科技部補助專題研究計畫報告

免疫耐受期慢性B型肝炎病人臨床表徵與預後之性別差異

報 告 類 別 ： 成果報告
計 畫 類 別 ： 個別型計畫
計 畫 編 號 ： MOST 108-2629-B-182A-006-
執 行 期 間 ： 108年08月01日至109年10月31日
執 行 單 位 ： 長庚醫療財團法人胃腸肝膽科

計 畫 主 持 人： 陳益程
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本研究具有政策應用參考價值：■否 □是，建議提供機關
（勾選「是」者，請列舉建議可提供施政參考之業務主管機關）
本研究具影響公共利益之重大發現：□否 □是

中 華 民 國 110 年 01 月 29 日

中 文 摘 要 ： 研究背景
慢性B型肝炎自然病程分為免疫耐受期、免疫清除期以及不活動期，若不活動期病人因病毒活化造成肝細胞損傷，則進入非典型免疫廓清期。免疫耐受期病人特性包含e抗原陽性、極高病毒量(>10^6-10^7 IU/mL)與正常/略為上升的肝生化(ALT)指數，肝切片檢查通常只有微弱組織變化。與女性病人比較，過去研究顯示慢性B型肝炎男性病人常有ALT指數異常，e抗原陽性率較低，且於e抗原轉換後有較高機會肝炎復發。且肝癌發生率男性亦高於女性。但免疫耐受期中臨床表徵與預後的男女差異未曾被探討。
研究目標
探討免疫耐受期臨床表徵與預後的男女差異
研究設計
這是前瞻性觀察研究，研究期間為2019/8/1到2020/10/31，將經由胃腸肝膽科門診收案。符合下列條件的慢性B型肝炎病人將被納入研究：HBsAg與HBeAg陽性超過6個月，20歲以上男性或女性，HBV DNA超過 10^7 IU/mL，收案前未曾接受過抗病毒藥物治療，正常肝生化指數(ALT 36 U/L)至少1年。研究期間收集生化檢驗，B型肝炎病毒血清學檢驗，非侵入性肝纖維化評估資料。
結果評估
分析肝生化檢驗，B型肝炎病毒血清學檢驗，非侵入性肝纖維化狀態，臨床預後於男女性間的差別。
統計分析
所有的統計評估會以雙尾檢定方式進行，其顯著水準為0.05。連續變項則以t-test或ANOVA分析，類別變項以卡方檢定或Fisher’s exact檢定方式進行分析，存活分析以Kaplan-Meier analysis進行。若資料不為常態分布型式，則以無母數統計方法、Wilcoxon rank-sum或sign-rank檢定方式進行連續及類別變項分析。

中 文 關 鍵 詞 ： 慢性B型肝炎，免疫耐受，B型肝炎病毒量，e抗原

英 文 摘 要 ： Background
The natural course of perinatally acquired chronic hepatitis B (CHB) infection consists of three distinct phases: immune tolerant, immune clearance and inactive residual phases and a variant phase of immune clearance when reactivation and immune-mediated liver injury occur in patients with inactive disease. Patients of immune tolerant phase are characterized by positive HBeAg, very high HBV DNA (>10^6-10^7 IU/mL) and normal or minimally elevated alanine aminotransferase (ALT). Liver biopsy results often show no or mild fibrosis and minimal inflammation. Past studies have reported that male CHB patients had abnormal ALT more frequently, lower positive rate of HBeAg and a higher relapse rate of hepatitis after HBeAg seroconversion when compared to females. The risk of HBV-associated hepatocellular carcinoma (HCC) was higher in men than in women. Sex difference in the clinical features and prognosis of immune-tolerant CHB patients has not been

explored.
Aim
To investigate the clinical characteristics and prognosis in immune-tolerant CHB patients between males and females.
Study design
This is a prospective observational study. Study period is 2019/8/1 to 2012/7/31. Patient number is 200. CHB patients will be recruited with the criteria of (1) HBsAg and HBeAg positive for at least 6 months; (2) Males and females of age >20; (3) HBV DNA>10^7 IU/mL; (4) Antiviral treatment naïve before entry; (5) persistent ALT (36 U/L) 1 year. Laboratory tests, HBV serological profiles and liver fibrosis evaluated by noninvasive methods will be collected.
Study endpoints
The difference in liver biochemistry, liver fibrosis, HBV profiles and clinical prognosis between males and females will be analyzed.

英文關鍵詞：Chronic hepatitis B, immune tolerance, HBV DNA, HBeAg

報告內容:

Gender difference in chronic hepatitis B patients in mimicking immune tolerant phase

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Introduction

There are estimated 248 million persons with chronic infection of hepatitis B virus (HBV) worldwide and the prevalence is up to 8.6% in East Asia (1,2). The natural course of perinatally acquired chronic hepatitis B (CHB) infection consists of three distinct phases: immune tolerant, immune clearance and inactive residual phases and a variant phase of immune clearance when reactivation and immune-mediated liver injury occur in patients with inactive disease (3). Patients of immune tolerant phase are characterized by positive HBeAg, very high HBV DNA (>10^6-10^7 IU/mL) and normal or minimally elevated alanine aminotransferase (ALT) (4-6).

In two previous studies including 57 and 40 Asian patients with positive HBeAg, persistently normal ALT (PNALT) and high serum HBV DNA (>10^7 copies/mL), liver biopsy showed fibrosis score F0-F1 (7,8). In patients remaining in the immune tolerant phase, only 6.3% had fibrosis progression on follow-up liver biopsy at five years (7). In a study from Taiwan on 30 CHB patients with positive HBeAg and high HBV DNA (>500 pg/mL), 90% showed only minor hepatic inflammatory activity (9). These findings suggest that liver biopsy results often show no or mild fibrosis and minimal inflammation in immune-tolerant patients. However, 23 HBeAg-positive patients with PNALT and HBV DNA >10^7 copies/mL in an Indian study had a median fibrosis score of 1.0 (0.0-3.0), which meant 50% of these immune tolerant patients had significant fibrosis (10). Therefore, large-scale studies to evaluate the stage of liver fibrosis in immune-tolerant CHB patients are needed.

Past studies have reported that male CHB patients had abnormal ALT more frequently, lower positive rate of HBeAg and a higher relapse rate of hepatitis after HBeAg seroconversion when compared to females (11-13). Although the reason for this phenomenon is still unclear, the results that the risk of HBV-associated hepatocellular carcinoma (HCC) was higher in men than in women may be explained by the impact of sex hormones in the virus-associated hepatocarcinogenesis (14). It has been reported that elevated serum

testosterone is associated with development of HCC and hepatic androgen receptor increases HBV-induced hepatocarcinogenesis in mice (15,16). Sex difference in the clinical features and prognosis of immune-tolerant CHB patients has not been explored.

Patients and Methods

Patients

From August 2019 to October 2020, 45 consecutive CHB patients were recruited into this study. They fulfilled the following criteria: (1) HBsAg positive for more than 6 months, (2) HBeAg positive for more than 6 months, (3) serum ALT within upper limit of normal (ULN, <36 U/L in men and women) for at least one year before entry, (4) HBV DNA ≥7 log IU/mL at the time of entry, (5) treatment naïve before entry. Patients with coinfection with hepatitis C virus (HCV), hepatitis D virus (HDV) or human immunodeficiency virus (HIV), concomitant alcoholic liver disease or autoimmune liver disease and those under immunomodulatory therapy or any other known medication of immunosuppression before entry were excluded. This study was conducted under the approval of institutional review board (IRB No. 201900165B0).

Clinical and laboratory assessments

Demographic information of age, gender, body mass index (BMI) was recorded from electronic medical records. Laboratory data including aspartate aminotransferase (AST), ALT, platelet count, fasting sugar, lipid profiles, homeostasis model assessment-insulin resistance (HOMA-IR) (17), HBeAg, anti-HBe, anti-HCV, anti-HDV, HBsAg, HBV DNA and HBV genotype were collected. Dyslipidemia was defined as at least one component of abnormal lipids (i.e. total cholesterol ≥240 mg/dL, low density lipoprotein cholesterol [LDL-C] ≥160 mg/dL, high density lipoprotein cholesterol [HDL-C] <40 mg/dL for men or <50 mg/dL for women, triglyceride ≥200 mg/dL) (18). HBV genotype was determined by polymerase chain reaction-restriction fragment length polymorphism of the surface gene of HBV. Serum HBsAg levels were quantified using the Roche Elecsys HBsAg II quant assay (detection limit, 0.05-52,000 IU/mL; Roche Diagnostics, Mannheim, Germany) according to the manufacturer's instructions. Serum HBV DNA was assayed by COBAS® AmpliPrep/COBAS® TaqMan® HBV Test, version 2.0 (lower limit of detection: 20 IU/mL, Roche Diagnostics, Mannheim, Germany). HBeAg, anti-HBe and anti-HCV were tested with

electrochemiluminescence immunoassay (ECLIA, Roche Diagnostics GmbH, Mannheim, Germany). Anti-HDV was assayed with enzyme immunoassay kit (Abbott Diagnostics, North Chicago, IL or General Biologicals Corp., Hsinchu, Taiwan after 2018 June).

Noninvasive assessments of liver fibrosis

Liver stiffness measurement (LSM, kPa) and controlled attenuation parameter (CAP, dB/m) were performed using vibration-controlled transient elastography (VCTE, Fibroscan® 502, Echosens, Paris, France). Both LSM and CAP values were expressed as the median of at least 10 successful measurements. Severe fibrosis was defined as LSM >9.0 kPa in patients with normal ALT or LSM >12.0 kPa in those with ALT 1-5x ULN (19). Hepatic steatosis was defined as CAP ≥248 dB/m. Mild, moderate, and severe steatosis were defined as CAP 248–267 dB/m, CAP 268–279 dB/m, and CAP ≥280 dB/m, respectively, based on a recent meta-analysis correlating CAP measurements with histologic steatosis grading (20). Fibrosis-4 (FIB-4) score was calculated by [age (years) × AST (IU/L)]/[platelet count (10^9/L)] × [ALT (IU/L)]$^{1/2}$. A FIB-4 score <1.45 had a negative predictive value of 90% for advanced fibrosis (Ishak fibrosis score 4-6 which includes early bridging fibrosis to cirrhosis). In contrast, a FIB-4 >3.25 would have a 97% specificity and a positive predictive value of 65% for advanced fibrosis (21).

Statistical analysis

Continuous variables were expressed as means and standard deviations (S.D.) or medians and interquartile ranges (IQR) as appropriate after testing for normal distribution using the Kolmogorov-Smirnov test and were compared by independent Student's *t*-test or Mann-Whitney-U test between two different groups. Categorical variables were presented as the number of cases (proportions) and compared by Chi-squared or Fisher's exact tests when appropriate. The serum HBsAg and HBV DNA levels were logarithmically transformed for analysis. Statistical analysis was performed by IBM SPSS Statistics for Windows, version 22.0 (IBM Corp., Armonk, NY, USA). A two-tailed p <0.05 was considered statistically significant.

Results

The mean age was 44.6±9.5 years and median BMI was 21.9 kg/m^2. There were 34 females (75.6%) and 25 (59.5%, genotype available in 42 patients) genotype C. The baseline clinical characteristics was shown in Table 1. Male patients had significantly lower mean platelet count (219 vs 282.6 10^9/L, p=0.008), higher mean total bilirubin level (0.9 vs 0.6 mg/dL, p=0.034), and median levels of r-GT (19 vs 12 U/L, p=0.006), creatinine (0.84 vs 0.56 mg/dL, p<0.001), and FIB-4 score (1.39 vs 0.81, p=0.022) than female patients. The remaining clinical characters were comparable between males and females.

Table 1. The clinical characteristics and comparison between male and female HBeAg-positive patients

	overall	male	female	p
No	45	11	34	
Age, years	44.6±9.5	46.6±13.5	43.9±8.0	0.416
BMI, kg/m^2	21.9 (20.4-23.5)	21.3 (19.5-24.0)	22.0 (20.7-23.4)	0.630
Genotype (%)				1.000
B	17 (40.5)	4 (44.4)	13 (39.4)	
C	25 (59.5)	5 (55.6)	20 (60.6)	
Platelet, $10^9/L$	268±68	219±44.5	282.6±67.1	0.008
Albumin, g/dL	4.4±0.2	4.5±0.3	4.4±0.2	0.269
AST, U/L	25.9±5.3	28.0±4.8	25.3±5.3	0.138
ALT, U/L	24.0±6.4	26.4±8.4	23.2±5.5	0.152
ALK-p, U/L	57.1±15.8	59.5±20.1	56.4±11.7	0.590
Bil T, mg/dL	0.6 (0.5-0.8)	0.9 (0.5-1.6)	0.6 (0.5-0.7)	0.034
r-GT, U/L	14 (10-17)	19 (14-30)	12 (9-16)	0.006
Cr, mg/dL	0.6 (0.51-0.67)	0.84 (0.68-0.97)	0.56 (0.49-0.61)	<0.001
Cholesterol, mg/dL	211 (183-247)	229 (197-266)	202 (182-225)	0.184
Triglyceride, mg/dL	83 (69-144)	112 (70-205)	83 (63-123)	0.209
HDL	59.1±13.4	62.6±19.1	58.2±11.7	0.414
LDL	127 (103-152)	128 (116-174)	123 (102-148)	0.621
Sugar, mg/dL	87 (82-89)	89 (86-94)	86 (80-89)	0.102
HbA1c, %	5.5 (5.3-5.7)	5.6 (5.4-5.8)	5.4 (5.3-5.5)	0.125
HOMA-IR	1.36 (0.84-1.98)	1.11 (0.78-1.77)	1.40 (0.85-2.15)	0.520
FIB-4	0.85 (0.63-1.39)	1.39 (0.85-1.59)	0.81 (0.59-1.03)	0.022
HBV DNA, log IU/mL	8.41±0.67	8.35±0.65	8.44±0.68	0.706
HBsAg, log IU/mL	4.63 (4.40-4.90)	4.64 (4.37-4.93)	4.61 (4.37-4.89)	0.629
LSM, kPa	4.5 (3.9-5.3)	5.0 (4.3-6.0)	4.3 (3.8-5.2)	0.150
CAP, dB/m	220.5±33.4	215.7±36.6	221.8±33.0	0.629
Steatosis	10 (22.2)	1 (9.1)	9 (26.5)	0.409

Of the 45 patients, 31 patients (7 males and 24 females) had follow-up tests after a median period of 11.3 (8.8-12.6) months. The mean follow-up ALT level was 30.6 U/L in males and 23.4 U/L in females (p=0.052). The median follow-up FIB-4 score and LSM were 1.31 and 4.4 kPa in males and 0.75 and 4.4 kPa in females, respectively (p=0.008 in FIB-4 score and

p=0.854 in LSM). The paired comparison of ALT, FIB-4 score and LSM was not significantly different in males (p=0.139, p=0.735, and p=0.917, respectively) and females (p=0.642, p=0.502, and p=0.764, respectively).

Discussion

The interpretation of our data was limited by small patient number. Interestingly, there were around 76% females in this study population with status of mimicking immune tolerance. As expected, the majority were genotype C in this study cohort. Male patients had a lower mean platelet count, a higher mean total bilirubin level, and median levels of r-GT, creatinine, and FIB-4 score. However, LSM by VCTE was not different between male and female patients. The recruitment of more patients is needed to validate the findings in our study.

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108年度專題研究計畫成果彙整表

計畫主持人：陳益程	**計畫編號：**108-2629-B-182A-006-
計畫名稱：免疫耐受期慢性B型肝炎病人臨床表徵與預後之性別差異	

成果項目			量化	單位	質化 （說明：各成果項目請附佐證資料或細項說明，如期刊名稱、年份、卷期、起訖頁數、證號...等）
國內	學術性論文	期刊論文	0	篇	
		研討會論文	0		
		專書	0	本	
		專書論文	0	章	
		技術報告	0	篇	
		其他	0	篇	
國外	學術性論文	期刊論文	0	篇	
		研討會論文	0		
		專書	0	本	
		專書論文	0	章	
		技術報告	0	篇	
		其他	0	篇	
參與計畫人力	本國籍	大專生	0	人次	
		碩士生	0		
		博士生	0		
		博士級研究人員	0		
		專任人員	1		林以涵
	非本國籍	大專生	0		
		碩士生	0		
		博士生	0		
		博士級研究人員	0		
		專任人員	0		
其他成果 （無法以量化表達之成果如辦理學術活動、獲得獎項、重要國際合作、研究成果國際影響力及其他協助產業技術發展之具體效益事項等，請以文字敘述填列。）			無		

附件九

科技部補助研究計畫涉及臨床試驗之性別分析報告

日期：2021 年 01 月 29 日

計畫編號	MOST 108-2629-B-182A-006-		
研究人員姓名	陳益程		
任職機關系所	長庚醫療財團法人胃腸肝膽科	職稱	主治醫師
計畫名稱	免疫耐受期慢性 B 型肝炎病人臨床表徵與預後之性別差異		

說明：

本年度專題研究計畫涉及臨床試驗且進行性別分析，請於計畫成果報告(期中進度報告/期末報告)時一併繳交「性別分析報告」。

項次	項目	說明	備註
1	本計畫之研究結果已進行性別分析。	比較男女性病人於收案時肝生化指數代謝性指數，肝纖維化評估與追蹤後變化	
2	本計畫之收案件數及其性別比例。	男性11人，女性34人	
3	本計畫研究結果之性別差異說明。 如無性別差異，亦請說明。	研究族群以女性居多，而FIB-4 score在男性病患較高	