PNALT, persistently normal serum alanine aminotransferase level; ULN, upper limit of normal.

From the ¹Department of Gastroenterology, Ren-Ai branch, Taipei City Hospital; the ²Department of Internal Medicine, National Taiwan University Hospital; the ³Department of Public Health and Graduate Institute of Epidemiology; the ⁴Graduate Institute of Clinical Medicine; the ⁵Hepatitis Research Center; and the ⁶Department of Medical Research, National Taiwan University College of Medicine and National Taiwan University Hospital, Taipei, Taiwan.

Received July 25, 2006; accepted December 14, 2006.

Supported by grants from the Taipei City Hospital (to Liao LY), and the Department of Health, and the National Science Council (both to Kao JH), Executive Yuan, Taiwan.

*These authors contributed equally to this work.

Address reprint requests to: Dr. Jia-Horng Kao, Director and Professor, Hepatitis Research Center and Graduate Institute of Clinical Medicine, National Taiwan University College of Medicine, 7 Chung-Shan South Road, Taipei 100, Taiwan. E-mail: kjh@ha.mc.ntu.edu.tw; fax: (886) 2-23825962.

Copyright © 2007 by the American Association for the Study of Liver Diseases.

Published online in Wiley InterScience (www.interscience.wiley.com).

DOI 10.1002/hep.21585

Potential conflict of interest: Nothing to report.

at risk of progressive liver disease. Unfortunately, the biological and virological basis of high-normal ALT in these patients remains largely unknown. Recently, persistent HBV viremia has been shown as a factor associated with liver disease progression.⁷ A recent large community cohort study from Taiwan confirmed that serum HBV DNA level greater than 10,000 copies/ml was significantly associated with risk of HCC development.⁶ Furthermore, the roles of HBV genotype and common HBV mutants including precore/core promoter and pre-S deletion mutations in the progression of chronic hepatitis B have become increasingly recognized. HBV genotypes C and basal core promoter mutants are shown to correlate with progression of liver disease.⁸⁻¹³ These lines of evidence prompted us to investigate the role of hepatitis B viral factors that have been associated with adverse clinical outcomes in HBeAg-negative carriers with persistently normal serum ALT level (PNALT) in Taiwan.

Patients and Methods

Patients. A total of 414 HBeAg-negative/anti-HBe-positive carriers with PNALT were consecutively enrolled. PNALT was defined by persistently normal serum ALT levels for at least 2 years in periodic biochemical examinations before enrollment. Serum ALT level was measured by using routine automated method. According to the instructions of the manufacturer, the definition of upper limit of normal (ULN) of ALT level is 40 and 30 IU/L for men and women, respectively. All of them were negative for HBeAg, negative for antibodies to HCV, hepatitis D virus, or human immunodeficiency virus, and none had received antiviral treatment before or during the follow-up period. Serum samples were collected and stored at -70°C until use.

Serological Testing. The biochemical tests were measured by using routine automated methods. The hepatitis B surface antigen, HBeAg, anti-HBe, and antibodies to HCV were assayed by commercial kits (General Biological hepatitis B surface antigen RIA and HBeAg/Anti-HBe RIA, General Biological Cooperation, Taiwan. HCV EIA II, Abbot Laboratories, North Chicago, IL).

HBV DNA Quantitative Assay and Genotyping. The identification of HBV viral load and genotype were performed by the real-time PCR-based single-tube assay as previously described.¹⁴ Briefly, the method contains two consecutive steps. The first step used real-time PCR for quantification of HBV DNA, and the second step used melting curve analysis for HBV genotyping. The detection limit of HBV DNA level was 100 copies/ml, ranging from 10^2 to 10^{11} copies/ml.

Amplification and Sequencing of Precore/Basal Core Promoter Genes. For sequence analysis, precore and core promoter genes were amplified by PCR as previously described.⁸ Nucleotide sequences of the amplified products were directly determined by using fluorescence-labeled primers with a 377 Automatic Sequencer (Applied Biosystems, Foster City, CA). Sequencing conditions were specified in the protocol for Taq DyeDeoxy Terminator Cycle Sequencing Kit (Applied Biosystems).

Statistical Analysis. Data were analyzed by chi-square test, Fisher's exact test, Student *t* test, and the multiple logistic regression where appropriate. All of the tests of significance were two-tailed, and a *P* value of less than 0.05 was considered statistically significant.

Results

Patient Characteristics. The baseline clinical and virological characteristics of the 414 HBeAg-negative carriers with PNALT are shown in Table 1. There were 229 men and 185 women. The mean age was 39 ± 10 years (range, 17-71 years). The mean of serum ALT level was 20 ± 8 IU/L (range, 4-40 IU/L). Of 414 patients, 353 (85.3%) had detectable serum HBV DNA level (>100 copies/ml). The mean and median HBV DNA level of 353 patients was 4.7 ± 1.5 and 4.5 $\log_{10}$ copies/ml (range, 2.3-11 $\log_{10}$ copies/ml), respectively. The distribution of HBV genotypes was genotype B in 276 (78.2%) and genotype C in 77 (21.8%). The overall prevalence of precore A1896 mutant and basal core promoter T1762/A1764 mutant was 71.4% and 28.2%, respectively. The yield rate of amplification and sequencing of precore/basal core promoter genes were 350/353 (99.2%) and 302/353 (85.6%), respectively.

Comparisons Between HBeAg-Negative HBV Carriers with Low- and High-Normal Alanine Aminotransferase Levels. Because the distribution of serum ALT level is continuous, it is difficult to arbitrarily define the cutoff concentration of ALT to separate the clinically significant HBV patients from inactive carriers. A recent study on Chinese chronic hepatitis B patients showed that patients with serum ALT levels of 0.5 to $1 \times \text{ULN}$ had a significantly increased risk of complications compared with patients with serum ALT levels $< 0.5 \times \text{ULN}$.⁵ Thus, we adopted their definition, and two groups of patients (low- and high-normal ALT) were stratified based on serum ALT level of $0.5 \times \text{ULN}$. The baseline clinical features in 176 patients (42.5%) with low-normal ALT and 238 (57.5%) with high-normal ALT were compared (Table 1). The mean age of patients with high-normal ALT was significantly higher than that of those with low-normal ALT (41 years vs. 37 years, $P < 0.001$).

Table 1. Baseline Characteristics of 414 HBeAg-Negative HBV Carriers with Low-Normal and High-Normal Alanine Aminotransferase

	Total	Alanine Aminotransferase		P
		Low-Normal	High-Normal	
No	414	176 (42.5%)	238 (57.5%)	
Gender				0.55
Male	229 (55.3%)	94 (53.4%)	135 (56.7%)	
Female	185 (44.7%)	82 (46.6%)	103 (43.3%)	
Age (years, mean $\pm$ SD)	39 $\pm$ 10	37 $\pm$ 10	41 $\pm$ 10	<0.001
10-year categories of age				<0.001
<30 years	77 (18.6%)	48 (27.3%)	29 (12.2%)	
30-39 years	134 (32.4%)	58 (33%)	76 (31.9%)	
40-49 years	133 (32.1%)	49 (27.8%)	84 (35.3%)	
$\geq$ 50 years	70 (16.9%)	21 (11.9%)	49 (20.6%)	
ALT	20 $\pm$ 8	13 $\pm$ 4	25 $\pm$ 6	<0.001
HBV DNA				0.48
Detectable	353 (85.3%)	153 (86.9%)	200 (84%)	
Undetectable	61 (14.7%)	23 (13.1%)	38 (16%)	
Log ₁₀ HBV DNA level§	4.7 $\pm$ 1.5	4.3 $\pm$ 1.4	5.0 $\pm$ 1.5	<0.001
(copies/ml)				0.001*
<4	122 (34.6%)	70 (45.8%)	52 (26%)	
4-5	100 (28.3%)	38 (24.8%)	62 (31%)	
5-6(<6)	74 (21%)	28 (18.3%)	46 (23%)	
6-7(<7)	37 (10.5%)	14 (9.2%)	23 (11.5%)	
$\geq$ 7	20 (5.7%)	3 (2.0%)	17 (8.5%)	
Genotype§				1.0
B	276 (78.2%)	120 (78.4%)	156 (78%)	
C	77 (21.8%)	33 (21.6%)	44 (22%)	
Precore 1896§				0.23†
Mutant	252 (71.4%)	104 (68%)	148 (74%)	
Wild	98 (27.8%)	48 (31.4%)	50 (25%)	
NA	3 (0.8%)	1 (0.6%)	2 (1.0%)	
Basal core promoter 1762/1764§				0.01‡
Mutant	110 (28.2%)	37 (24.2%)	73 (36.5%)	
Wild	192 (54.4%)	94 (61.4%)	98 (49%)	
NA	51 (14.4%)	22 (14.4%)	29 (14.5%)	

Abbreviation: ALT: alanine aminotransferase.

*Patients had HBV DNA level less than 4 Log₁₀ or undetectable versus patients had HBV DNA level greater than 4 Log₁₀.

†Precore 1896 mutant versus wild-type.

‡Basal core promoter 1762/1764 mutant versus wild type.

§The percentage was based on those patients with detectable HBV DNA.

Among patients with high-normal ALT, 148 (62.2%) had HBV DNA level greater than 10⁴ copies/ml, which was significantly higher than 83 (47.2%) in patients with low-normal ALT level ($P < 0.001$). In addition, patients with high-normal ALT had significantly higher prevalence of basal core promoter T1762/A1764 mutant than patients with low-normal ALT (73/200, 36.5% vs. 37/153, 24.2%, $P = 0.01$).

Factors Associated with High-Normal Serum ALT Level. The factors associated with high-normal serum ALT level were further analyzed by multiple logistic regression (Table 2). Male sex [odds ratio (OR), 1.82; 95% confidence interval (CI), 1.10-3.01, $P = 0.019$], increasing age (OR, <30 years: 1, reference; 30-39 years: 2.43, 95% CI, 1.18-5.03, $P = 0.016$; 40-49 years: 4.22, 95% CI, 1.99-8.93, $P < 0.001$; $\geq$ 50 years: 4.06, 95% CI, 1.69-9.78, $P = 0.002$), and serum HBV DNA level >

10⁴ copies/ml (OR, 1.83; 95% CI, 1.07-3.13, $P = 0.027$) were independently associated with high-normal ALT.

Association Between Serum Alanine Aminotransferase Level and Viral Factor in 10-Year Categories of Age. As shown in Table 3, in the 10-year age group, the younger patients (<50 years) with high-normal ALT had significantly higher mean HBV DNA level than those with low-normal ALT. In addition, the youngest patient (<30 years) with high-normal ALT had significantly higher prevalence of basal core promoter T1762/A1764 mutant than those with low-normal ALT (7/17, 41.2% vs. 3/38, 7.9%, $P = 0.01$). The prevalence of basal core promoter T1762/A1764 mutant was positively correlated with increasing 10-year categories of age (Table 4). Patients with precore G1896 wild-type strain and basal core promoter T1762/A1764 mutant had a significantly higher serum HBV DNA level than those with both pre-

Table 2. Multiple Logistic Regression Analysis of Factors Associated with High-Normal Alanine Aminotransferase Level

	OR	95% CI	P
Sex			0.019
Female	1		
Male	1.82	1.10-3.01	
Age			
< 30 years	1		
30-39 years	2.43	1.18-5.03	0.016
40-49 years	4.22	1.99-8.93	<0.001
≥50 years	4.06	1.69-9.78	0.002
HBV DNA level			0.027
< 4 Log ₁₀	1		
≥ 4 Log ₁₀	1.83	1.07-3.13	
Genotype			0.62
B	1		
C	1.17	0.66-2.16	
Precore 1896			0.31
Wild	1		
Mutant	1.33	0.77-2.29	
Basal core promoter 1762/1764			0.20
Wild	1		
Mutant	1.42	0.84-2.40	

core G1896 and basal core promoter A1762/G1764 wild-type strain as well as both precore A1896 and basal core promoter T1762/A1764 mutant (Fig. 1).

Changes of Serum ALT Level During Follow-Up.

Eighty-nine patients had regular follow-up longer than 1 year after enrollment. Mean duration of follow-up was 30 ± 16 months (range, 12-77 months). We further examined the association between HBV DNA and changes of serum ALT level at study entry and last follow-up. Of 76 patients having detectable HBV DNA at study entry, 12 patients who went from low-normal ALT at study entry to high-normal ALT at last follow-up had significantly higher baseline serum HBV DNA levels than 15 with persistent low-normal ALT during the follow-up (Table 5).

Discussion

Most patients who have undergone HBeAg seroconversion are considered to have low-replicative HBV infection as well as normal serum ALT levels which indicate

favorable clinical outcomes.¹⁵ Unfortunately, several HBeAg-negative viral mutant strains are now known to be responsible for continuous HBV DNA replication after HBeAg seroconversion.¹⁶ In this study, 31.6% of HBeAg-negative carriers with PNALT had an HBV DNA level greater than 10^5 copies/ml, which has been considered a level indicative of clinical significance.¹⁷⁻¹⁹ This finding was consistent with previous reports that a normal ALT level alone was not an accurate indicator of inactive disease.^{20,21} Our findings suggested that the inactive range of serum ALT level should be redefined.²² In addition, serum HBV DNA should be monitored even in HBeAg-negative carriers with PNALT.

According to a large long-term follow-up study from Taiwan, elevated serum ALT level was significantly associated with an increased risk of hepatocellular carcinoma.²³ Recently, a large hospital-based cohort study on Chinese chronic hepatitis B patients also showed that patients with high-normal ALT had significantly higher risk for the development of cirrhotic complications and HCC compared with patients with low-normal ALT.⁵ However, the virological mechanisms involved in liver disease progression in HBeAg-negative carriers with PNALT remains unknown. In this study, patients with high-normal ALT had significantly higher serum HBV DNA levels than those with low-normal ALT. Multiple logistic regression analysis further showed that male sex, advanced age, and serum HBV DNA level greater than 10^4 copies/ml were independently associated with high-normal ALT. In addition, the viral load was also significantly correlated with serum ALT level in different categories of age. Because a quantitative HBV DNA test is expensive and cannot be routinely performed in many parts of the world, the serum ALT level may serve as a more feasible and cheaper surrogate marker. Furthermore, during the course of chronic HBV infection, serum HBV DNA level usually remains stable in HBeAg-negative carriers with PNALT.²⁴ Collectively, our findings provide strong evidence that serum ALT level is an important marker, in addition to other viral or host factors such as HBV DNA

Table 3. Association Between Serum Alanine Aminotransferase Level and Viral Factors in 353 HBeAg-Negative Carriers With Detectable HBV DNA Stratified for Low-Normal and High-Normal Alanine Aminotransferase in Each 10-Year Age Group

ALT	Log ₁₀ HBV DNA Level				P	Precore 1896 Mutant (%)			Basal Core Promoter Mutant (%)			P value
	No(%)	Low-Normal	No(%)	High-Normal		Low-Normal	High-Normal	P	low-normal	high-normal		
<30 years	43 (28.1)	3.9 ± 1.4	21 (10.5)	4.8 ± 1.5	0.03	26/43 (60.5)	17/21 (81)	0.16	3/38 (7.9)	7/17 (41.2)		0.01
30-39 years	50 (32.7)	4.6 ± 1.4	63 (31.5)	5.3 ± 1.8	0.03	31/50 (62)	46/63 (73)	0.23	16/45 (35.6)	20/54 (37)		1.0
40-49 years	42 (27.5)	4.1 ± 1.1	72 (36)	4.8 ± 1.3	0.003	33/41 (80.5)	49/72 (68.1)	0.19	11/33 (33.3)	27/63 (42.9)		0.39
≥50 years	18 (11.8)	5.0 ± 1.4	44 (22)	5.1 ± 1.2	0.69	14/18 (77.8)	36/42 (85.7)	0.47	7/15 (46.7)	19/37 (51.4)		1.0

Table 4. Association Between HBV Genotypes and Status of Precore/Basal Core Promoter Genes in 353 HBeAg-Negative Carriers with Detectable HBV DNA by 10-Year Categories of Age

	10-Year Categories of Age				P
	<30 Years (%)	30–39 Years(%)	40–49 Years(%)	≥50 Years(%)	
Genotype					0.96
B	50 (78.1)	88 (77.9)	88 (77.2)	50 (80.6)	
C	14 (20.9)	25 (22.1)	26 (22.8)	12 (19.4)	
Precore 1896					0.14
Wild	21 (32.8)	36 (31.9)	31 (27.4)	10 (16.7)	
Mutant	43 (67.2)	77 (68.1)	82 (72.6)	50 (83.3)	
Basal core promoter 1762/1764					0.01
Wild	45 (81.8)	63 (63.6)	58 (60.4)	26 (50)	
Mutant	10 (18.2)	36 (36.4)	38 (39.6)	26 (50)	

and age, for the evaluation and follow-up of patients with chronic HBV infection.

We further investigated the influence of persistent HBV viremia on the changes of serum ALT level during a long-term follow-up. In the subgroup analysis of patients with regular follow-up, hepatitis flare of serum ALT level $> 5 \times \text{ULN}$ was not found in HBeAg-negative carriers with PNALT. However, nearly 60% of patients had high-normal ALT at last follow-up. Baseline serum HBV DNA levels of patients that went from low-normal ALT at study entry to high-normal ALT at last follow-up had significantly higher baseline serum HBV DNA levels than those with persistently low-normal ALT during the

follow-up. The lack of long-term outcome of large HBV carriers or histology data is the major limitation of our study; however, our findings indicated that persistent HBV viremia may cause subclinical yet continuous disease activity in HBeAg-negative carriers with PNALT. This observation were supported by recent large cohort studies from Taiwan, indicating that serum HBV DNA level greater than 10^4 copies/ml was significantly associated with increased risks of cirrhosis and HCC development.^{6,25} In addition, serum HBV DNA level had been found to be associated with histological severity,¹⁸ and fluctuations in serum HBV load may imply intermittent immune-mediated viral control and hepatocyte damage.²⁶ Taken together, high-normal ALT was possibly associated with liver disease progression, and a serum HBV DNA level of 10^4 copies/ml might serve as a critical cutoff level to predict disease progression.

Recently, several HBV mutant strains including mutations in precore, core promoter, and deletion in pre-S genes have been reported to be associated with the pathogenesis of progressive liver disease, including cirrhosis and HCC.^{8–13} However, the association between viral mutants and progression of liver disease in HBeAg-negative carriers with PNALT remains unclear. In this study, com-

Fig. 1. Box plot for hepatitis B virus DNA levels and status of precore/basal core promoter (BCP) genes in 299 HBeAg-negative carriers. The data are presented as the median (horizontal line) and the range from the 25th to the 75th percentiles. Mean HBV DNA level is indicated as a black dot. *P* values are derived for the comparison of the mean levels between patients with different status of precore/basal core promoter genes. Group 3 versus 1, *P* = 0.019; Group 3 versus 2, *P* = 0.039; Group 3 versus 4, *P* = 0.046.

Table 5. Association Between HBV DNA and Changes of Serum Alanine Aminotransferase Level at Study Entry and Last Follow-up in 76 Patients with Detectable HBV DNA at Study Entry

ALT Level		No (%)	Log ₁₀ HBV DNA Level
At Study Entry	At Last Follow-up		
Low-normal	Low-normal	15 (19.7)	5.35 ± 0.86 ^a
Low-normal	High-normal	12 (15.8)	6.39 ± 1.69 ^b
High-normal	Low-normal	17 (22.4)	5.76 ± 0.86 ^c
High-normal	High-normal	32 (42.1)	5.75 ± 0.95 ^d

b versus a, *P* = 0.049.

b versus c, *P* = 0.19.

b versus d, *P* = 0.12.

pared with patients with low-normal ALT, patients with high-normal ALT had significantly higher mean age as well as the prevalence of basal core promoter T1762/A1764 mutant. In addition, the prevalence of basal core promoter T1762/A1764 mutant was in parallel with increasing 10-year categories of age. Furthermore, patients with basal core promoter T1762/A1764 mutant alone had a significantly higher serum HBV DNA level than those with basal core promoter A1762/G1764 wild-type strain, regardless of precore 1896 status. These results confirmed the *in vitro* data that basal core promoter T1762/A1764 mutant may increase HBV replication.^{27,28} The impact of basal core promoter mutant T1762/A1764 on the replication of HBV may be modulated by precore A1896 mutant. Accordingly, we proposed a hypothesis that when age increases, basal core promoter T1762/A1764 mutant may emerge and lead to the increase of HBV viral load in a certain proportion of HBeAg-negative carriers. Collectively, our findings support that, in addition to HBV DNA level, basal core promoter mutant also plays an important role in the pathogenesis of liver disease progression in HBeAg-negative carriers with PNALT, as is the case of those with chronic hepatitis activity.

In conclusion, our data indicate that HBeAg-negative patients with persistently normal ALT are not a homogeneous group, and those with high-normal ALT share some of the characteristics that have been associated with adverse long-term outcomes. These findings also highlight the fact that the distinction is not always clearcut between inactive carriers and patients with HBeAg-negative hepatitis.

References

- Kao JH, Chen DS. Global control of hepatitis B virus infection. *Lancet Infect Dis* 2002;2:395-403.
- Bonino F, Brunetto MR. Chronic hepatitis B e antigen (HBeAg) negative, anti-HBe positive hepatitis B: an overview. *J Hepatol* 2003;39:S160-S163.
- Papatheodoridis GV, Hadziyannis SJ. Diagnosis and management of precore mutant chronic hepatitis B. *J Viral Hepatol* 2001;8:311-321.
- Kim HC, Nam CM, Jee SH, Han KH, Oh DK, Suh I. Normal serum aminotransferase concentration and risk of mortality from liver diseases: prospective cohort study. *BMJ* 2004;328:983-988.
- Yuen MF, Yuan HJ, Wong DK, Yuen JC, Wong WM, Chan AO, et al. Prognostic determinants for chronic hepatitis B in Asians: therapeutic implications. *Gut* 2005;54:1610-1614.
- Chen CJ, Yang HI, Su J, Jen CL, You SL, Lu SN, et al. Risk of hepatocellular carcinoma across a biological gradient of serum hepatitis B virus DNA level. *JAMA* 2006;295:65-73.
- Brunetto MR, Oliveri F, Coco B, Leandro G, Colombatto P, Gorin JM, et al. The outcome of chronic anti-HBe positive chronic hepatitis B in alpha interferon treated and untreated patients: a long term cohort study. *J Hepatol* 2002;36:263-270.
- Kao JH, Chen PJ, Lai MY, Chen DS. Basal core promoter mutations of hepatitis B virus increase the risk of hepatocellular carcinoma in hepatitis B carriers. *Gastroenterology* 2003;124:327-334.
- Lin CL, Liao LY, Wang CS, Chen PJ, Lai MY, Chen DS, et al. Basal core promoter mutant of hepatitis B virus and progression of liver disease in hepatitis B e antigen-negative chronic hepatitis B. *Liver Int* 2005;25:564-570.
- Liu CJ, Chen BF, Chen PJ, Lai MY, Huang WL, Kao JH, et al. Role of hepatitis B viral load and basal core promoter mutation in hepatocellular carcinoma in hepatitis B carriers. *J Infect Dis* 2006;193:1258-1265.
- Liu CJ, Chen BF, Chen PJ, Lai MY, Huang WL, Kao JH, et al. Role of hepatitis B virus precore/core promoter mutations and serum viral load on non-cirrhotic hepatocellular carcinoma: a case control study. *J Infect Dis* 2006;194:594-599.
- Chen BF, Liu CJ, Jow GM, Chen PJ, Kao JH, Chen DS. High prevalence and mapping of pre-S deletion in hepatitis B virus carriers with progressive liver diseases. *Gastroenterology* 2006;130:1153-1168.
- Yang HI, Chen PJ, Yeh SH, Jen CL, You SL, Chen DS, et al. Risk of hepatocellular carcinoma associated with genotypes and mutants of hepatitis B virus: a community-based prospective cohort study [Abstract]. *J Hepatol* 2006;42(Suppl):27A.
- Yeh SH, Tsai CY, Kao JH, Liu CJ, Kuo TJ, Lin MW, et al. Quantification and genotyping of hepatitis B virus in a single reaction by real-time PCR and melting curve analysis. *J Hepatol* 2004;41:659-666.
- Chu CM, Karayiannis P, Fowler MJ, Monjardino J, Liaw YF, Thomas HC. Natural history of chronic hepatitis B virus infection in Taiwan: studies of hepatitis B virus DNA in serum. *HEPATOLOGY* 1985;5:431-434.
- Tong S, Kim KH, Chante C, Wands J, Li J. Hepatitis B virus e antigen variants. *Int J Med Sci* 2005;2:2-7.
- Gerken G, Gomes J, Lampertico P, Colombo M, Rothaar T, Trippler M, et al. Clinical evaluation and applications of the amplicor HBV Monitor test, a quantitative HBV DNA PCR assay. *J Virol Methods* 1998;74:155-165.
- Lindh M, Horal P, Dhillon AP, Norkrans G. Hepatitis B virus DNA levels, precore mutations, genotypes and histological activity of chronic hepatitis B. *J Viral Hep* 2000;7:258-267.
- Lok AS, McMahon BJ. Practice Guidelines Committee, American Association for the Study of Liver Diseases: chronic hepatitis B. *HEPATOLOGY* 2001;34:1225-1241.
- Chan HL, Tsang SW, Liew CT, Tse CH, Wong ML, Ching JY, et al. Viral genotype and hepatitis B virus DNA levels are correlated with histologic liver damage in HBeAg-negative chronic hepatitis B virus infection. *Am J Gastroenterol* 2002;97:406-412.
- Sung JJ, Chan HL, Wong ML, Tse CH, Yuen SC, Tam JS, et al. Relationship of clinical and virological factors with hepatitis activity in hepatitis B e antigen-negative chronic hepatitis B virus-infected patients. *J Viral Hepatol* 2002;9:229-234.
- Prati D, Taioli E, Zanella A, Della Torre E, Butelli S, Del Vecchio E, et al. Updated definitions of healthy ranges for serum alanine aminotransferase levels. *Ann Intern Med* 2002;137:1-10.
- Geier A, Garton C, Dietrich CG, Chen CJ, Yang HI, You SL. Hepatitis B e antigen and the risk of hepatocellular carcinoma. *N Engl J Med* 2002;347:1721-1722.
- Chu CJ, Hussain M, Lok AS. Quantitative serum HBV DNA levels during different stages of chronic hepatitis B infection. *HEPATOLOGY* 2002;36:1408-1415.
- Iloeje UH, Yang HI, Su J, Jen CL, You SL, Chen CJ. Predicting cirrhosis risk based on the level of circulating hepatitis B viral load. *Gastroenterology* 2006;130:678-686.
- Lee KM, Cho SW, Kim SW, Kim HJ, Hahn KB, Kim JH. Effect of virological response on post-treatment durability of lamivudine-induced HBeAg seroconversion. *J Viral Hepatol* 2002;9:208-212.
- Kidd AH, Kidd-Ljunggren K. A revised secondary structure model for the 3'-end of hepatitis B virus pregenomic RNA. *Nucl Acids Res* 1996; 24: 3295-3301.
- Li J, Buckwold VE, Hon MW, Ou JH. Mechanism of suppression of hepatitis B virus precore RNA transcription by a frequent double mutation. *J Virol* 1999;73:1239-1244.