國立台灣大學公共衛生學院職業醫學與工業衛生研究所

博士論文

Institute of Occupational Medicine and Industrial Hygiene

College of Public Health

National Taiwan University

Doctoral Dissertation

B 型、C 型肝炎患者藥物使用與病程發展之關連性

The association between drug use and disease progression among patients with hepatitis B virus or hepatitis C virus infection

楊曜旭

Yao-Hsu Yang

指導老師: 陳保中 博士

Advisor: Pau-Chung Chen, Ph.D.

中華民國 106 年 5 月

May 2017

doi:10.6342/NTU201701710

目錄

doi:10.6342/NTU201701710

doi:10.6342/NTU201701710

致謝

一轉眼五年過去了，這五年來搭著高鐵台北嘉義兩地跑的日子總算是有點成果。不過拿到博士學位只是另一個新的起點，這也代表著以後不再具有學生身分，在學術這條路上會被更嚴格的檢視。

當初 2006 年大學畢業時，有幸踏入公共衛生的領域並且接受王榮德教授跟陳保中教授的指導，除了兩位恩師外，賴榮年主任、長興學長、淑卿學姊與佳容學姊對我指導與照顧甚多，這兩年的碩士歷程也奠定了我往後做研究的基礎。之後雖然重返臨床工作，但是仍一直心繫著研究，非常感謝科內吳清源主任、林吟霙學姊、施瑋泰醫師以及其他同事們的協助，讓我可以的在臨床工作上毫無後顧之憂，全心全力的攻讀博士學位。也非常感謝我博士班的同學唯珊跟涵涵，雖然平常大家都是各忙各的，但是有你們的陪伴讓我的博士班生活過得更充實。

在嘉義長庚工作的第五年時，很幸運的有機會可以建置健康資料卓越研究中心，感謝蔡燉煌院長跟陳文政主任給了我一個可以自由發揮的舞台，也非常感謝他們兩位對於健康資料卓越研究中心的指導與協助。對於健康資料卓越研究中心一起努力的夥伴們，我也要獻上十二萬分的謝意:李全濱博士、林孟宏博士、郭庭妤小姐、黃如君小姐、劉佳妍小姐、陳麒任先生、陳科榕先生，因為你們的努力付出，健康資料卓越研究中心才有今天的成果。

非常感謝我的父母親這一路來對我的栽培，也感謝黃士洋叔叔跟洪秀粧阿姨從碩士班起對我的支持與照顧。感謝這些年來幫忙與照顧過我的貴人。最後要感謝我的兩位恩師:陳保中教授跟王榮德教授。這些年來，他們兩位不但給我知識上的指導，更給了我最好的身教示範。僅以這本論文，對於生命中所有的貴人致上最高的謝意，也期許自己能夠在公共衛生跟醫療健康的領域裡有更多的貢獻。

106 年 7 月

doi:10.6342/NTU201701710

一、中英文摘要：

(一)中文摘要

截至目前為止對於 B 型肝炎與 C 型肝炎之治療仍以干擾素或抗病毒藥物為主，可以大幅的減少肝癌與肝硬化等病程發展。除了傳統的治療方式以外，也有不少的藥物具有 chemoprevntion 之作用，本研究之主要分析藥物有 statin 跟中藥小柴胡湯兩種。台灣的健保資料庫是全世界唯一有包含中醫藥使用之資料庫，而長庚醫學研究資料庫除了藥物處置等紀錄以外，還有檢查結果、檢驗報告等資料。透過分析健保資料庫跟長庚醫學研究資料庫，我們可以提供更多 statin 與小柴胡湯對於 B 型肝炎與 C 型肝炎治療療效的實證。

A. Statin

目前許多研究已證實 statin 可以降低 B 型肝炎與 C 型肝炎患者罹患肝癌之風險，本研究之目的是使用健保資料庫跟長庚醫學研究資料庫分析 statin 是否可以降低 B 型肝炎與 C 型肝炎患者發展到肝硬化之風險。

健保資料庫的分析指出，根據受試者的 Statin 使用量將 C 型肝炎患者分為四組：小於 28-83 個藥物耗用標準化之定義每日劑量(defined daily dose, DDD)

84-365DDD 跟大於 365DDD，未達 28DDD 的患者當作對照組。相對於對照組，各組別罹患肝硬化的風險比值分別為 28-83DDD: 0.33 (95% CI:0.31-0.36)、

84-365DDD: 0.24 (95% CI:0.22-0.25)、>365DDD:0.13 (95% CI:0.12-0.15)。

長庚醫學研究資料庫的分析指出根據 B 型肝炎患者的 Statin 使用量將患者分為四組：小於 28-83 個 DDD、84-365DDD 跟大於 365DDD，未達 28DDD 的患者當作對照組。相對於對照組，各組別罹患肝硬化的風險比值分別為 28-83DDD: 0.65 (95% CI, 0.53 to 0.80)、 84-365DDD: 0.53 (95% CI, 0.45 to 0.63)、>365DDD: 0.42 (95% CI, 0.34 to 0.51)。

doi:10.6342/NTU201701710

B. 小柴胡湯

先前的研究指出小柴胡湯對於 B 型肝炎與 C 型肝炎的患者也有其保護作用。日本有小型的臨床試驗研究證實小柴胡湯可以降低肝硬化病患罹患肝癌之風險。目前對於小柴胡湯之療效鮮少有臨床資料提供相關資料，本計畫擬使用健保資料庫跟長庚醫學研究資料庫分析 B 型肝炎之小柴胡湯使用與肝癌風險。

從健保資料庫 1997-2010 年的資料中挑選出 89,466 位肝硬化且有 B 型肝炎之病患當作研究族群，資料分析指出，根據 B 型肝炎患者的小柴胡湯使用量將患者分為四組：35-69g、70-139g 跟大於 140g，並以用量於 35g 的病患當作對照組。各組別罹患肝癌的風險比值分別為 0.79 (95% CI, 0.65 to 0.97), 0.73 (95% CI, 0.57 to 0.94), and 0.67 (95% CI, 0.52 to 0.86)。

關鍵字: 小柴胡湯, 史他汀, 肝硬化, 肝癌, 健保資料庫,長庚醫學研究資料庫

doi:10.6342/NTU201701710

(二)英文摘要

Both hepatitis B virus (HBV) and hepatitis C virus (HCV) infection are well-known etiological factors of Hepatocellular carcinoma (HCC) and cirrhosis. This study investigated the association between the use of statin and xiao-chai-hu-tang(XCHT) and the disease progression in patients with HBV or HCV infection. There are two parts of this research:

A. Statin

Recently, statins have been investigated for their antiproliferative, antiangiogenic, antiinflammatory, and antineoplastic effects. Several animal studies have shown that statins can inhibit the progression of cirrhosis; however, few clinical studies have been conducted. This study investigated the association between the use of statin and the risk of cirrhosis development in patients with HBV or HCV infection by analyzing the data from Taiwan National Health Insurance Research Database (NHIRD) and Chang Gung Research Database (CGRD).

From NHIRD, A dose-response relationship between statin use and cirrhosis risk was observed among HCV infected patients. The adjusted hazard ratios were 0.33 (95% CI, 0.31 to 0.36), 0.24 (95% CI, 0.22 to 0.25), and 0.13 (95% CI, 0.12 to 0.15) for statin use of 28 to 83, 84 to 365, and more than 365 cDDD, respectively, relative to no statin use (< 28 cDDD).

The adjusted hazard ratios of cirrhosis were 0.65 (95% CI, 0.53 to 0.80), 0.53 (95% CI, 0.45 to 0.63), and 0.42 (95% CI, 0.34 to 0.51) for statin use of 28 to 83, 84 to 365, and more than 365 cDDD, respectively, relative to no statin use (< 28 cDDD) among HBV infected patients recruited from CGRD .

doi:10.6342/NTU201701710

B. XCHT

We conducted a population-based cohort study by using the Taiwan National Health Insurance Research Database. A total of 89,466 HBV-infected patients with cirrhosis were included as the study cohort. Each patient was individually tracked from 1997 to 2010 to identify incident cases of HCC. A Cox proportional hazards regression with time-dependent covariates for drug exposure was employed to evaluate the association between XCHT use and HCC risk.

A dose–response relationship between XCHT use and HCC risk was observed. The adjusted hazard ratios were 0.79 (95% CI, 0.65 to 0.97), 0.73 (95% CI, 0.57 to 0.94), and 0.67 (95% CI, 0.52 to 0.86) for patients using 35 to 69, 70 to 139, and ≧140 grams per year, respectively, relative to less than 35 g.

Key words: Xiao-chai-hu-tang, statin, cirrhosis, hepatocellular carcinoma, National Health Insurance Research Database (NHIRD), Chang Gung Research Database (CGRD)

doi:10.6342/NTU201701710

Introduction

Background

Hepatitis B virus (HBV) and Hepatitis C virus (HCV) infection is endemic in Taiwan. Previous studies showed that high prevalence of chronic hepatitis B infection in the general population (15–20%), and 80–90% of the chronic liver diseases and hepatocellular carcinoma were caused by chronic infection with the HBV(1). Hepatitis C virus (HCV) infection has become a critical public health problem in Taiwan, it has been estimated that more than 300,000 people have HCV infection. Both HBV and HCV infection are well-known etiological factors of Hepatocellular carcinoma (HCC), which is the second of the ten leading cancer death in Taiwan.

The primary treatment goals for patients with hepatitis B (HBV) infection are to prevent progression of the disease, particularly to cirrhosis, liver failure, and HCC(2). Treatment includes the use of antiviral treatment using pegylated interferon (PEG-IFN) or nucleos(t)ide analogues. A synergistic approach of suppressing viral load and boosting the patient's immune response with immunotherapeutic interventions is needed for the best prognosis. The current recommended therapy for chronic hepatitis C is the combination of interferon

doi:10.6342/NTU201701710

alpha (IFN-α) and ribavirin, which results in a sustained clearance of hepatitis C virus in 40-50% of patients(3). Both treatments mentioned above for HBV and HCV can prolong the disease progression including cirrhosis and HCC. Beside antiviral treatment, there also some other drug can prolong the disease progression of HBV or HCV infection(4-10).

Statin

Statins (3-hydroxy-3-methyl-glutaryl-CoA reductase inhibitors) are used to treat and prevent coronary heart disease and stroke in patients with hyperlipidemia(11). Recently, statins have been investigated for their antiproliferative, antiangiogenic, antiinflammatory, and antineoplastic effects. Previous studies have also revealed the protective effect of statins on HCC in chronic hepatitis C (CHC), as well as in chronic hepatitis B (CHB) patients(8-10, 12, 13). The use of statins has been shown to reduce fibrosis progression and cirrhosis in patients with chronic hepatitis C. However, the protective effect of statins in the development of cirrhosis in patients with CHB or CHC is limited.

Xiao-chai-hu-tang

Traditional Chinese Medicine (TCM) has been commonly used in treating liver diseases in Taiwan(14). xiao-chai-hu-tang (XCHT, Sho-saiko-to or TJ-9 in

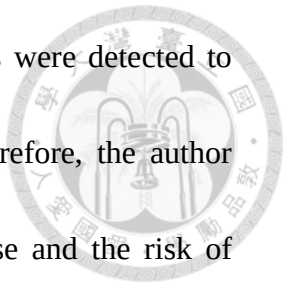
doi:10.6342/NTU201701710

Japanese) is a well-known Chinese traditional medicine that has been used for thousands of years. It consists of seven medicinal herbs (Bupleurum falcatum, Glycyrrhiza glabra, Panax ginseng, Pinellia ternate, Scutellaria baicalensis, Zizyphus jujuba, and Zingiber officinale) and is currently prescribed to treat chronic hepatitis(6). Many in vitro and in vivo studies have indicated that XCHT may have protective effects against HCC and cirrhosis(6, 15-22), the possible mechanisms are as follow.

1. ANTI-HEPATIC FIBROSIS
2. PROTECTION AGAINST HEPATOTOXIC INJURY
3. ANTI-HEPATIC CARCINOGENESIS
4. IMMUNE-MODULATING EFFECT
5. ANTI VIRUS

Clinical studies on XCHT use and HCC development in HBV or HCV patients are limited. A prospective, randomised, nonblind controlled study evaluated the long-term potential of XCHT to prevent HCC in patients with cirrhosis in Japan(23). The cumulative incidence of HCC was lower in the XCHT group than in the control group. There are three limitations for this research, First, they only use Kaplan-Meier method to examine differences in the risk of HCC, and they cannot adjust the effect of other risk factors. Second,

doi:10.6342/NTU201701710



they only include 260 patients in this trial. Only 37 patients were detected to have the surface antigen of HBV, HBsAg, in this trial; therefore, the author could not further analyse the association between XCHT use and the risk of HCC in patients with HBV-related cirrhosis. And they cannot complete subgroup analysis and sensitivity analysis. Third, they didn't adjust the influence of other drugs.

We try to use National Health Insurance Research Database (NHIRD) and Chang Gung Research Database (CGRD) to analysis the association between statin or XCHT use and disease progression among patients with HBV or HCV infection.

National Health Insurance Research Database (NHIRD)

Since the National Health Insurance is a compulsory programme for all residents in Taiwan, the NHIRD is a comprehensive healthcare database that covers almost all of the 23.7 million residents of Taiwan. We used databases for admissions and outpatient visits, both of which contained information on patient characteristics and medical records, including up to five discharge diagnoses or three outpatient visit diagnoses according to the International Classification of Diseases, Ninth Revision (ICD-9) classification, date of admission, date of discharge, dates of visits, sex, and date of birth for patients. The data files also

doi:10.6342/NTU201701710

contained information on patient prescriptions, including the names of prescribed drugs, dosages, durations, and total expenditures. Previous epidemiologic research has used these databases, and medical records of prescriptions, diagnoses, and hospitalisations are of high quality. FHPs are fully reimbursed under the current National Health Insurance system of Taiwan. The NHIRD is the only computerised reimbursement database in the world that stores longitudinal prescription data for both Western and Chinese medicines, including FHPs. Thus, the NHIRD provides an optimal platform for determining the effect of XCHT in reducing the risk of HCC in patients with HBV or HCV infection

Chang Gung Research Database (CGRD)

The Chang Gung Research Database (CGRD) is a de-identified database derived from medical records of Chang Gung Memorial Hospital (CGMH), and it is systematically updated annually to include new data generated in CGMH. CGMH, founded in 1976, is currently the largest hospital system in Taiwan, and it comprises seven medical institutes, which are located from the northeast to southern regions of Taiwan: Keelung CGMH, Taipei CGMH, Linkou CGMH, Taoyuan CGMH, Yunlin CGMH, Chiayi CGMH, and Kaohsiung CGMH. CGMH has 10,070 beds and admits more than 280,000 patients each year. The

doi:10.6342/NTU201701710

outpatient department visits and emergency department visits to CGMH were over 8,500,000 and 500,000, respectively in 2015. In recent years, the CGRD promoted clinical and scientific studies to a considerable extent. In 2015, more than 1800 studies were conducted by CGMH staff, and the studies were published in a diverse range of reputed journals. Some of these studies are based on the CGRD as multicenter research studies with relatively large sample sizes. There are 44 different dataset in CGRD and we will conducted a hospital-based cohort study by using CGRD, Lack of laboratory data or medical examination reports is the limitation of NHIRD, but we can get the information of laboratory data or medical examination reports from CGRD.

Table1: List of Chang Gung Research Database

序號	系統名稱	數量	序號	系統名稱	數量
01	門診作業系統	5	08	批價作業系統	4
02	急診作業系統	7	09	申報作業系統	4
03	住院作業系統	2	10	掛號作業系統	1
04	護理作業系統	6	11	住出院作業系統	2
05	住診護理系統	5	12	疾病分類系統	1
06	檢驗作業系統	4	13	癌症中心系統	2
07	病理報告系統	1	14		

doi:10.6342/NTU201701710

Objectives

Aim I

This study investigated the association between the use of statins and the risk of cirrhosis development in patients with hepatitis C infection.

Aim II

This study investigated the association between the use of statins and the risk of cirrhosis development in patients with hepatitis B infection.

Aim III

The purpose of this study was to investigate the association between use of XCHT and the risk of HCC in patients with HBV-related cirrhosis.

doi:10.6342/NTU201701710

Reference

1. El-Serag HB. Epidemiology of viral hepatitis and hepatocellular carcinoma. Gastroenterology. 2012;142(6):1264-73 e1.

2. Lun-Gen L. Antiviral Therapy of Liver Cirrhosis Related to Hepatitis B Virus Infection. J Clin Transl Hepatol. 2014;2(3):197-201.

3. Palumbo E. Pegylated interferon and ribavirin treatment for hepatitis C virus infection. Ther Adv Chronic Dis. 2011;2(1):39-45.

4. Okuno M, Kojima S, Moriwaki H. Chemoprevention of hepatocellular carcinoma: concept, progress and perspectives. J Gastroenterol Hepatol. 2001;16(12):1329-35.

5. Singh S, Singh PP, Roberts LR, Sanchez W. Chemopreventive strategies in hepatocellular carcinoma. Nat Rev Gastroenterol Hepatol. 2014;11(1):45-54.

6. Zheng N, Dai J, Cao H, Sun S, Fang J, Li Q, et al. Current understanding on antihepatocarcinoma effects of xiao chai hu tang and its constituents. Evid Based Complement Alternat Med. 2013;2013:529458.

7. Butt AA, Yan P, Bonilla H, Abou-Samra AB, Shaikh OS, Simon TG, et al. Effect of addition of statins to antiviral therapy in hepatitis C virus-infected persons: Results from ERCHIVES. Hepatology. 2015;62(2):365-74.

doi:10.6342/NTU201701710

8. Yang YH, Chen WC, Tsan YT, Chen MJ, Shih WT, Tsai YH, et al. Statin use and the risk of cirrhosis development in patients with hepatitis C virus infection. J Hepatol. 2015;63(5):1111-7.

9. Tsan YT, Lee CH, Ho WC, Lin MH, Wang JD, Chen PC. Statins and the risk of hepatocellular carcinoma in patients with hepatitis C virus infection. J Clin Oncol. 2013;31(12):1514-21.

10. Tsan YT, Lee CH, Wang JD, Chen PC. Statins and the risk of hepatocellular carcinoma in patients with hepatitis B virus infection. J Clin Oncol. 2012;30(6):623-30.

11. Baigent C, Keech A, Kearney PM, Blackwell L, Buck G, Pollicino C, et al. Efficacy and safety of cholesterol-lowering treatment: prospective meta-analysis of data from 90,056 participants in 14 randomised trials of statins. Lancet. 2005;366(9493):1267-78.

12. Huang YW, Lee CL, Yang SS, Fu SC, Chen YY, Wang TC, et al. Statins Reduce the Risk of Cirrhosis and Its Decompensation in Chronic Hepatitis B Patients: A Nationwide Cohort Study. Am J Gastroenterol. 2016;111(7):976-85.

13. Chang FM, Wang YP, Lang HC, Tsai CF, Hou MC, Lee FY, et al. Statins decrease the risk of decompensation in HBV- and HCV-related cirrhosis: A population-based study. Hepatology. 2017.

doi:10.6342/NTU201701710

14. Chen FP, Kung YY, Chen YC, Jong MS, Chen TJ, Chen FJ, et al. Frequency and pattern of Chinese herbal medicine prescriptions for chronic hepatitis in Taiwan. J Ethnopharmacol. 2008;117(1):84-91.

15. Kayano K, Sakaida I, Uchida K, Okita K. Inhibitory effects of the herbal medicine Sho-saiko-to (TJ-9) on cell proliferation and procollagen gene expressions in cultured rat hepatic stellate cells. J Hepatol. 1998;29(4):642-9.

16. Lee JK, Kim JH, Shin HK. Therapeutic effects of the oriental herbal medicine Sho-saiko-to on liver cirrhosis and carcinoma. Hepatol Res. 2011;41(9):825-37.

17. Ohtake N, Yamamoto M, Takeda S, Aburada M, Ishige A, Watanabe K, et al. The herbal medicine Sho-saiko-to selectively inhibits CD8+ T-cell proliferation. Eur J Pharmacol. 2005;507(1-3):301-10.

18. Shimizu I, Ma YR, Mizobuchi Y, Liu F, Miura T, Nakai Y, et al. Effects of Sho-saiko-to, a Japanese herbal medicine, on hepatic fibrosis in rats. Hepatology. 1999;29(1):149-60.

19. Shiota G, Maeta Y, Mukoyama T, Yanagidani A, Udagawa A, Oyama K, et al. Effects of Sho-Saiko-to on hepatocarcinogenesis and 8-hydroxy-2'-deoxyguanosine formation. Hepatology. 2002;35(5):1125-33.

20. Watanabe S, Kitade Y, Masaki T, Nishioka M, Satoh K, Nishino H. Effects of lycopene and Sho-saiko-to on hepatocarcinogenesis in a rat model of spontaneous

doi:10.6342/NTU201701710

liver cancer. Nutr Cancer. 2001;39(1):96-101.

21. Yano H, Mizoguchi A, Fukuda K, Haramaki M, Ogasawara S, Momosaki S, et al. The herbal medicine sho-saiko-to inhibits proliferation of cancer cell lines by inducing apoptosis and arrest at the G0/G1 phase. Cancer Res. 1994;54(2):448-54.

22. Chen MH, Chen JC, Tsai CC, Wang WC, Chang DC, Tu DG, et al. The role of TGF-beta 1 and cytokines in the modulation of liver fibrosis by Sho-saiko-to in rat's bile duct ligated model. J Ethnopharmacol. 2005;97(1):7-13.

23. Oka H, Yamamoto S, Kuroki T, Harihara S, Marumo T, Kim SR, et al. Prospective study of chemoprevention of hepatocellular carcinoma with Sho-saiko-to (TJ-9). Cancer. 1995;76(5):743-9.

24. Chou WC, Wang F, Cheng YF, Chen MF, Lu CH, Wang CH, et al. A simple risk stratification model that predicts 1-year postoperative mortality rate in patients with solid-organ cancer. Cancer Med. 2015;4(11):1687-96.

25. Chou WC, Liu KH, Lu CH, Hung YS, Chen MF, Cheng YF, et al. To Operate or Not: Prediction of 3-Month Postoperative Mortality in Geriatric Cancer Patients. J Cancer. 2016;7(1):14-21.

doi:10.6342/NTU201701710

Part I: Statin use and the risk of cirrhosis development in patients with hepatitis C virus infection.

doi:10.6342/NTU201701710

Title page

Statin use and the risk of cirrhosis development in patients with hepatitis C virus infection

Yao-Hsu Yang[1,2,3], Wen-Cheng Chen[2,4], Yu-Tse Tsan[3,5,6], Mei-Jyh Chen[3,7], Wei-Tai Shih[1,3], Ying-Huang Tsai[8,9*] and Pau-Chung Chen[3,10*]

1. Department for Traditional Chinese Medicine, Chang Gung Memorial Hospital, Chia-Yi, Taiwan
2. Center of Excellence for Chang Gung Research Datalink, Chang Gung Memorial Hospital, Chiayi, Taiwan
3. Institute of Occupational Medicine and Industrial Hygiene, National Taiwan University College of Public Health, Taipei, Taiwan
4. Department of Radiation Oncology, Chang Gung Memorial Hospital, Chiayi, Taiwan
5. Institute of Occupational Medicine, Department of Emergency Medicine, Taichung Veterans General Hospital, Taichung, Taiwan
6. School of Medicine, Chung Shan Medical University, Taichung, Taiwan;
7. Departments of Internal Medicine, Integrated Diagnostics and Therapeutics, National Taiwan University Hospital and National Taiwan University College of Medicine, Taipei, Taiwan;
8. Division of Pulmonary and Critical Care Medicine and Department of Respiratory Care, Chang Gung Memorial Hospital, Chiayi, Taiwan
9. Department of Respiratory Therapy, Chang Gung University, Taoyuan, Taiwan
10. Department of Environmental and Occupational Medicine, National Taiwan University Hospital and National Taiwan University College of Medicine, Taipei, Taiwan

*Correspondence to:

Dr. Pau-Chung Chen, Institute of Occupational Medicine and Industrial Hygiene, National Taiwan University College of Public Health, 17 Syujhou Road, Taipei 10055, Taiwan. Phone No.: +886-2-3322 8088; Fax No.: +886-2-2358 2402; and Email: pchen@ntu.edu.tw

Ying-Huang Tsai, Division of Pulmonary and Critical Care Medicine and Department of Respiratory Care, Chang Gung Memorial Hospital, Chiayi, Taiwan

No.6, W. Sec., Jiapu Rd., Puzih City, Chiayi County 613, Taiwan. Phone No.: +886-5-3621000

doi:10.6342/NTU201701710

ext:2004 ; Fax No.: +886-5-3622188; and Email: chestmed@cgmh.org.tw

Keywords: statin, hepatitis C virus, cirrhosis

Conflict of Interest: The authors have no conflict of interest to disclose.

Financial Support: This study was supported by a grant from Chia-yi Chang Gung Memorial Hospital, Taiwan (CORPG6D0161).

Author's Contributions:

Yao-Hsu Yang: conception of study design, statistical analysis, interpretation of the data, literature review and wrote the manuscript.

Wen-Cheng Chen: conception of study design, interpretation of the data and critical revision.

Yu-Tse Tsan: conception of study design, interpretation of the data , literature review and critical revision.

Mei-Jyh Chen: conception of study design, interpretation of the data and critical revision.

Wei-Tai Shih: conception of study design, statistical analysis, interpretation of the data.

Ying-Huang Tsai: conception of study design, interpretation of the data, critical revision and study supervision.

Pau-Chung Chen: conception of study design, statistical analysis, interpretation of the

doi:10.6342/NTU201701710

data, critical revision and study supervision.

Abstract

Background & Aims:

Several animal studies have shown that statins can inhibit the progression of cirrhosis; however, few clinical studies have been conducted. Previous study has indicated that statins can prevent the progression of hepatic fibrosis in patients with hepatitis C virus (HCV) infection and advanced hepatic fibrosis but data for human not progress to cirrhosis yet is lacking. This study investigated the association between the use of statin and the risk of cirrhosis development in patients with HCV infection.

Study Population and Methods:

We conducted a population-based cohort study by using the Taiwan National Health Insurance Research Database. A total of 226,856 patients with HCV infection were included as the study cohort. Each patient was followed from 1997 to 2010 to identify incident cases of cirrhosis. A Cox proportional hazard regression was performed to evaluate the association between statin use and cirrhosis risk.

Results:

A total of 34,273 cases of cirrhosis were identified in the cohort with HCV infection during the follow-up period of 2,874,031.7 person-years. The incidence rate was 445.5 cases of cirrhosis per 100,000 person-years (95% confidence interval (CI),

doi:10.6342/NTU201701710

423.3 to 465.7) for statin users (defined as those who used more than 28 cumulative defined daily doses (cDDD)), and 1311.2 cirrhosis cases per 100,000 person-years (95% CI, 1,297.1 to 1,325.6) for nonusers. A dose-response relationship between statin use and cirrhosis risk was observed. The adjusted hazard ratios were 0.33 (95% CI, 0.31 to 0.36), 0.24 (95% CI, 0.22 to 0.25), and 0.13 (95% CI, 0.12 to 0.15) for statin use of 28 to 83, 84 to 365, and more than 365 cDDD, respectively, relative to no statin use (< 28 cDDD).

Conclusion:

Among the patients with HCV infection, statin use was associated with a reduced risk of cirrhosis development in a dose-dependent manner. Further clinical research is required.

doi:10.6342/NTU201701710

Introduction

Hepatitis C virus (HCV) infection typically leads to chronic hepatitis, and often follows a progressive course over many years, finally resulting in cirrhosis, hepatocellular carcinoma (HCC)[1, 2]a, and even the need for liver transplantation. The objective of antiviral treatment of chronic HCV is to achieve sustained eradication of HCV, which is defined as the persistent absence of HCV RNA in serum for at least 6 months after treatment completion[3]. Another objective is to stop or delay the progression to cirrhosis, decompensated liver disease, or HCC[4].

Statins (3-hydroxy-3-methyl-glutaryl-CoA reductase inhibitors) are used to treat and prevent coronary heart disease and stroke in patients with hyperlipidemia[5]. Recently, statins have been investigated for their antiproliferative, antiangiogenic, antiinflammatory, and antineoplastic effects[6-8]. In addition, some animal studies have shown that statins can inhibit the progression of cirrhosis[9-12], but clinical studies are limited. A previous study of the Hepatitis C Antiviral Long-term Treatment Against Cirrhosis (HALT-C) trial cohort indicated that statin use can reduce the risk of fibrosis progression in patients with chronic hepatitis C infection and advanced hepatic fibrosis who had previously failed to respond to antiviral therapy[13].

Data regarding the antifibrogenic actions of statins for patients with chronic hepatitis

doi:10.6342/NTU201701710

C infection that has not yet progressed to cirrhosis are limited. The purpose of this study was to investigate the association between statin use and the risk of cirrhosis development in patients with hepatitis C infection.

Materials and Methods

Data Sources

We conducted a nationwide cohort study by using population-based data from the Taiwan National Health Insurance Research Database (NHIRD). Because National Health Insurance (NHI) is a compulsory universal program for all residents in Taiwan, the NHIRD is a comprehensive health care database that covers nearly the entire 23.7 million population of this country. We used databases for admissions and outpatient visits, both of which included information on patient characteristics such as sex, date of birth, date of admission, date of discharge, dates of visits, and up to five discharge diagnoses or three outpatient visit diagnoses (according to International Classification of Diseases, Ninth Revision (ICD-9) codes). The data files also contained information on patient prescriptions, including the names of prescribed drugs, dosage, duration, and total expenditure. These databases have previously been used for epidemiologic research, and the information on prescription use, diagnoses, and hospitalizations is of high quality[6, 8, 14].

doi:10.6342/NTU201701710

Following strict confidentiality guidelines in accordance with personal electronic data protection regulations, the National Health Research Institutes of Taiwan maintains an anonymous database of NHI reimbursement data that is suitable for research. In addition, this study was approved by the Ethics Review Board of Chang Gung Memorial Hospital, Chia-Yi Branch, Taiwan.

Study population and outcomes

We conducted a population-based cohort study that included all patients older than 18 years who were newly diagnosed with HCV infection (ICD-9 codes 070.7, 070.41, 070.44, 070.51, 070.54, V02.62) without hepatitis B virus infection (ICD-9 codes 070.2, 070.3, V02.61) or HCC (ICD-9 code 155.0) between 1 January 1999 and 31 December 2010, ensuring more than 2 years of prior exposure to HCV with complete admission, outpatient visit, and drug data. Patients with follow-up duration of less than 1 year or missing data on sex, age, income, or level of urbanization were excluded. Cirrhosis cases in this study were identified using the ICD code (ICD-9 codes 571.2, 571.5, 571.6, 572.2, 572.3, 572.4, 572.8, or 573.0) with at least three records of outpatient visits within one year or one admission diagnosis during study period, and the date of the initial cirrhosis diagnosis was defined as the index date of cirrhosis. A total of 226,856 patients were included in the final analyses.

doi:10.6342/NTU201701710

Statin Exposure

We identified patients who received prescriptions for statins in the outpatient visits database from 1 January 1997 to 1 year before the date of cirrhosis diagnosis or the end of follow-up.

The defined daily dose (DDD) recommended by the World Health Organization is a unit for measuring a prescribed amount of drug; it is the assumed average maintenance dose per day of a drug consumed for its main indication in adults. By using the following formula, we could compare any statins on the basis of the same standard: (total amount of drug)/(amount of drug in a DDD) = number of DDDs[15]. Cumulative DDD (cDDD), which indicates the total exposed dosage, was estimated as the sum of dispensed DDD of any statins to compare their use to the risk of cirrhosis. We collected similar information on nonstatin lipid-lowering medications (cholestyramine, colestipol, colextran, niceritrol, nicofuranose, acipimox, probucol, and ezetimibe).

To examine the dose–effect relationship, we categorized the statins into four groups in each cohort (<28, 28 to 83, 84 to 365, and >365 cDDDs). Patients who used statins for less than 28 cDDDs were defined as statin nonusers, and patient who used statins for more than 28 cDDDs were defined as statin users.

doi:10.6342/NTU201701710

Potential Confounders

We systematically identified the potential confounding risk factors for cirrhosis as the following diagnoses recorded during the study period: alcohol-related disease (ARD; ICD-9 codes 291, 303.0, 303.9, 305.0, 571.0, 571.1, 571.2, or 571.3), nonalcoholic steatohepatitis (NASH; ICD-9 code 571.8, 571.9) and diabetes (ICD-9 code 250). The information of the Charlson comorbidity index (CCI) was also collected and considered as one possible confounding risk[16].

We collected exposure information of other drugs that might alter the risk of cirrhosis, such as anti-HCV treatment (interferon or ribavirin)[3], aspirin[17], angiotensin-converting enzymeinhibitors (ACEIs) (captopril, enalapril, lisinopril, perindopril, ramipril, quinapril, benazepril, cilazapril, and fosinopril)[18], and metformin[19]. We also considered sociodemographic characteristics (age, sex, income, and level of urbanization) in the modeling.

Statistical Analysis

The distribution of demographic factors and the proportions of comorbidities between the statin users and nonusers in the study cohort and matched cohort were compared. The incidence rates and 95% confidence interval (95% CI) of cirrhosis were

doi:10.6342/NTU201701710

calculated for the entire follow-up period. We used the Kaplan–Meier method to estimate cirrhosis cumulative incidences. The log-rank test was performed to examine differences in the risk for cirrhosis in the cohort. Finally, Cox proportional hazards models were used to compute the hazard ratios (HRs) accompanying 95% CIs after adjustment for age, sex, urbanization, income, and diabetes. Two-tailed $P = 0.05$ was considered significant. Patients with a death date in the admission file and those from the beneficiaries register who were lost to follow-up were censored. All of these analyses were conducted using SAS statistical software (Version 9.4; SAS Institute, Cary, NC, USA).

Sensitivity Analyses

Many medicines have shown positive results in chemoprevention. To examine potential effect modifiers, we conducted analyses stratified by groups with and without the use of anti-HCV treatment, metformin, ACEIs, and aspirin. We also examined the outcome stratified by groups according to sex and age and with or without ARD, NASH, diabetes and receiving liver biopsy. These sensitivity analyses were applied to evaluate the difference and consistency between statin use and the risk of cirrhosis.

doi:10.6342/NTU201701710

Matched Cohort

To further examine the effect of statin use, we analyzed the data by using an alternative method. The statin users and nonusers were frequency matched randomly by age, sex, income, urbanization, diabetes, and the year of HCV infection diagnosis at a ratio of 2:1 (nonuser versus user). Overall, 84,213 insured adults (28,071 matched sets) were included in the matched cohort (Fig. 1). The results of the analysis for both the study cohort and matched cohort are illustrated in the tables and Fig. 2.

Results

We included a total of 226,856 patients (116,491 women and 110,465 men) diagnosed with HCV infection during the study period (Fig. 1). The basic demographic characteristics of the patient population are summarized in Table 1. A total of 12.9% (29,204) of the patients had used statins for more than 28 cDDDs, and they tended to be elderly, female, have a high CCI, and a high percentage of diabetes, hypertension, NASH, chronic kidney disease and biliary stone. Statin users received more nonstatin lipid-lowering drugs, fibrates, ACEIs, and aspirin, but less anti-HCV treatment. There were 34,303 cirrhosis cases in the HCV infection cohort during the follow-up period of 2874031.7 person-years. The overall incidence rate (95% CI) was 1193.5 (1181.0-1206.2) cirrhosis cases per 100,000 person-years. The incidence rates (95% CI) of cirrhosis were 445.5 (425.1-466.8) and 1311.2 (1297.1-1325.6) among patients

doi:10.6342/NTU201701710

with HCV infection who were statin users and nonusers, respectively.

Figures 2a and 2b illustrate the results of the Kaplan–Meier method for the study cohort and matched cohort. The risk reduction exhibited a progressive dose-response relationship in the study cohort and matched cohort. The log-rank test revealed a significant difference over the entire Kaplan–Meier curve.

Table 2 shows that there was a dose-response relationship between statin use and the risk of cirrhosis development. In study cohort, the adjusted HRs were 0.33 (95% CI, 0.31-0.36), 0.24 (95% CI, 0.22-0.25), and 0.13 (95% CI, 0.12-0.15) for patients with statin use of 28 to 83 cDDDs, 84 to 365 cDDDs, and >365 cDDDs, respectively. The sensitivity analysis adjustments exhibited little effect on the estimates of the association between statin use and the risk of cirrhosis development according to different models. Although the HRs of subgroup according to NASH did not decrease monotonically with increasing statin use, the effects of statins remained significant and the P-value for trend <0.0001. The effects of statins remained dose-response relationship in the patients of different subgroups in diabetes, ARD, receiving liver biopsy, sex, age.

As patients received anti-HCV treatment, the effect of statin decreased to 0.56 (95% CI, 0.35-0.89), 0.51 (95% CI, 0.34-0.77), and 0.37 (95% CI, 0.20-0.71) for patients with statin use of 28 to 83 cDDDs, 84 to 365 cDDDs, and >365 cDDDs, respectively.

doi:10.6342/NTU201701710

Table 2 also demonstrates that the effect of statin keep the same trend of dose-response relationship in each subgroup stratified by different cDDD of metformin, aspirin, and ACEIs use.

A total of 28,071 matched sets of statin users and nonusers were selected from the study cohort after matching by sex, age, income, urbanization, diabetes, and the year of HCV infection diagnosis. The trend of dose-response relationship between statin use and the risk reduction of cirrhosis development did not alter when the matched cohort was used.

Discussion

To our knowledge, this study was the first study to document a dose-response relationship between the use of statins and the risk of cirrhosis development after controlling for the confounding effects of age, sex, income, urbanization, diabetes, and other medication. This study had several strengths. The study cohort was mainly obtained from a computerized database, which is population-based and includes all HCV-infected patients in Taiwan; we can therefore eliminate the possibility of selection bias. In addition, because the data on statin and other medicine use were obtained from a historical database that collects all available prescription information during the study period, we can eliminate the possibility of recall bias.

doi:10.6342/NTU201701710

If physicians are less likely to prescribe statins because of their hepatotoxicity, patients with liver disease are less likely to be prescribed statins. We took several steps to avoid possible confounding effects of contraindication. First, we excluded patients with HCC. Second, we excluded statin use recorded within 1 year before cirrhosis diagnosis or the end of follow up, assuming that in this time period liver disease is likely to be severe and overt. A sensitivity analysis of statin exposure at least 2 years, and 3 years before the diagnosis of cirrhosis or the end of follow up were conducted and we still find a statistical significance and dose-response relationship of on different time exposed to statins. In addition, we conducted sensitivity analyses by stratification to clarify the misclassifications and potential confounders, and the results revealed no significant changes in the HRs of the different subgroups.

We also used an alternative study design to examine whether the result was consistent. After matching statin nonusers and users in a ratio of 2:1 according to variables of age, sex, income, urbanization, diabetes, and the year of HCV infection diagnosis, we found no significant changes in the results between different study designs.

Simon et al analyzed the association between statin use and liver fibrosis progression in a well-characterized cohort of chronic hepatitis C patients enrolled in the HALT-C trial[13]. However, there were some limitations in that study. First, only patients with

doi:10.6342/NTU201701710

advanced liver fibrosis (Ishak fibrosis score ≥3) at entry with a history of virological nonresponse to standard interferon therapies were enrolled in this study; patients who did not progress to cirrhosis or who responded to standard interferon therapies were excluded. Second, only 29 patients had received continuous statin therapy throughout the study, resulting in limited power to detect the association within the larger multivariable model. Third, the study did not consider other drugs that might reduce the risk of cirrhosis as confounding factors [20].

The mechanism by which statin use may reduce the risk of cirrhosis development is not clearly understood. Several possible mechanisms have been investigated in previous studies. Statins can downregulate expression of profibrotic cytokines including transforming growth factor-β, connective tissue growth factor, and platelet-derived growth factor, which can stimulate the activation of hepatic stellate cells, resulting in further fibrogenesis[21-24]. Another potential mechanism is that the upregulation of kruppel-like factor 2 expression results in vasodilation and the improvement of liver microcirculation[25-27]. Statins may also inhibit fibrogenesis of hepatic myofibroblasts and the replication of HCV [11, 28, 29].

The potential limitations of this study should be noted. First, we did not obtain any histological data from liver biopsy, reports of liver ultrasound examination or any other laboratory data related to HCV infection. Cirrhosis cases in this study were

doi:10.6342/NTU201701710

identified only by using the ICD-9 code. However, we find that statin users were more likely to receive liver ultrasound examination or liver biopsy than statin nonusers. So that there was no detection bias exists among stain nonusers and it is less likely to underestimate cirrhosis cases in stain users. The diagnosis of cirrhosis may be less accurate than liver biopsy and only those with obvious cirrhosis would have been captured in our study. However, errors in cirrhosis diagnosis tend to occur in random as a result of the same definition being used in both groups of statin user and nonuser, which might have limited effect on the result. In our study population, there were 23,660 patients with admission record of liver cirrhosis. While we defied cirrhosis cases by using admission record which is stricter than original definition, the results didn't alter with cirrhosis definition change. Second, several unmeasured confounders, including body mass index, smoking habit, alcohol intake, and other over-the-counter drug use, which are associated with cirrhosis, were not included in our database. Third, there is no way to verify the exact dosage that the study participants actually took. We presumed that all prescribed medications were taken by patients as prescribed; this may overestimate the actual ingested dosage because some degree of noncompliance is always expected. Finally, because the data on drug prescription were not complete in 1996, we included statin use after 1997; the use of these drugs before 1997 could not be included in our analysis. This could have underestimated the cDDD and

doi:10.6342/NTU201701710

dose-response effects.

In conclusion, statin use may reduce the risk for cirrhosis development in HCV-infected patients in a dose-dependent manner. Further mechanistic research is required.

doi:10.6342/NTU201701710

Acknowledgments

The author's would like to thank Center of Excellence for Chang Gung Research Datalink for the comments and assistance in data analysis. This study was supported by a grant from Chia-yi Chang Gung Memorial Hospital, Taiwan (CORPG6D0161). This funding body played no role in study design, analysis, and interpretation of data in this paper. This study was based on a portion of data from the National Health Insurance Research Database provided by the Bureau of National Health Insurance, Department of Health, and was managed by the National Health Insurance Research Institutes, Taiwan. The statistical results and conclusions presented in this paper do not represent those of the Bureau of National Health Insurance, Department of Health, or the National Health Insurance Research Institutes.

doi:10.6342/NTU201701710

References:

[1] Yano M, Kumada H, Kage M, Ikeda K, Shimamatsu K, Inoue O, et al. The long-term pathological evolution of chronic hepatitis C. Hepatology 1996;23:1334-1340.

[2] Niederau C, Lange S, Heintges T, Erhardt A, Buschkamp M, Hurter D, et al. Prognosis of chronic hepatitis C: results of a large, prospective cohort study. Hepatology 1998;28:1687-1695.

[3] Ng V, Saab S. Effects of a sustained virologic response on outcomes of patients with chronic hepatitis C. Clin Gastroenterol Hepatol 2011;9:923-930.

[4] Koh C, Heller T, Haynes-Williams V, Hara K, Zhao X, Feld JJ, et al. Long-term outcome of chronic hepatitis C after sustained virological response to interferon-based therapy. Aliment Pharmacol Ther 2013;37:887-894.

[5] Baigent C, Keech A, Kearney PM, Blackwell L, Buck G, Pollicino C, et al. Efficacy and safety of cholesterol-lowering treatment: prospective meta-analysis of data from 90,056 participants in 14 randomised trials of statins. Lancet 2005;366:1267-1278.

[6] Tsan YT, Lee CH, Ho WC, Lin MH, Wang JD, Chen PC. Statins and the risk of hepatocellular carcinoma in patients with hepatitis C virus infection. Journal of

doi:10.6342/NTU201701710

clinical oncology : official journal of the American Society of Clinical Oncology 2013;31:1514-1521.

[7] Demierre MF, Higgins PD, Gruber SB, Hawk E, Lippman SM. Statins and cancer prevention. Nature reviews Cancer 2005;5:930-942.

[8] Tsan YT, Lee CH, Wang JD, Chen PC. Statins and the risk of hepatocellular carcinoma in patients with hepatitis B virus infection. Journal of clinical oncology : official journal of the American Society of Clinical Oncology 2012;30:623-630.

[9] Rombouts K, Kisanga E, Hellemans K, Wielant A, Schuppan D, Geerts A. Effect of HMG-CoA reductase inhibitors on proliferation and protein synthesis by rat hepatic stellate cells. Journal of hepatology 2003;38:564-572.

[10] Miyaki T, Nojiri S, Shinkai N, Kusakabe A, Matsuura K, Iio E, et al. Pitavastatin inhibits hepatic steatosis and fibrosis in non-alcoholic steatohepatitis model rats. Hepatology research : the official journal of the Japan Society of Hepatology 2011;41:375-385.

[11] Klein S, Klosel J, Schierwagen R, Korner C, Granzow M, Huss S, et al. Atorvastatin inhibits proliferation and apoptosis, but induces senescence in hepatic myofibroblasts and thereby attenuates hepatic fibrosis in rats. Laboratory investigation; a journal of technical methods and pathology 2012;92:1440-1450.

[12] Trebicka J, Hennenberg M, Odenthal M, Shir K, Klein S, Granzow M, et al.

doi:10.6342/NTU201701710

Atorvastatin attenuates hepatic fibrosis in rats after bile duct ligation via decreased turnover of hepatic stellate cells. Journal of hepatology 2010;53:702-712.

[13] Simon TG, King LY, Zheng H, Chung RT. Statin Use is Associated with a Reduced Risk of Fibrosis Progression in Chronic Hepatitis C. Journal of hepatology 2014.

[14] Lai MN, Wang SM, Chen PC, Chen YY, Wang JD. Population-based case-control study of Chinese herbal products containing aristolochic acid and urinary tract cancer risk. J Natl Cancer Inst 2010;102:179-186.

[15] WHO Collaborating Center for Drugs Statistics Methodology: ATC/DDD Index 2013. http://www.whocc.no/atc_ddd_index/.

[16] Kieszak SM, Flanders WD, Kosinski AS, Shipp CC, Karp H. A comparison of the Charlson comorbidity index derived from medical record data and administrative billing data. J Clin Epidemiol 1999;52:137-142.

[17] Denda A, Endoh T, Kitayama W, Tang Q, Noguchi O, Kobayashi Y, et al. Inhibition by piroxicam of oxidative DNA damage, liver cirrhosis and development of enzyme-altered nodules caused by a choline-deficient, L-amino acid-defined diet in rats. Carcinogenesis 1997;18:1921-1930.

[18] Yoshiji H, Kuriyama S, Fukui H. Angiotensin-I-converting enzyme inhibitors may be an alternative anti-angiogenic strategy in the treatment of liver fibrosis and

doi:10.6342/NTU201701710

hepatocellular carcinoma. Possible role of vascular endothelial growth factor. Tumour Biol 2002;23:348-356.

[19] Nkontchou G, Cosson E, Aout M, Mahmoudi A, Bourcier V, Charif I, et al. Impact of metformin on the prognosis of cirrhosis induced by viral hepatitis C in diabetic patients. J Clin Endocrinol Metab 2011;96:2601-2608.

[20] Negro F. Are statins a remedy for all seasons? Journal of hepatology 2015;62:8-10.

[21] Kisseleva T, Brenner DA. Anti-fibrogenic strategies and the regression of fibrosis. Best practice & research Clinical gastroenterology 2011;25:305-317.

[22] Eberlein M, Heusinger-Ribeiro J, Goppelt-Struebe M. Rho-dependent inhibition of the induction of connective tissue growth factor (CTGF) by HMG CoA reductase inhibitors (statins). Br J Pharmacol 2001;133:1172-1180.

[23] Paradis V, Dargere D, Bonvoust F, Vidaud M, Segarini P, Bedossa P. Effects and regulation of connective tissue growth factor on hepatic stellate cells. Laboratory investigation; a journal of technical methods and pathology 2002;82:767-774.

[24] Iwakiri Y, Shah V, Rockey DC. Vascular pathobiology in chronic liver disease and cirrhosis - current status and future directions. Journal of hepatology 2014;61:912-924.

[25] SenBanerjee S, Lin Z, Atkins GB, Greif DM, Rao RM, Kumar A, et al. KLF2 Is

doi:10.6342/NTU201701710

a novel transcriptional regulator of endothelial proinflammatory activation. The Journal of experimental medicine 2004;199:1305-1315.

[26] Marrone G, Russo L, Rosado E, Hide D, Garcia-Cardena G, Garcia-Pagan JC, et al. The transcription factor KLF2 mediates hepatic endothelial protection and paracrine endothelial-stellate cell deactivation induced by statins. Journal of hepatology 2013;58:98-103.

[27] Sen-Banerjee S, Mir S, Lin Z, Hamik A, Atkins GB, Das H, et al. Kruppel-like factor 2 as a novel mediator of statin effects in endothelial cells. Circulation 2005;112:720-726.

[28] Kapadia SB, Chisari FV. Hepatitis C virus RNA replication is regulated by host geranylgeranylation and fatty acids. Proceedings of the National Academy of Sciences of the United States of America 2005;102:2561-2566.

[29] Ye J, Wang C, Sumpter R, Jr., Brown MS, Goldstein JL, Gale M, Jr. Disruption of hepatitis C virus RNA replication through inhibition of host protein geranylgeranylation. Proceedings of the National Academy of Sciences of the United States of America 2003;100:15865-15870.

doi:10.6342/NTU201701710

Table 1. Patient demographics and clinical characteristics of study cohort and matched cohort.

	study cohort			Matched cohort		
	Non-user	User	P-value	Non-user	User	P-value
Total follow-up person-year	2479856.8	394174.9		687559.1	378848.3	
No. of cirrhosis	32517 (16.5%)	1756 (6.0%)	<.0001	12297(21.9%)	1682(6.0%)	<.0001
Incidence	1311.2	445.5		1788.5	444.0	
95% CI	1297.1-1325.6	425.1-466.8		1757.2-1820.4	423.3-465.7	
Gender						
Female	99232(50.2%)	17159(58.8%)	<.0001	32540(58.0%)	16270(58.0%)	1.000
Male	98420(49.8%)	12045(41.2%)		23602(42.0%)	11801(42.0%)	
charlson comorbidity index (CCI) Mean (S.D.)	1.58 (2.03)	2.26(2.15)		2.11(2.25)	2.23(2.14)	
Age						
18-29	31552(16.0%)	1029(3.5%)	<.0001	1922(3.4%)	961(3.4%)	1.000
30-39	40221(20.4%)	3646(12.5%)		7066(12.6%)	3533(12.6%)	
40-49	47566(24.1%)	8676(29.7%)		16696(29.7%)	8348(29.7%)	

doi:10.6342/NTU201701710

50-59	37327(18.9%)	8431(28.9%)		16034(28.6%)	8017(28.6%)	
>=60	40986(20.7%)	7422(25.4%)		14424(25.7%)	7212(25.7%)	
Comorbidity						
Diabetes	47501(24.0%)	16718(57.3%)	<.0001	31200(55.6%)	15600(55.6%)	1.000
Hypertension	98913(50.0%)	23869(81.7%)	<.0001	36535(65.1%)	22906(81.6%)	<.0001
NASH	5474(2.7%)	1351(4.6%)	<.0001	1842(3.3%)	1299(4.6%)	<.0001
ARD	21453(10.9%)	2197(7.5%)	<.0001	5816(10.4%)	2118(7.6%)	<.0001
CKD	19203(9.7%)	6225(21.3%)	<.0001	7484(13.3%)	5934(21.1%)	<.0001
Biliary stones	37399(18.9%)	6630(22.7%)	<.0001	12722(22.7%)	6367(22.7%)	0.9443
Nonstatin lipid-lowering drug						
0-27 cDDD	196488(99.4%)	27226(93.2%)	<.0001	55630(99.1%)	26187(93.3%)	<.0001
28-83 cDDD	628(0.3%)	761(2.6%)		263(0.5%)	720(2.6%)	
84-365 cDDD	453(0.2%)	936(3.2%)		212(0.4%)	898(3.2%)	
>365 cDDD	83(0.1%)	281(1.0%)		37(0.1%)	266(1.0%)	
fibrate						

doi:10.6342/NTU201701710

0-27 cDDD	188164(95.2%)	19146(65.6%)	<.0001	52014(92.7%)	18451(65.7%)	<.0001
28-83 cDDD	4495(2.3%)	3067(10.5%)		1801(3.2%)	2954(10.5%)	
84-365 cDDD	3739(1.9%)	4371(15.0%)		1702(3.0%)	4183(14.9%)	
>365 cDDD	1254(0.6%)	2620(9.0%)		625(1.1%)	2483(8.9%)	
anti-HCV treatment						
No	186175(94.2%)	27608(94.5%)	0.0112	52934(94.3%)	26523(94.5%)	0.2975
Partial treatment	2193(1.1%)	273(0.9%)		588(1.1%)	265(0.9%)	
Complete treatment	9284(4.7%)	1323(4.5%)		2620(4.7%)	1283(4.6%)	
Metformin						
0-27 cDDD	175753(88.9%)	17066(58.4%)	<.0001	41708(74.3%)	16738(59.6%)	<.0001
28-365 cDDD	10747(5.4%)	4288(14.7%)		6968(12.4%)	4005(14.3%)	
>365 cDDD	11152(5.6%)	7850(26.9%)		7466(13.3%)	7328(26.1%)	
ACEI						
0-27 cDDD	151484(76.6%)	12835(44.0%)	<.0001	37382(66.6%)	12408(44.2%)	<.0001
28-365 cDDD	29098(14.7%)	8399(28.8%)		11381(20.3%)	8056(28.7%)	

doi:10.6342/NTU201701710

>365 cDDD	17070(8.6%)	7970(27.3%)		7379(13.1%)	7607(27.1%)	
Aspirin						
0-27 cDDD	152997(77.4%)	12001(41.1%)	<.0001	38558(68.7%)	11571(41.2%)	<.0001
28-365 cDDD	27654(14.0%)	7430(25.4%)		10341(18.4%)	7127(25.4%)	
>365 cDDD	17001(8.6%)	9773(33.5%)		7243(12.9%)	9373(33.4%)	

Abbreviations: ACEI, angiotensin converting enzyme inhibitor; ARD, alcohol-related disease; cDDD, cumulative defined daily dose; CI, confidence interval; CKD, chronic kidney disease; HCV, hepatitis C virus; ; NASH, Nonalcoholic Steatohepatitis

doi:10.6342/NTU201701710

Table 2. Adjusted hazard ratios (HRs) of cirrhosis development associated with statin use during the follow-up period in study cohort and matched cohort.

	study cohort						Matched cohort					
	28-83cDDD		84-365cDDD		>365cDDD		28-83cDDD		84-365cDDD		>365cDDD	
	HR	95% CI	HR	95% CI	HR	95% CI	HR	95% CI	HR	95% CI	HR	95% CI
Main model*	0.33	0.31-0.36	0.24	0.22-0.25	0.13	0.12-0.15	0.33	0.31-0.36	0.24	0.22-0.26	0.13	0.12-0.15
Additional covariates†												
Main model+ARD	0.33	0.31-0.36	0.24	0.22-0.26	0.14	0.14-0.16	0.34	0.31-0.36	0.24	0.23-0.26	0.14	0.12-0.15
Main model+NASH	0.33	0.31-0.36	0.24	0.22-0.25	0.13	0.12-0.15	0.33	0.31-0.6	0.24	0.22-0.26	0.13	0.12-0.15
Main model+CCI	0.32	0.30-0.34	0.22	0.20-0.24	0.12	0.11-0.14	0.32	0.30-0.35	0.22	0.21-0.24	0.10	0.11-0.13
Main model+Nonstatin lipid-lowering drug	0.33	0.31-0.36	0.24	0.22-0.26	0.14	0.12-0.16	0.33	0.31-0.36	0.24	0.22-0.26	0.14	0.12-0.15
Main model+fibrate	0.35	0.32-.38	0.26	0.24-0.28	0.15	0.14-0.17	0.35	0.32-0.38	0.26	0.24-0.28	0.14	0.13-0.17
Main model+anti-HCV treatment	0.33	0.31-0.36	0.24	0.22-0.25	0.13	0.12-0.15	0.33	0.31-0.36	0.24	0.22-0.6	0.13	0.12-0.15
Main model+metformine	0.36	0.33-0.39	0.27	0.25-0.29	0.16	0.14-0.18	0.36	0.33-0.39	0.27	0.25-0.29	0.16	0.14-0.18
Main model+ACEI	0.36	0.34-0.39	0.26	0.25-0.28	0.16	0.14-0.18	0.36	0.33-0.39	0.27	0.25-0.29	0.15	0.13-0.17
Main model+aspirin	0.38	0.36-0.41	0.30	0.27-0.32	0.19	0.17-0.21	0.38	0.35-0.41	0.30	0.27-0.32	0.18	0.16-0.20
Subgroup effects												
ARD												
NO	0.34	0.31-0.36	0.25	0.23-0.27	0.15	0.13-0.17	0.34	0.31-0.37	0.25	0.23-0.27	0.14	0.12-0.16
YES	0.32	0.27-0.38	0.21	0.17-0.25	0.10	0.07-0.15	0.33	0.28-0.39	0.21	0.18-0.25	0.11	0.08-0.16
Diabetes												

doi:10.6342/NTU201701710

NO	0.34	0.30-0.38	0.22	0.20-0.26	0.16	0.13-0.20	0.33	0.29-0.38	0.22	0.19-0.25	0.15	0.12-0.19
YES	0.32	0.30-0.36	0.24	0.22-026	0.13	0.11-0.15	0.33	0.30-0.37	0.25	0.23-0.27	0.12	0.11-0.14
NASH												
NO	0.33	0.31-0.36	0.23	0.22-0.25	0.14	0.12-0.15	0.33	0.31-0.36	0.24	0.22-0.25	0.13	0.12-0.15
YES	0.28	0.19-0.42	0.31	0.23-0.42	0.12	0.07-0.20	0.27	0.18-0.41	0.30	0.22-0.41	0.11	0.06-0.19
liver biopsy												
NO	0.33	0.31-0.36	0.24	0.22-0.26	0.14	0.12-0.16	0.34	0.31-0.36	0.24	0.22-0.26	0.14	0.12-0.16
YES	0.31	0.23-0.40	0.21	0.17-0.27	0.08	0.05-0.13	0.30	0.23-0.40	0.22	0.17-0.29	0.08	0.05-0.13
Gender												
Male	0.35	0.31-0.39	0.24	0.22-0.27	0.13	0.11-0.16	0.35	0.32-0.40	0.25	0.22-0.27	0.14	0.11-0.17
Female	0.31	0.28-0.35	0.23	0.21-0.25	0.13	0.11-0.15	0.31	0.28-0.35	0.23	0.21-0.26	0.13	0.11-0.15
Age, years												
≧50	0.34	0.31-0.37	0.24	0.22-0.27	0.13	0.11-0.15	0.35	0.32-0.38	0.25	0.23-0.28	0.13	0.11-0.15
<50	0.32	0.28-0.37	0.22	0.20-0.25	0.15	0.12-0.18	0.30	0.27-0.35	0.22	0.19-0.25	0.13	0.11-0.16
anti-HCV treatment												
No	0.33	0.30-0.35	0.23	0.21-0.25	0.13	0.12-0.15	0.33	0.30-0.35	0.23	0.22-0.25	0.13	0.11-0.14
Yes	0.56	0.35-0.89	0.51	0.34-0.77	0.38	0.20-0.71	0.62	0.39-0.99	0.56	0.37-0.85	0.42	0.22-0.79
Metformin												
0-27 cDDD	0.30	0.27-0.33	0.21	0.19-0.24	0.13	0.11-0.16	0.30	0.27-0.33	0.22	0.19-0.24	0.13	0.11-0.15
28-365 cDDD	0.42	0.35-0.50	0.31	0.26-0.37	0.21	0.16-0.28	0.44	0.36-0.53	0.34	0.28-0.40	0.23	0.18-0.31
>365 cDDD	0.58	0.50-0.68	0.40	0.35-0.46	0.19	0.16-0.24	0.59	0.50-0.69	0.40	0.35-0.46	0.19	0.15-0.23
ACEI												

doi:10.6342/NTU201701710

0-27 cDDD	0.26	0.23-0.29	0.18	0.16-0.21	0.09	0.07-0.11	0.25	0.22-0.29	0.18	0.16-0.21	0.09	0.07-0.12
28-365 cDDD	0.44	0.39-0.51	0.33	0.29-0.38	0.17	0.14-0.21	0.45	0.40-0.52	0.33	0.29-0.38	0.16	0.13-0.20
>365 cDDD	0.61	0.53-0.71	0.39	0.34-0.45	0.26	0.21-0.31	0.65	0.55-0.75	0.42	0.37-0.49	0.26	0.22-0.32
Aspirin												
0-27 cDDD	0.30	0.27-0.34	0.20	0.18-0.23	0.12	0.09-0.14	0.32	0.28-0.34	0.21	0.18-0.23	0.11	0.09-0.14
28-365 cDDD	0.48	0.42-0.56	0.41	0.36-0.46	0.21	0.16-0.27	0.47	0.41-0.55	0.41	0.36-0.48	0.20	0.15-0.26
>365 cDDD	0.63	0.53-0.73	0.45	0.39-0.52	0.29	0.25-0.35	0.64	0.54-0.76	0.47	0.40-0.54	0.30	0.25-0.36

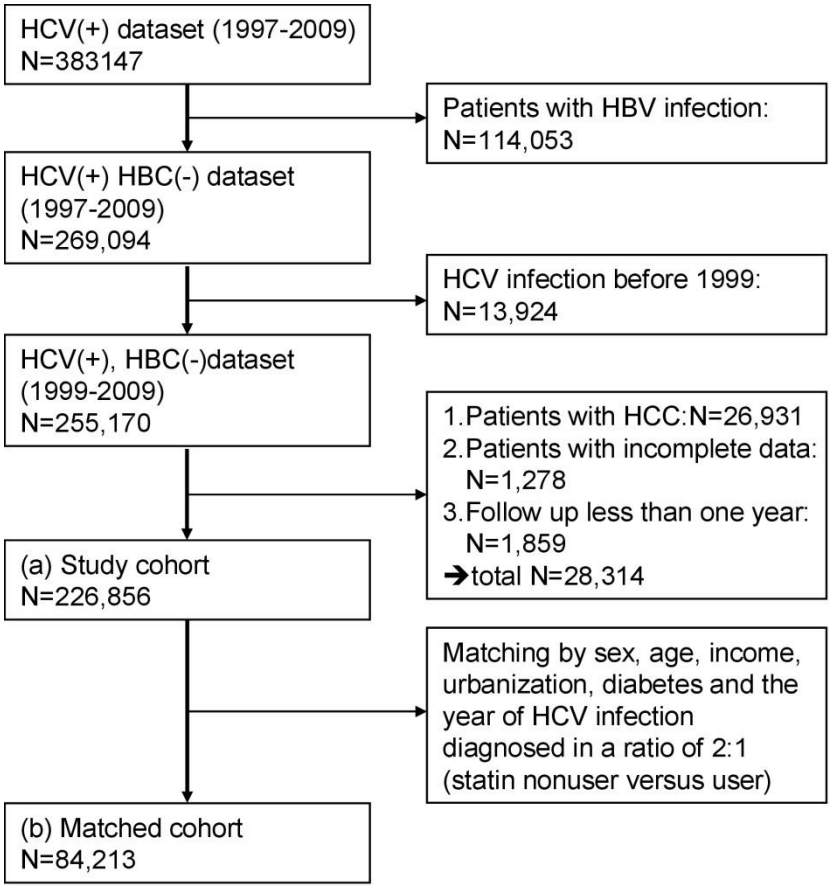
Abbreviations: ACEI, angiotensin converting enzyme inhibitor; ARD, alcohol-related disease; cDDD, cumulative defined daily dose; CCI, charlson comorbidity index; CI, confidence interval; HCV, hepatitis C virus; HR, hazard ratio; NASH, Nonalcoholic Steatohepatitis

*Main model is adjusted for age, sex, urbanization, income, diabetes.

†The models were adjusted for covariates in the main model as well as each additional listed covariate.

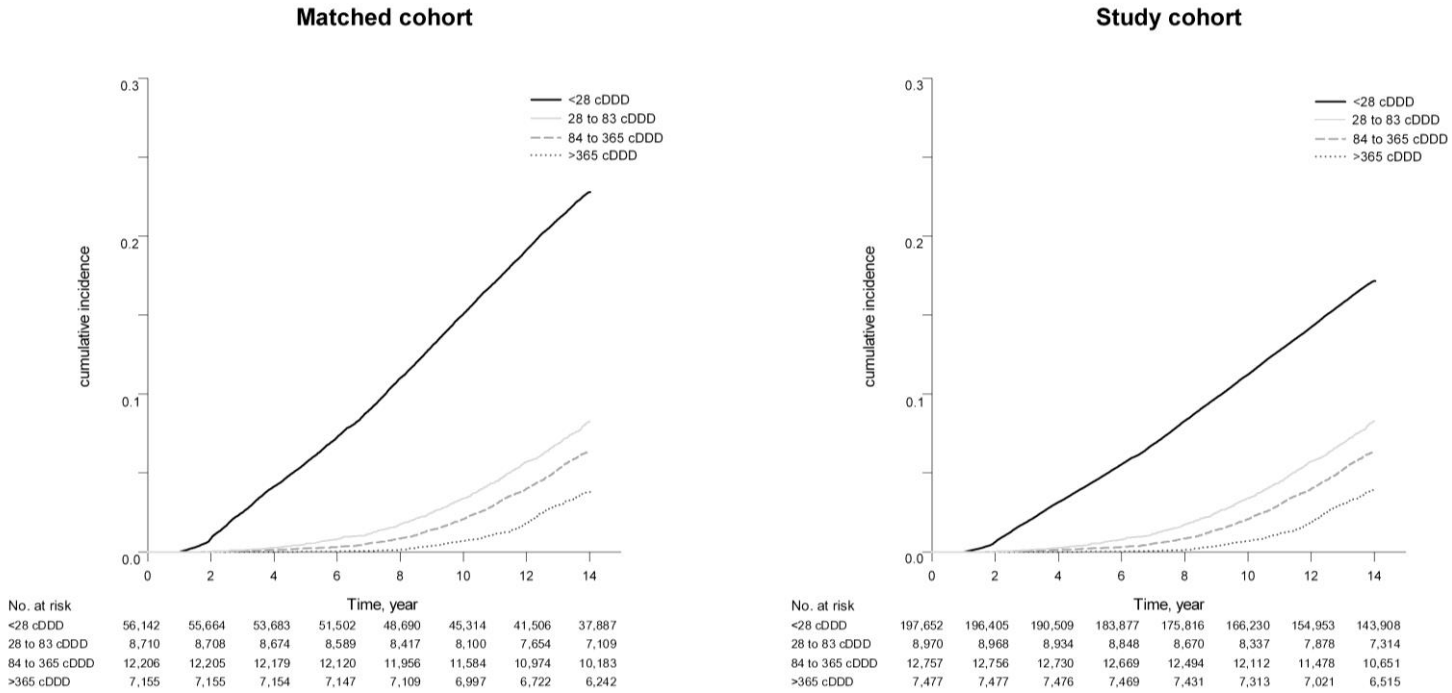
doi:10.6342/NTU201701710

Figure1 Flowchart of the patient enrollment process of study cohort and matched cohort.



doi:10.6342/NTU201701710

Figure 2 Cumulative incidence of cirrhosis development by cumulative defined daily dose (cDDD) of statin use during the follow-up period from the study (a) and matched (b) cohorts.



doi:10.6342/NTU201701710

Part II: Statin use and the risk of cirrhosis development in patients with hepatitis B virus infection: a hospital based cohort study

doi:10.6342/NTU201701710

Title page

Statin use and the risk of cirrhosis development in patients with hepatitis B virus infection: a hospital based cohort study

Yao-Hsu Yang[1,2,3,4], Wen-Cheng Chen[2,5], Yu-Tse Tsan[3,6,7], Mei-Jyh Chen[3,8], Wei-Tai Shih[1,3], Ying-Huang Tsai[9,10*] and Pau-Chung Chen[3,11*]

1. Department for Traditional Chinese Medicine, Chang Gung Memorial Hospital, Chia-Yi, Taiwan
2. Center of Excellence for Chang Gung Research Datalink, Chang Gung Memorial Hospital, Chiayi, Taiwan
3. Institute of Occupational Medicine and Industrial Hygiene, National Taiwan University College of Public Health, Taipei, Taiwan
4. School of Traditional Chinese Medicine, College of Medicine, Chang Gung University, Taoyuan, Taiwan;
5. Department of Radiation Oncology, Chang Gung Memorial Hospital, Chiayi, Taiwan
6. Institute of Occupational Medicine, Department of Emergency Medicine, Taichung Veterans General Hospital, Taichung, Taiwan
7. School of Medicine, Chung Shan Medical University, Taichung, Taiwan;
8. Departments of Internal Medicine, Integrated Diagnostics and Therapeutics, National Taiwan University Hospital and National Taiwan University College of Medicine, Taipei, Taiwan;
9. Division of Pulmonary and Critical Care Medicine and Department of Respiratory Care, Chang Gung Memorial Hospital, Chiayi, Taiwan
10. Department of Respiratory Therapy, Chang Gung University, Taoyuan, Taiwan
11. Department of Environmental and Occupational Medicine, National Taiwan University Hospital and National Taiwan University College of Medicine, Taipei, Taiwan

*Correspondence to:

Dr. Pau-Chung Chen, Institute of Occupational Medicine and Industrial Hygiene, National Taiwan University College of Public Health, 17 Syujhou Road, Taipei 10055, Taiwan. Phone No.: +886-2-3322 8088; Fax No.: +886-2-2358 2402; and Email: pchen@ntu.edu.tw

Ying-Huang Tsai, Division of Pulmonary and Critical Care Medicine and Department of Respiratory Care, Chang Gung Memorial Hospital, Chiayi, Taiwan

No.6, W. Sec., Jiapu Rd., Puzih City, Chiayi County 613, Taiwan. Phone No.: +886-5-3621000

doi:10.6342/NTU201701710

ext:2004 ; Fax No.: +886-5-3622188; and Email: chestmed@cgmh.org.tw

Keywords: statin, hepatitis B virus, cirrhosis

Conflict of Interest: The authors have no conflict of interest to disclose.

Financial Support: This study was supported by a grant from Chia-yi Chang Gung Memorial Hospital, Taiwan (CORPG6D0161, CGRPG6F0011).

doi:10.6342/NTU201701710

Abstract

Background & Aims:

The use of statins has been shown to reduce fibrosis progression and cirrhosis in patients with chronic hepatitis C. Recent studies have also revealed the protective effect of statins on HCC in chronic hepatitis C, as well as in chronic hepatitis B (CHB) patients. However, the protective effect of statins in the development of cirrhosis in patients with CHB is limited. This study investigated the association between the use of statins and the risk of cirrhosis development in patients with CHB.

Study Population and Methods:

We conducted a population-based cohort study by using the Chang Gung Research database (CGRD). A total of 16,355 patients with HBV infection were included as the study cohort. Each patient was followed from 2001 to 2010 to identify incident cases of cirrhosis. A Cox proportional hazard regression was performed to evaluate the association between statin use and cirrhosis risk.

Results:

A total of 2,960 cases of cirrhosis were identified in the cohort with 16,355 HBV infection patients during the follow-up period. There were 159 cirrhosis cases (13.6%) of cirrhosis among 1,173 statin users (defined as those who used more than 28 cumulative defined daily doses (cDDD), and 2,801 cirrhosis cases (18.5%) among

doi:10.6342/NTU201701710

15,182 statin nonusers. A dose-response relationship between statin use and cirrhosis risk was observed. The adjusted hazard ratios were 0.65 (95% CI, 0.53 to 0.80), 0.53 (95% CI, 0.45 to 0.63), and 0.42 (95% CI, 0.34 to 0.51) for statin use of 28 to 83, 84 to 365, and more than 365 cDDD, respectively, relative to no statin use (< 28 cDDD).

Conclusion:

Among the patients with HBV infection, statin use was associated with a reduced risk of cirrhosis development in a dose-dependent manner. Further clinical research is required.

doi:10.6342/NTU201701710

Introduction

Hepatitis B virus (HBV) infection typically leads to chronic hepatitis, and often follows a progressive course over many years, finally resulting in cirrhosis, hepatocellular carcinoma (HCC), and even the need for liver transplantation(1-3). The objective of antiviral treatment of chronic HBV is to achieve sustained eradication of HBV, which is defined as the persistent absence of HBV RNA in serum for at least 6 months after treatment completion. Another objective is to stop or delay the progression to cirrhosis, decompensated liver disease, or HCC(4).

Statins (3-hydroxy-3-methyl-glutaryl-CoA reductase inhibitors) are used to treat and prevent coronary heart disease and stroke in patients with hyperlipidemia(5). Recently, statins have been investigated for their antiproliferative, antiangiogenic, antiinflammatory, and antineoplastic effects(6-9). In addition, some animal studies have shown that statins can inhibit the progression of cirrhosis, but clinical studies are limited. Previous studies indicated that statin use can decrease the risk of liver cirrhosis development and its and its decompensation in chronic hepatitis B patients(10). The results show that statin is an independent protector against the development of cirrhosis and its decompensation in patients with HBV infection. However, they only included patients with positivity of hepatitis B surface antigen for more than 6 months. Another study indicate that statin use can decreases the

doi:10.6342/NTU201701710

decompensation rate in both HBV- and HCV-related cirrhosis(11). Data regarding the antifibrogenic actions of statins for patients with HBV infection that has not yet progressed to cirrhosis are limited. The purpose of this study was to investigate the association between statin use and the risk of cirrhosis development in patients with HBV infection.

Materials and Methods

Data Sources

The Chang Gung Research Database (CGRD) is a de-identified database derived from medical records of Chang Gung Memorial Hospital (CGMH), and it is systematically updated annually to include new data generated in CGMH. CGMH, founded in 1976, is currently the largest hospital system in Taiwan, and it comprises seven medical institutes, which are located from the northeast to southern regions of Taiwan: Keelung CGMH, Taipei CGMH, Linkou CGMH, Taoyuan CGMH, Yunlin CGMH, Chiayi CGMH, and Kaohsiung CGMH. CGMH has 10,070 beds and admits more than 280,000 patients each year. The outpatient department visits and emergency department visits to CGMH were over 8,500,000 and 500,000, respectively in 2015. In recent years, the CGRD promoted clinical and scientific studies to a considerable extent. In 2015, more than 1800 studies were conducted by CGMH staff, and the

doi:10.6342/NTU201701710

studies were published in a diverse range of reputed journals. Some of these studies are based on the CGRD as multicenter research studies with relatively large sample sizes. There are 44 different dataset in CGRD and we will conducted a hospital-based cohort study by using CGRD, Lack of laboratory data or medical examination reports is the limitation of National Health Insurance Research Database (NHIRD), but we can get the information of laboratory data or medical examination reports from CGRD.

We conducted a nationwide cohort study by using population-based data from the CGRD. We used databases for admissions and outpatient visits, both of which included information on patient characteristics such as sex, date of birth, date of admission, date of discharge, dates of visits, and up to five discharge diagnoses or three outpatient visit diagnoses (according to International Classification of Diseases, Ninth Revision (ICD-9) codes). The data files also contained information on patient prescriptions, including the names of prescribed drugs, dosage and duration. Following strict confidentiality guidelines in accordance with personal electronic data protection regulations, the National Health Research Institutes of Taiwan maintains an anonymous database of NHI reimbursement data that is suitable for research. In addition, this study was approved by the Ethics Review Board of Chang Gung Memorial Hospital, Chia-Yi Branch, Taiwan.

doi:10.6342/NTU201701710

Study population

We conducted a population-based cohort study that included all patients older than 18 years who were newly diagnosed with HBV infection (ICD-9 codes070.2, 070.3, V02.61 or HbsAg positive or Anti-HBc positive) without hepatitis C virus infection (ICD-9 codes070.7, 070.41, 070.44, 070.51, 070.54, V02.62) or HCC (ICD-9 code 155.0) between 1 January 2001 and 31 December 2010.

FIB-4 index:

The FIB-4 index is a simple formula to predict liver fibrosis based on the standard biochemical values (AST, ALT and platelet count) and age. FIB-4 was calculated as: FIB-4 = age [years] × AST [IU/L]/platelet count [× 10^9/L] × ($ALT^{1/2}$[IU/L])(12). We defined FIB -4>3.5 as cirrhosis development. Patients with less than two FIB-4 record, Fib-4>3.5 at baseline or missing data on sex, age, income, or level of urbanization were excluded. A total of 16,355 patients were included in the final analyses.

Statin Exposure

We identified patients who received prescriptions for statins in the outpatient visits database from 1 January 2001 to cirrhosis diagnosis or the end of follow-up.

doi:10.6342/NTU201701710

The defined daily dose (DDD) recommended by the World Health Organization is a unit for measuring a prescribed amount of drug; it is the assumed average maintenance dose per day of a drug consumed for its main indication in adults. By using the following formula, we could compare any statins on the basis of the same standard: (total amount of drug)/(amount of drug in a DDD) = number of DDDs. Cumulative DDD (cDDD), which indicates the total exposed dosage, was estimated as the sum of dispensed DDD of any statins to compare their use to the risk of cirrhosis. We collected similar information on nonstatin lipid-lowering medications (cholestyramine, colestipol, colextran, niceritrol, nicofuranose, acipimox, probucol, and ezetimibe) and fibrate.

To examine the dose–effect relationship, we categorized the statins into four groups in each cohort (<28, 28 to 83, 84 to 365, and >365 cDDDs). Patients who used statins for less than 28 cDDDs were defined as statin nonusers, and patient who used statins for more than 28 cDDDs were defined as statin users.

Potential Confounders

We systematically identified the potential confounding risk factors for cirrhosis as the following diagnoses recorded during the study period: alcohol-related disease (ARD; ICD-9 codes 291, 303.0, 303.9, 305.0, 571.0, 571.1, 571.2, or 571.3), nonalcoholic

doi:10.6342/NTU201701710

steatohepatitis (NASH; ICD-9 code 571.8, 571.9) and diabetes (ICD-9 code 250). The information of the Charlson comorbidity index (CCI) was also collected and considered as one possible confounding risk(13). We also used logistic regression to calculate propensity score of all subjects according to their statin treatment. Calculation involved all covariates list in table1, including age, gender, comorbidity and other drug usages. Propensity score were used as one possible confounder in further analysis.

We collected exposure information of other drugs that might alter the risk of cirrhosis, such as anti-HBV treatment(4), aspirin(14, 15), angiotensin-converting enzymeinhibitors (ACEIs) (captopril, enalapril, lisinopril, perindopril, ramipril, quinapril, benazepril, cilazapril, and fosinopril)(16), and metformin(17, 18). We also considered sociodemographic characteristics (age, sex, income, and level of urbanization) in the modeling.

Statistical Analysis

The distribution of demographic factors and the proportions of comorbidities between the statin users and nonusers in the study cohort and matched cohort were compared. Cox proportional hazards models were used to compute the hazard ratios (HRs) accompanying 95% CIs after adjustment for age, sex, urbanization, income, and

doi:10.6342/NTU201701710

diabetes. Two-tailed $P = 0.05$ was considered significant. Patients with a death date in the admission file and those from the beneficiaries register who were lost to follow-up were censored. All of these analyses were conducted using SAS statistical software (Version 9.4; SAS Institute, Cary, NC, USA).

Sensitivity analyses and subgroup analyses

Many medicines have shown positive results in chemoprevention. To examine potential effect modifiers, we conducted subgroup analyses stratified by groups with and without the use of anti-HBV treatment, metformin, ACEIs, and aspirin. We also examined the outcome stratified by groups according to sex and age and with or without ARD, NASH, diabetes and receiving liver biopsy. These sensitivity analyses and subgroup analyses were applied to evaluate the difference and consistency between statin use and the risk of cirrhosis.

Results

We included a total of 16,355 patients (5,302 women and 11,053 men) diagnosed with HBV infection during the study period. The basic demographic characteristics of the patient population are summarized in Table 1. A total of 7.2% (1,173) of the patients had used statins for more than 28 cDDDs, and they tended to be elderly, female, have

doi:10.6342/NTU201701710

a high CCI, and a high percentage of diabetes, hypertension, NASH, chronic kidney disease and biliary stone. Statin users received more nonstatin lipid-lowering drugs, fibrates, ACEIs, and aspirin, but less anti-HBV treatment.

Table 2 shows that there was a dose-response relationship between statin use and the risk of cirrhosis development. In study cohort, the adjusted HRs were 0.65 (95% CI, 0.53-0.80), 0.53 (95% CI, 0.45-0.63), and 0.42 (95% CI, 0.34-0.51) for patients with statin use of 28 to 83 cDDDs, 84 to 365 cDDDs, and >365 cDDDs, respectively. The sensitivity analysis adjustments exhibited little effect on the estimates of the association between statin use and the risk of cirrhosis development according to different models after adding other covariates, such as propensity score, CCI, comorbidity and other drug with chemoprevention effect. The effects of statins remained dose-response relationship in the patients of different subgroups in diabetes, ARD, receiving liver biopsy, sex, age.

Table 3 also demonstrates that the effect of statin keep the same trend of dose-response relationship in each subgroup stratified by different cDDD of metformin, aspirin, and ACEIs use. However, as patients received anti-HBV treatment, the effect of statin decreased to 0.92 (95% CI, 0.45-1.88), 0.65 (95% CI, 0.37-1.15), and 0.60 (95% CI, 0.28-1.29) for patients with statin use of 28 to 83 cDDDs, 84 to 365 cDDDs, and >365 cDDDs, respectively. For patients with NASH, the effect of

doi:10.6342/NTU201701710

statin also did not reach statistical significant.

Discussion

To our knowledge, this study was the first hospital based cohort study to document a dose-response relationship between the use of statins and the risk of cirrhosis development after controlling for the confounding effects of age, sex, income, urbanization, diabetes, and other medication. This study had several strengths. The study cohort was mainly obtained from a hospital based database, we could obtain laboratory data from CGRD, which is the limitation of National Health Insurance Research Database (NHIRD). In addition, because the data on statin and other medicine use were obtained from a historical database that collects all available prescription information during the study period, we can eliminate the possibility of recall bias.

If physicians are less likely to prescribe statins because of their hepatotoxicity, patients with liver disease are less likely to be prescribed statins. We took several steps to avoid possible confounding effects of contraindication. First, we excluded patients with HCC. Second, we excluded statin use recorded within 1 year before cirrhosis defined by fib-4 >3.5 or the end of follow up, assuming that in this time period liver disease is likely to be severe and overt. In addition, we conducted

doi:10.6342/NTU201701710

sensitivity analyses by stratification to clarify the misclassifications and potential confounders, and the results revealed no significant changes in the HRs of the different subgroups except for patient with NASH or anti-HBV treatment. The result of patient with NASH or anti-HBV treatment did not reach statistical significant might contributing to the insignificant effect measured of statin use on each subgroup. We also conducted a population-based cohort study by using the Taiwan National Health Insurance Research Database. A total of 891,136 patients with HBV infection were included as the study cohort. Each patient was followed from 1997 to 2010 to identify incident cases of cirrhosis. The results were demonstrated at supplement table1 and 2, and a dose-response relationship between statin use and cirrhosis risk was observed. The adjusted hazard ratios were 0.31 (95% CI, 0.29 to 0.32), 0.23 (95% CI, 0.21 to 0.24), and 0.11 (95% CI, 0.10 to 0.12) for statin use of 28 to 83, 84 to 365, and more than 365 cDDD, respectively, relative to no statin use (< 28 cDDD). The further analysis of sensitivity analyses and subgroup analyses show that the trend of dose-response relationship between statin use and the risk reduction of cirrhosis development did not alter.

Huang et al. analyzed the association between statin use and liver cirrhosis development by using NHIRD(10). The results show that statin is an independent protector against the development of cirrhosis and its decompensation in patients with

doi:10.6342/NTU201701710

HBV infection and the protective effect reveal dose–response relationship. The results were consistent with our analysis in both CGRD cohort and NHIRD cohort. However, there were some limitations in that study. First, this study only includes patients with positivity of hepatitis B surface antigen for more than 6 months. Only patients after propensity matching were enrolled in final analysis, more than 70% statin user were excluded. Second, the baseline characteristics still quite heterogeneous after propensity score matching. Third, the study did not consider other drugs that might reduce the risk of cirrhosis as confounding factors(14-17).

The mechanism by which statin use may reduce the risk of cirrhosis development is not clearly understood. Several possible mechanisms have been investigated in previous studies. Statins can down regulate expression of profibrotic cytokines including transforming growth factor-β, connective tissue growth factor, and platelet-derived growth factor, which can stimulate the activation of hepatic stellate cells, resulting in further fibrogenesis(19-21). Another potential mechanism is that the upregulation of kruppel-like factor 2 expression results in vasodilation and the improvement of liver microcirculation(22, 23). Statins may also inhibit fibrogenesis of hepatic myofibroblasts and the replication of HBV(24).

The potential limitations of this study should be noted. First, we did not obtain any histological data from liver biopsy, reports of liver ultrasound examination. Cirrhosis

doi:10.6342/NTU201701710

cases in this study were identified only by using the Fib-4. Previous studies indicate that Fib-4 is a simple, accurate, non-invasive and economical assessment of predicting cirrhosis in patient with HBV infection(12). We had investigated the association between statin use and the risk of cirrhosis development in patients with hepatitis B infection by using NHIRD. The cirrhosis were defined by using ICD code (571.2, 571.5, 571.6, 572.2, 572.3, 572.4, 572.8, or 573.0) with at least three records of outpatient visits within one year or one admission diagnosis during study period. The results were consistent between CGRD cohort and NHIRD cohort. The diagnosis of cirrhosis may be less accurate than liver biopsy and only those with obvious cirrhosis would have been captured in our study. However, errors in cirrhosis diagnosis tend to occur in random as a result of the same definition being used in both groups of statin user and nonuser, which might have limited effect on the result. The results of subgroup analysis stratified by groups according to liver biopsy shows consistent in both receiving or not. Second, several unmeasured confounders, including body mass index, smoking habit, alcohol intake, and other over-the-counter drug use, which are associated with cirrhosis, were not included in our database. Third, there is no way to verify the exact dosage that the study participants actually took. We presumed that all prescribed medications were taken by patients as prescribed; this may overestimate the actual ingested dosage because some degree of noncompliance is always expected.

doi:10.6342/NTU201701710

In conclusion, statin use may reduce the risk for cirrhosis development in HBV-infected patients in a dose-dependent manner. Further mechanistic research is required.

doi:10.6342/NTU201701710

Acknowledgments

The author's would like to thank Center of Excellence for Chang Gung Research Datalink for the comments and assistance in data analysis. This study was supported by a grant from Chia-yi Chang Gung Memorial Hospital, Taiwan (CORPG6D0161, CGRPG6F0011). This funding body played no role in study design, analysis, and interpretation of data in this paper.

doi:10.6342/NTU201701710

References:

1. Chen CJ, Wang LY, Yu MW. Epidemiology of hepatitis B virus infection in the Asia-Pacific region. J Gastroenterol Hepatol. 2000;15 Suppl:E3-6.

2. El-Serag HB. Epidemiology of viral hepatitis and hepatocellular carcinoma. Gastroenterology. 2012;142(6):1264-73 e1.

3. Pan CQ, Zhang JX. Natural History and Clinical Consequences of Hepatitis B Virus Infection. Int J Med Sci. 2005;2(1):36-40.

4. Lun-Gen L. Antiviral Therapy of Liver Cirrhosis Related to Hepatitis B Virus Infection. J Clin Transl Hepatol. 2014;2(3):197-201.

5. Baigent C, Keech A, Kearney PM, Blackwell L, Buck G, Pollicino C, et al. Efficacy and safety of cholesterol-lowering treatment: prospective meta-analysis of data from 90,056 participants in 14 randomised trials of statins. Lancet. 2005;366(9493):1267-78.

6. Tsan YT, Lee CH, Ho WC, Lin MH, Wang JD, Chen PC. Statins and the risk of hepatocellular carcinoma in patients with hepatitis C virus infection. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2013;31(12):1514-21.

7. Tsan YT, Lee CH, Wang JD, Chen PC. Statins and the risk of hepatocellular carcinoma in patients with hepatitis B virus infection. Journal of clinical oncology :

doi:10.6342/NTU201701710

official journal of the American Society of Clinical Oncology. 2012;30(6):623-30.

8. Demierre MF, Higgins PD, Gruber SB, Hawk E, Lippman SM. Statins and cancer prevention. Nature reviews Cancer. 2005;5(12):930-42.

9. Yang YH, Chen WC, Tsan YT, Chen MJ, Shih WT, Tsai YH, et al. Statin use and the risk of cirrhosis development in patients with hepatitis C virus infection. J Hepatol. 2015;63(5):1111-7.

10. Huang YW, Lee CL, Yang SS, Fu SC, Chen YY, Wang TC, et al. Statins Reduce the Risk of Cirrhosis and Its Decompensation in Chronic Hepatitis B Patients: A Nationwide Cohort Study. Am J Gastroenterol. 2016;111(7):976-85.

11. Chang FM, Wang YP, Lang HC, Tsai CF, Hou MC, Lee FY, et al. Statins decrease the risk of decompensation in HBV- and HCV-related cirrhosis: A population-based study. Hepatology. 2017.

12. Kim BK, Kim DY, Park JY, Ahn SH, Chon CY, Kim JK, et al. Validation of FIB-4 and comparison with other simple noninvasive indices for predicting liver fibrosis and cirrhosis in hepatitis B virus-infected patients. Liver Int. 2010;30(4):546-53.

13. Kieszak SM, Flanders WD, Kosinski AS, Shipp CC, Karp H. A comparison of the Charlson comorbidity index derived from medical record data and administrative billing data. J Clin Epidemiol. 1999;52(2):137-42.

doi:10.6342/NTU201701710

14. Jiang ZG, Feldbrugge L, Tapper EB, Popov Y, Ghaziani T, Afdhal N, et al. Aspirin use is associated with lower indices of liver fibrosis among adults in the United States. Aliment Pharmacol Ther. 2016;43(6):734-43.

15. Sitia G, Iannacone M, Guidotti LG. Anti-platelet therapy in the prevention of hepatitis B virus-associated hepatocellular carcinoma. J Hepatol. 2013;59(5):1135-8.

16. Yoshiji H, Kuriyama S, Fukui H. Angiotensin-I-converting enzyme inhibitors may be an alternative anti-angiogenic strategy in the treatment of liver fibrosis and hepatocellular carcinoma. Possible role of vascular endothelial growth factor. Tumour Biol. 2002;23(6):348-56.

17. DePeralta DK, Wei L, Ghoshal S, Schmidt B, Lauwers GY, Lanuti M, et al. Metformin prevents hepatocellular carcinoma development by suppressing hepatic progenitor cell activation in a rat model of cirrhosis. Cancer. 2016;122(8):1216-27.

18. Kita Y, Takamura T, Misu H, Ota T, Kurita S, Takeshita Y, et al. Metformin prevents and reverses inflammation in a non-diabetic mouse model of nonalcoholic steatohepatitis. PLoS One. 2012;7(9):e43056.

19. Eberlein M, Heusinger-Ribeiro J, Goppelt-Struebe M. Rho-dependent inhibition of the induction of connective tissue growth factor (CTGF) by HMG CoA reductase inhibitors (statins). Br J Pharmacol. 2001;133(7):1172-80.

20. Iwakiri Y, Shah V, Rockey DC. Vascular pathobiology in chronic liver disease

doi:10.6342/NTU201701710

and cirrhosis - current status and future directions. J Hepatol. 2014;61(4):912-24.

21. Paradis V, Dargere D, Bonvoust F, Vidaud M, Segarini P, Bedossa P. Effects and regulation of connective tissue growth factor on hepatic stellate cells. Lab Invest. 2002;82(6):767-74.

22. Marrone G, Russo L, Rosado E, Hide D, Garcia-Cardena G, Garcia-Pagan JC, et al. The transcription factor KLF2 mediates hepatic endothelial protection and paracrine endothelial-stellate cell deactivation induced by statins. J Hepatol. 2013;58(1):98-103.

23. SenBanerjee S, Lin Z, Atkins GB, Greif DM, Rao RM, Kumar A, et al. KLF2 Is a novel transcriptional regulator of endothelial proinflammatory activation. The Journal of experimental medicine. 2004;199(10):1305-15.

24. Bader T, Korba B. Simvastatin potentiates the anti-hepatitis B virus activity of FDA-approved nucleoside analogue inhibitors in vitro. Antiviral Res. 2010;86(3):241-5.

doi:10.6342/NTU201701710

Table1 Patient demographics and clinical characteristics of study cohort .

	Non-user	Statin User	P-value
First Fib-4 index	1.52	1.53	
No. of cirrhosis	2801(18.5)	159(13.6)	**<.0001**
Gender			**<.0001**
Female	4863(32.0)	439(37.4)	
Male	10319(68.0)	734(62.6)	
Propensity score	0.88	0.62	
CCI	1.93	2.61	
Age			**<.0001**
18-29	3231(21.3)	87(7.4)	
30-39	4335(28.6)	206(17.6)	
40-49	4360(28.7)	420(35.8)	
50-59	2094(13.8)	304(25.9)	
>=60	1162(7.7)	156(13.3)	
Comorbidity			
DM	3147(20.7)	599(51.1)	**<.0001**
HTN	4683(30.9)	803(68.5)	**<.0001**
ARD	938(6.2)	65(5.5)	**0.3810**
CKD	823(5.4)	231(19.7)	**<.0001**
Biliary stones	1549(10.2)	142(12.11)	**0.0392**
NASH	457(3.0)	55(4.7)	**<.0001**
Nonstatin lipid-lowering drug			**<.0001**
0-27 cDDD	15132(99.7)	1094(93.3)	
28-83 cDDD	17(0.1)	22(1.9)	
84-365 cDDD	30(0.2)	42(3.6)	
>365 cDDD	3(0.0)	15(1.3)	
fibrate			**<.0001**
0-27 cDDD	14952(98.5)	991(84.5)	
28-83 cDDD	90(0.6)	58(4.9)	
84-365 cDDD	103(0.7)	85(7.3)	
>365 cDDD	37(0.2)	39(3.3)	
HB tx			**0.1630**
No	14306(94.2)	1107(94.4)	
Partial treatment	412(2.7)	23(2.0)	
Complete treatment	464(3.1)	43(3.7)	

doi:10.6342/NTU201701710

ACEI			**<.0001**
0-27 cDDD	14321(94.3)	850(72.5)	
28-365 cDDD	602(4.0)	211(18.0)	
>365 cDDD	259(1.7)	112(9.6)	
Aspirin			**<.0001**
0-27 cDDD	14170(93.3)	651(55.5)	
28-365 cDDD	655(4.3)	267(22.8)	
>365 cDDD	357(2.4)	255(21.7)	
Metformine			**<.0001**
0-27 cDDD	14394(94.8)	813(69.3)	
28-365 cDDD	510(3.4)	190(16.2)	
>365 cDDD	**278(1.8)**	**170(14.5)**	

doi:10.6342/NTU201701710

Table 2. Adjusted hazard ratios (HRs) of cirrhosis development associated with statin use during the follow-up period.

	Cumulative defined daily dose of Statin					
	28-83cDDD		84-365cDDD		>365cDDD	
	HR	95% CI	HR	95% CI	HR	95% CI
Main model*	0.65	0.53-0.80	0.53	0.45-0.63	0.42	0.34-0.51
Additional covariates†						
Main model+ Fib-4 score	0.71	0.58-0.87	0.60	0.50-0.70	0.51	0.42-0.63
Main model+ propensity score	0.68	0.52-0.88	0.61	0.49-0.75	0.52	0.41-0.67
Main model+CCI	0.66	0.54-0.80	0.50	0.43-0.60	0.41	0.33-0.50
Main model+ARD	0.64	0.52-0.78	0.54	0.45-0.64	0.42	0.34-0.51
Main model+NASH	0.66	0.54-0.80	0.53	0.45-0.63	0.42	0.34-0.52
Main model+ Nonstatin lipid-lowering drug	0.66	0.54-0.81	0.54	0.45-0.64	0.43	0.35-0.53
Main model+fibrate	0.68	0.55-0.83	0.55	0.46-0.65	0.43	0.35-0.54
Main model+ anti-HBV treatment	0.65	0.53-0.79	0.53	0.45-0.63	0.42	0.34-0.51
Main model+metformin	0.64	0.53-0.79	0.53	0.44-0.62	0.51	0.34-0.51
Main model+ACEI	0.66	0.54-0.81	0.54	0.45-0.64	0.51	0.34-0.52
Main model+aspirin	0.70	0.57-0.85	0.58	0.48-0.68	0.46	0.37-0.56

Abbreviations: ACEI, angiotensin converting enzyme inhibitor; ARD, alcohol-related disease; cDDD, cumulative defined daily dose; CCI, charlson comorbidity index; CI, confidence interval; HCV, hepatitis C virus; HR, hazard ratio; NASH, Nonalcoholic Steatohepatitis

*Main model is adjusted for age, sex, urbanization, income, diabetes.

†The models were adjusted for covariates in the main model as well as each additional listed covariate.

doi:10.6342/NTU201701710

Table 3. Subgroup analysis of adjusted hazard ratios (HRs) for cirrhosis development associated with statin use during the follow-up period.

	Cumulative defined daily dose of Statin					
	28-83cDDD		84-365cDDD		>365cDDD	
Subgroup effect*	HR	95% CI	HR	95% CI	HR	95% CI
Age, years						
≧50	0.73	0.56-0.95	0.61	0.48-0.76	0.43	0.33-0.57
<50	0.57	0.42-0.77	0.48	0.37-0.63	0.44	0.32-0.61
Gender						
Male	0.75	0.59-0.95	0.58	0.48-0.71	0.41	0.32-0.54
Female	0.57	0.39-0.84	0.57	0.43-0.77	0.52	0.38-0.72
ARD						
NO	0.65	0.53-0.81	0.54	0.45-0.64	0.43	0.34-053
YES	0.50	0.28-0.90	0.49	0.27-0.90	0.33	0.16-0.71
Diabetes						
NO	0.50	0.36-0.71	0.48	0.36-0.64	0.31	0.21-0.46
YES	0.78	0.61-1.01	0.56	0.46-0.70	0.48	0.37-0.61
NASH						
NO	0.64	0.52-0.78	0.52	0.44-0.62	0.42	0.34-0.51
YES	1.46	0.55-3.88	1.20	0.55-3.00	0.53	0.18-1.59
liver biopsy						
NO	0.66	0.52-0.83	0.53	0.44-0.65	0.44	0.35-0.55
YES	0.69	0.46-1.05	0.51	0.37-0.71	0.38	0.24-0.61
anti-HBV treatment						
No	0.64	0.52-0.78	0.52	0.44-0.62	0.40	0.33-0.50
Yes	0.92	0.45-1.88	0.65	0.37-1.15	0.60	0.28-1.29
Metformin						
0-27 cDDD	0.68	0.53-0.88	0.47	0.37-0.59	0.35	0.25-0.47
28-365 cDDD	0.57	0.34-0.96	0.58	0.34-0.88	0.52	0.31-0.87
>365 cDDD	0.64	0.41-1.01	0.60	0.44-0.82	0.42	0.30-0.60
ACEI						
0-27 cDDD	0.59	0.44-0.78	0.47	0.36-0.60	0.37	0.26-0.51
28-365 cDDD	0.71	0.48-1.05	0.51	0.36-0.73	0.45	0.30-0.67
>365 cDDD	0.85	0.56-1.31	0.71	0.52-0.98	0.46	0.32-0.67
Aspirin						
0-27 cDDD	0.63	0.47-0.83	0.56	0.44-0.71	0.40	0.28-0.56
28-365 cDDD	0.90	0.61-1.33	0.53	0.37-0.77	0.36	0.19-0.65

doi:10.6342/NTU201701710

>365 cDDD	0.69	0.44-1.09	0.65	0.47-0.91	0.54	0.40-0.74

Abbreviations: ACEI, angiotensin converting enzyme inhibitor; ARD, alcohol-related disease; cDDD, cumulative defined daily dose; CCI, charlson comorbidity index; CI, confidence interval; HCV, hepatitis C virus; HR, hazard ratio; NASH, Nonalcoholic Steatohepatitis

*All models were adjusted for age, sex, urbanization, income, diabetes.

doi:10.6342/NTU201701710

Supplement Table1 Patient demographics and clinical characteristics of NHIRD cohort and CGRD cohort.

	NHIRD			CGRD		
	Non-user	User	P-value	Non-user	User	P-value
No. of cirrhosis	56392(6.3)	3254(3.1)	<.0001	1568(13.0)	172(11.3)	0.0663
Gender			<.0001			0.0204
Female	336068(42.7)	46670(44.9)		4321(35.8)	590(38.9)	
Male	451056(57.3)	57342(55.1)		7738(64.2)	928(61.1)	
Age			<.0001			
18-29	310297(39.4)	10601(10.2)		2918(24.2)	126(8.3)	
30-39	229902(29.2)	24755(23.8)		3578(29.7)	288(19.0)	
40-49	146143(18.6)	35142(33.8)		3322(27.6)	535(35.2)	
50-59	59338(7.5)	20947(20.1)		1448(12.0)	369(24.3)	
>=60	44144(5.3)	12567(12.1)		793(6.6)	200(13.2)	
Comorbidity						
DM	83810(10.7)	48072(44.2)	<.0001	2231(18.5)	749(49.3)	<.0001
HTN	145091(18.4)	62342(59.9)	<.0001	3524(29.2)	1011(66.6)	<.0001
ARD	22786(2.9)	3011(2.9)	0.9998	727(6.0)	81(5.3)	0.3810

doi:10.6342/NTU201701710

CKD	10935(1.4)	6286(6.0)	<.0001	627(5.2)	270(17.8)	<.0001
Biliary stones	36255(4.6)	7649(7.4)	<.0001	1252(10.4)	183(12.1)	0.0392
NASH	17751(2.3)	5914(5.7)	<.0001	457(3.0)	55(4.7)	<.0001
Nonstatin lipid-lowering drug			<.0001			<.0001
0-27 cDDD	783993(99.6)	96340(92.6)		11991(99.4)	1394(91.8)	
28-83 cDDD	1614(0.2)	2690(2.6)		25(0.2)	34(2.2)	
84-365 cDDD	1266(0.2)	3705(3.6)		34(0.3)	65(4.3)	
>365 cDDD	251(0.0)	1277(1.2)		9(0.1)	25(1.7)	
fibrate			<.0001			<.0001
0-27 cDDD	758303(96.3)	70425(67.7)		11775(97.6)	1268(83.5)	
28-83 cDDD	13313(1.7)	9819(9.4)		114(1.0)	72(4.7)	
84-365 cDDD	11474(1.5)	14838(14.3)		129(1.1)	130(5.6)	
>365 cDDD	4034(0.5)	8930(8.6)		41(0.3)	48(3.2)	
HB tx			<.0001			0.1630
No	760287(96.6)	100812(96.9)		10777(89.4)	1411(93.0)	

doi:10.6342/NTU201701710

Partial treatment	12809(1.6)	1625(1.6)		621(5.1)	54(3.6)	
Complete treatment	14028(1.8)	1575(1.5)		661(5.5)	53(3.5)	
ACEI			<.0001			<.0001
0-27 cDDD	705933(89.7)	60312(58.0)		11101(92.1)	1125(74.1)	
28-365 cDDD	52828(6.7)	23635(22.7)		663(5.5)	268(17.7)	
>365 cDDD	28363(3.6)	20065(19.3)		295(2.5)	125(8.2)	
Aspirin			<.0001			<.0001
0-27 cDDD	703805(89.4)	54794(52.7)		10893(90.3)	809(53.3)	
28-365 cDDD	54810(7.0)	22377(21.5)		800(6.6)	362(23.9)	
>365 cDDD	28509(3.6)	26841(25.8)		366(3.0)	347(22.9)	
Metformine			<.0001			<.0001
0-27 cDDD	747182(94.9)	68567(65.9)		11210(93.0)	1035(68.2)	
28-365 cDDD	21105(2.7)	13938(13.4)		563(4.7)	254(16.7)	
>365 cDDD	18837(2.4)	21507(20.7)		286(2.4)	229(15.1)	

doi:10.6342/NTU201701710

Supplement Table 2. Adjusted hazard ratios (HRs) of cirrhosis development associated with statin use during the follow-up period in NHIRD cohort and CGRD cohort.

	NHIRD						CGRD					
	28-83cDDD		84-365cDDD		>365cDDD		28-83cDDD		84-365cDDD		>365cDDD	
	HR	95% CI	HR	95% CI	HR	95% CI	HR	95% CI	HR	95% CI	HR	95% CI
Main model*	0.31	0.29-0.32	0.23	0.21-0.24	0.11	0.10-0.12	0.55	0.41-0.75	0.50	0.40-0.63	0.45	0.34-0.59
Additional covariates†												
Main model+propensity score	0.38	0.36-0.4	0.32	0.31-0.34	0.19	0.18-0.21	0.59	0.43-0.80	0.55	0.43-0.69	0.49	0.37-0.66
Main model+CCI	0.32	0.3-0.34	0.23	0.22-0.24	0.11	0.1-0.12	0.54	0.40-0.73	0.50	0.40-0.63	0.41	0.31-0.54
Main model+ARD	0.31	0.29-0.32	0.23	0.22-0.24	0.12	0.11-0.13	0.55	0.40-0.74	0.51	0.40-0.64	0.46	0.34-0.60
Main model+NASH	0.31	0.29-0.32	0.23	0.21-0.24	0.11	0.1-0.12	0.55	0.41-0.75	0.50	0.40-0.64	0.44	0.34-0.59
Main model+Nonstatin lipid-lowering drug	0.31	0.29-0.33	0.23	0.22-0.24	0.11	0.11-0.13	0.56	0.41-0.76	0.51	0.41-0.65	0.46	0.35-0.62
Main model+fibrate	0.33	0.31-0.35	0.25	0.24-0.27	0.13	0.12-0.14	0.57	0.42-0.77	0.52	0.41-0.66	0.47	0.35-0.63
Main model+anti-HBV treatment	0.3	0.29-0.32	0.22	0.21-0.24	0.11	0.10-0.12	0.56	0.41-0.75	0.51	0.40-0.64	0.45	0.34-0.59
Main model+metformin	0.33	0.31-0.35	0.26	0.24-0.27	0.13	0.12-0.14	0.55	0.40-0.74	0.51	0.40-0.64	0.46	0.35-0.61
Main model+ACEI	0.33	0.31-0.34	0.25	0.23-0.26	0.13	0.12-0.14	0.56	0.41-0.76	0.52	0.41-0.65	0.46	0.35-0.61
Main model+aspirin	0.35	0.33-0.37	0.28	0.26-0.29	0.15	0.14-0.17	0.59	0.44-0.80	0.55	0.44-0.70	0.52	0.39-0.69
Subgroup effects												
Gender												
Male	0.29	0.27-0.31	0.21	0.2-0.23	0.11	0.1-0.12	0.50	0.33-0.76	0.51	0.38-0.68	0.45	0.31-0.64

doi:10.6342/NTU201701710

Female	0.32	0.29-0.35	0.23	0.22-0.26	0.1	0.09-0.12	0.58	0.37-0.91	0.46	0.31-0.67	0.40	0.26-0.62
Age, years												
≧50	0.3	0.28-0.33	0.22	0.21-0.24	0.11	0.09-0.12	0.60	0.41-0.90	0.64	0.48-0.85	0.44	0.30-0.63
<50	0.32	0.29-0.34	0.24	0.22-0.26	0.12	0.11-0.14	0.46	0.28-0.74	0.38	0.26-0.56	0.50	0.32-0.76
ARD												
NO	0.31	0.29-0.33	0.24	0.22-0.25	0.12	0.11-0.13	0.58	0.43-0.80	0.53	0.42-0.67	0.46	0.34-0.61
YES	0.26	0.22-0.3	0.18	0.16-0.21	0.08	0.06-0.11	0.28	0.09-0.88	0.34	0.14-0.83	0.40	0.13-1.26
Diabetes												
NO	0.32	0.29-0.35	0.22	0.2-0.24	0.12	0.11-0.15	0.45	0.28-0.73	0.4	0.28-0.63	0.30	0.17-0.52
YES	0.29	0.27-0.31	0.22	0.21-0.24	0.1	0.09-0.12	0.65	0.44-0.96	0.56	0.42-0.75	0.55	0.39-0.76
NASH												
NO	0.3	0.29-0.32	0.23	0.21-0.24	0.11	0.1-0.12	0.53	0.39-0.73	0.48	0.37-0.61	0.44	0.33-0.58
YES	0.34	0.26-0.43	0.23	0.18-0.29	0.11	0.08-0.16	0.90	0.28-2.91	1.58	0.66-3.75	0.65	0.14-2.96
liver biopsy												
NO	0.34	0.27-0.42	0.23	0.19-0.29	0.11	0.08-0.15	0.54	0.39-0.75	0.52	0.41-0.67	0.46	0.34-0.61
YES	0.3	0.29-0.32	0.23	0.21-0.24	0.11	0.1-0.12	0.64	0.30-1.36	0.39	0.21-0.73	0.42	0.19-0.96
anti-HBV treatment												
No	0.3	0.28-0.32	0.22	0.21-0.23	0.11	0.1-0.12	0.55	0.40-0.75	0.52	0.41-0.66	0.41	0.31-0.56
Yes	0.63	0.43-0.92	0.56	0.41-0.77	0.31	0.19-0.5	0.58	0.18-1.84	0.32	0.12-0.86	0.81	0.38-1.74
Metformin												
0-27 cDDD	0.28	0.26-0.3	0.21	0.19-0.22	0.11	0.09-0.12	0.56	0.39-0.80	0.46	0.34-0.63	0.36	0.24-0.54
28-365 cDDD	0.4	0.35-0.46	0.28	0.24-0.31	0.12	0.1-0.16	0.37	0.16-0.84	0.53	0.32-0.86	0.60	0.32-1.15

doi:10.6342/NTU201701710

>365 cDDD	0.54	0.47-0.62	0.42	0.38-0.46	0.19	0.16-0.22	0.93	0.42-2.06	0.67	0.39-1.14	0.68	0.39-1.18
ACEI												
0-27 cDDD	0.26	0.24-0.28	0.18	0.17-0.2	0.09	0.08-0.10	0.55	0.38-0.78	0.49	0.37-0.65	0.42	0.30-0.60
28-365 cDDD	0.44	0.39-0.49	0.32	0.29-0.35	0.16	0.13-0.18	0.56	0.28-1.11	0.50	0.29-0.87	0.50	0.27-0.94
>365 cDDD	0.51	0.44-0.58	0.41	0.36-0.45	0.19	0.16-0.22	0.67	0.20-2.20	0.73	0.36-1.50	0.56	0.26-1.20
Aspirin												
0-27 cDDD	0.29	0.27-0.31	0.2	0.19-0.22	0.09	0.08-0.11	0.67	0.47-0.96	0.48	0.34-0.67	0.43	0.28-0.67
28-365 cDDD	0.49	0.44-0.55	0.37	0.33-0.41	0.17	0.14-0.21	0.55	0.29-1.05	0.38	0.22-0.65	0.43	0.19-0.98
>365 cDDD	0.61	0.53-0.70	0.51	0.46-0.57	0.29	0.25-0.33	0.31	0.10-1.00	1.27	0.80-2.02	0.69	0.43-1.10

Abbreviations: ACEI, angiotensin converting enzyme inhibitor; ARD, alcohol-related disease; cDDD, cumulative defined daily dose; CCI, charlson comorbidity index; CI, confidence interval; HCV, hepatitis C virus; HR, hazard ratio; NASH, Nonalcoholic Steatohepatitis

*Main model is adjusted for age, sex, urbanization, income, diabetes.

†The models were adjusted for covariates in the main model as well as each additional listed covariate.

doi:10.6342/NTU201701710

Part III: Xiao-chai-hu-tang and the risk of hepatocellular carcinoma in patients with hepatitis B virus-related cirrhosis

doi:10.6342/NTU201701710

Title page

Xiao-chai-hu-tang and the risk of hepatocellular carcinoma in patients with hepatitis B virus-related cirrhosis

Yao-Hsu Yang[1,2,3,4], Chang-Hsing Lee[5], Yu-Tse Tsan[3,6,7], Mei-Jyh Chen[3,8], and Pau-Chung Chen[3,9*]

1. Department for Traditional Chinese Medicine, Chang Gung Memorial Hospital, Chia-Yi, Taiwan

2. Center of Excellence for Chang Gung Research Datalink, Chang Gung Memorial Hospital, Chiayi, Taiwan

3. Institute of Occupational Medicine and Industrial Hygiene, National Taiwan University College of Public Health, Taipei, Taiwan

4. School of Traditional Chinese Medicine, College of Medicine, Chang Gung University, Taoyuan, Taiwan

5. Department of Occupational Medicine, Ton Yen General Hospital, Hisnchu County, Taiwan

6. Institute of Occupational Medicine,Department of Emergency Medicine, Taichung Veterans General Hospital, Taichung, Taiwan

7. School of Medicine, Chung Shan Medical University, Taichung, Taiwan;

8. Departments of Internal Medicine, Integrated Diagnostics and Therapeutics, National Taiwan University Hospital and National Taiwan University College of Medicine, Taipei, Taiwan;

9. Department of Environmental and Occupational Medicine, National Taiwan University Hospital and National Taiwan University College of Medicine, Taipei, Taiwan

***Correspondence to:**

Dr. Pau-Chung Chen, Institute of Occupational Medicine and Industrial Hygiene, National Taiwan University College of Public Health, 17 Syujhou Road, Taipei 10055, Taiwan. Phone No.: +886-2-3322 8088; Fax No.: +886-2-2358 2402; and Email: pchen@ntu.edu.tw

Conflict of Interest: The authors have no conflict of interest to disclose.

Financial Support: This study was supported by a grant from Chia-yi Chang Gung Memorial Hospital, Taiwan (CORPG6D0161).

Author's Contributions:

Yao-Hsu Yang: conception of study design, statistical analysis, interpretation of the

doi:10.6342/NTU201701710

data, literature review and wrote the manuscript.

Chang-Hsing Lee: conception of study design, interpretation of the data and critical revision.

Yu-Tse Tsan: conception of study design, interpretation of the data , literature review and critical revision.

Mei-Jyh Chen: conception of study design, interpretation of the data and critical revision.

Pau-Chung Chen: conception of study design, statistical analysis, interpretation of the data, critical revision and study supervision.

doi:10.6342/NTU201701710

Abstract

Background & Aims:

Previous studies have indicated that among hepatitis B virus (HBV)-infected patients, the hepatocellular carcinoma (HCC) incidence rate was much higher in patients with cirrhosis than in those without cirrhosis. Many in vitro and in vivo studies have indicated that xiao-chai-hu-tang (XCHT) may have protective effects against HCC and cirrhosis, but clinical studies are lacking. The purpose of this study was to investigate the association between use of XCHT and the risk of HCC in patients with HBV-related cirrhosis.

Study Population and Methods:

We conducted a population-based cohort study by using the Taiwan National Health Insurance Research Database. A total of 89,466 HBV-infected patients with cirrhosis were included as the study cohort. Each patient was individually tracked from 1997 to 2010 to identify incident cases of HCC. A Cox proportional hazards regression with time-dependent covariates for drug exposure was employed to evaluate the association between XCHT use and HCC risk.

Results

There were 14,070 HCCs in the study cohort during the follow-up period of 549,399.1 person-years. A dose–response relationship between XCHT use and HCC risk was

doi:10.6342/NTU201701710

observed. The adjusted hazard ratios were 0.79 (95% CI, 0.65 to 0.97), 0.73 (95% CI, 0.57 to 0.94), and 0.67 (95% CI, 0.52 to 0.86) for patients using 35 to 69, 70 to 139, and ≧140g per year, respectively, relative to less than 35 g.

Conclusion

Among patients with HBV-related cirrhosis, XCHT use was associated with a reduced risk of HCC. Further mechanistic and clinical research is necessary.

Key words: Xiao-chai-hu-tang; hepatocellular carcinoma; hepatitis B virus; cirrhosis

doi:10.6342/NTU201701710

Introduction

Hepatitis B virus (HBV) infects millions of people worldwide, especially in the Asia-Pacific region, including China, Taiwan, and several other countries in Southeast Asia, where chronic HBV infection is highly prevalent.[1,2] Chronic HBV infection leads to chronic hepatitis, cirrhosis, and hepatocellular carcinoma (HCC).[3] Previous studies have indicated that the HCC incidence rate is much higher among HBV-infected patients with cirrhosis than among HBV-infected patients without cirrhosis.[2,4] Although interferon and nucleoside analogues have been clinically proven to be effective in curing chronic hepatitis B,[5] there are still no effective therapeutic drugs for HCC. Some commonly prescribed medications seem promising as chemopreventive agents against HCC, including statins, angiotensin-converting enzyme inhibitors (ACEIs), nonsteroidal anti-inflammatory drugs (NSAIDs), metformin, aspirin, and xiao-chai-hu-tang (XCHT, Sho-saiko-to or TJ-9 in Japanese).[6-8]

XCHT is a well-known Chinese traditional medicine that has been used for thousands of years. It consists of seven medicinal herbs (*Bupleurum falcatum, Glycyrrhiza glabra, Panax ginseng, Pinellia ternate, Scutellaria baicalensis, Zizyphus jujuba, and Zingiber officinale*) and is currently prescribed to treat chronic hepatitis. A prospective, randomised, nonblind controlled study evaluated the long-term potential of XCHT to

doi:10.6342/NTU201701710

prevent HCC in patients with cirrhosis in Japan. The cumulative incidence of HCC was lower in the XCHT group than in the control group.[6] However, only 37 patients were detected to have the surface antigen of HBV, HBsAg, in this trial; therefore, the author could not further analyse the association between XCHT use and the risk of HCC in patients with HBV-related cirrhosis. Many in vitro and in vivo studies have indicated that XCHT may have protective effects against HCC and cirrhosis,[9-16] but clinical studies on HCC development in HBV-related cirrhosis are limited. The purpose of this study was to investigate the association between use of XCHT and the risk of HCC in patients with HBV-related cirrhosis.

Methods

Data Sources

We conducted a nationwide cohort study by using population-based data from the Taiwan National Health Insurance Research Database (NHIRD). Since the National Health Insurance is a compulsory programme for all residents in Taiwan, the NHIRD is a comprehensive healthcare database that covers almost all of the 23.7 million residents of Taiwan. We used databases for admissions and outpatient visits, both of which contained information on patient characteristics and medical records, including up to five discharge diagnoses or three outpatient visit diagnoses according to the International Classification of Diseases, Ninth Revision (ICD-9) classification, date of

doi:10.6342/NTU201701710

admission, date of discharge, dates of visits, sex, and date of birth for patients. The data files also contained information on patient prescriptions, including the names of prescribed drugs, dosages, durations, and total expenditures. Previous epidemiologic research has used these databases, and medical records of prescriptions, diagnoses, and hospitalisations are of high quality.[17-19]

Finished herbal products (FHPs) are the modern form of Chinese herbal decoctions , of which herbal formulae or single herb are concentrated into granulated compounds. FHPs are fully reimbursed under the current National Health Insurance system of Taiwan. The NHIRD is the only computerised reimbursement database in the world that stores longitudinal prescription data for both Western and Chinese medicines, including FHPs. Thus, the NHIRD provides an optimal platform for determining the effect of XCHT in reducing the risk of HCC in HBV-infected patients with cirrhosis. In addition, this study adhered to strict confidentiality guidelines, in accordance with regulations regarding personal electronic data protection, and was approved by the ethics review board of Chang Gung Memorial Hospital, Chia-Yi Branch.

Study Population and Outcomes

We conducted a population-based cohort study that considered all patients older than 18 years who received a first-time diagnosis of HBV infection (ICD-9 codes 070.2, 070.3, and V02.61) between 1 January 1997, and 31 December 2010. We excluded

doi:10.6342/NTU201701710

patients with hepatitis C virus infection (ICD-9 codes 070.7, 070.41, 070.44, 070.51, 070.54, and V02.62) and without cirrhosis (ICD-9 codes 571.2, 571.5, 571.6, 572.2, 572.3, 572.4, 572.8, and 573.0). Patients with missing data for sex, age, income, or level of urbanisation were excluded. Those who had received a diagnosis of HCC before or follow-up period of less than 6 months were also excluded. Patients with HCC (ICD-9 code 155.0) were identified in the Registry for Catastrophic Illness Patient Database (RCIPD), with the first-time diagnosis date as the index date. If a patient is to qualify for a cancer catastrophic illness certificate, cytological or pathological reports or evidence including additional laboratory and imaging studies supporting the diagnosis of cancer must be provided. A total of 89,466 patients were included in the final analyses (Figure 1).

XCHT Exposure and Potential Confounders

We identified patients who filled prescriptions for XCHT in the outpatient visit database between the date of cirrhosis diagnosis and the index date for HCC or the end of follow-up. We defined a patient who used more than 140 g in cumulative doses of XCHT as an XCHT user, and a patient who did not use more than 140 g of XCHT as an XCHT nonuser. We also collected information regarding exposure to other drugs that might reduce the risk of HCC, such as anti-HBV drugs (interferon, lamivudine, entecavir, adefovir dipivoxil, and telbivudine), ACEIs (captopril, enalapril, lisinopril,

doi:10.6342/NTU201701710

perindopril, ramipril, quinapril, benazepril, cilazapril, and fosinopril), statins, aspirin, NSAIDs, and metformin.[7,8,17] The defined daily dose (DDD) was used to calculate exposed dosage of any previous drug and cumulative DDD (cDDD), which indicates the total exposed dosage, was estimated as the sum of dispensed DDD.

The severity levels of cirrhosis were classified as compensated or decompensated to evaluate severity. We defined patients with cirrhosis catastrophic illness certificates as having decompensated cirrhosis. In Taiwan, application for a cirrhosis catastrophic illness certificate requires clinically evident complications, such as refractory ascites, oesophagus or stomach varices, hepatic encephalopathy, or liver insufficiency. Thus, patients with a diagnosis of cirrhosis and listed in the RCIPD were regarded as having decompensated cirrhosis. Furthermore, diabetes (ICD-9 code 250) was regarded as another crucial confounding factor in the statistical model.[20,21]

We also considered sociodemographic characteristics, namely age, sex, income, and level of urbanisation in the model. Urbanisation levels in Taiwan are divided into four strata according to publications of the Taiwan National Health Research Institutes; level 1 refers to the most urbanised communities and level 4 refers to the least urbanised communities.

Statistical Analysis

We used the Kaplan-Meier method to estimate HCC cumulative incidences. The

doi:10.6342/NTU201701710

log-rank test was performed to examine differences in the risk of HCC in the cohort. Cox proportional hazards regression without time-dependent covariates for drug exposure was used to estimate to evaluate whether XCHT use can reduce the risk of HCC among patients with hepatitis B virus-related cirrhosis. Finally, we used cox proportional hazards regression with time-dependent covariates for drug exposure to avoid time-varying prescription changes. We determined the cumulative dosage of XCHT up to the start of each year during the follow-up period. This calculation was performed to reduce within-person variation and to estimate long-term prescriptions properly. To examine the dose-response relationship, we categorised patients into four groups on the basis of yearly XCHT use: <35 g (reference group), 35 g to 70 g, 70 g to 139 g, and ≥140 g. The hazard ratio (HR) for HCC development was calculated according to different levels of XCHT use per year compared with the reference group. Accompanying 95% confidence intervals (CIs) were calculated after adjustment for variables, including age, sex, urbanisation, income, severity of cirrhosis, and diabetes. A two-tailed *P* value of .05 was considered significant. All of these analyses were conducted using SAS statistical software (version 9.4; SAS Institute, Cary, NC).

Sensitivity Analyses and Subgroup Analyses

Many medicines have shown positive results in chemoprevention, such as anti-HBV drugs, ACEIs, aspirin, NSAIDs, metformin, and statins.[7,8,17] To examine the

doi:10.6342/NTU201701710

consistency of the risk, we conducted sensitivity analyses by adding the probabilities corresponding to these medicines to a statistical model. Furthermore, we examined the outcomes stratified by sex, age, and severity of cirrhosis. These sensitivity analyses and subgroup analyses were applied to evaluate the consistency of the correlation between the use of XCHT and the risk of HCC.

Results

We included a total of 89,466 patients (23,628 women, 65,838 men) with diagnoses of cirrhosis during the study period (Figure 1). The basic demographic characteristics of the patient population are summarised in Table 1. Of the patients, 3.7% (3,282) used more than 140 g of XCHT. Patients who took less than 140 g of XCHT tended to be elderly, and the percentages of patients with hypertension, diabetes mellitus, chronic kidney disease, alcohol-related disease, and decompensated cirrhosis were higher among the XCHT nonusers than among the XCHT users. The XCHT users were more likely to accept anti-HBV drugs, NSAIDs, but not metformin. For the prescription of other drugs with chemopreventive effects, including ACEIs, aspirin, and statins, there were no statistically significant differences between the XCHT users and nonusers. There were 14,070 HCCs in the HBV-related cirrhosis cohort during the follow-up period of 549,399.1 person-years. The incidence rates of HCC were 2,608.4 and

doi:10.6342/NTU201701710

1,607.2 patient cases per 100,000 person years among the XCHT nonusers and XCHT users, respectively. Figure 2a illustrates the results of the Kaplan-Meier analysis for the cohort. The log-rank test revealed a significant observed difference over the entire Kaplan-Meier curve. In analyses stratified by age, sex, and severity of cirrhosis, we consistently found that the incidence of HCC remained lower for the XCHT users (Figure 2b-g).

Figure 3 demonstrate that XCHT user had less risk for HCC (HR: 0.67 95%CI:0.61-0.74). The effect of XCHT keeps consistent in subgroup analysis and sensitivity analyses. Table 2 shows the dose–response relationship between XCHT use and HCC risk in patients with HBV-related cirrhosis when patients were divided into four groups according to the XCHT cumulative dosage per year. The adjusted HRs were 0.79 (95% CI, 0.65 to 0.97), 0.73 (95% CI, 0.57 to 0.94), and 0.67 (95% CI, 0.52 to 0.86) for patients with XCHT cumulative dosages per year of 35 g to 70 g, 70 g to 140 g, and higher than 140 g, relative to patients who took dosages lower than 35 g. Table 2 also shows that the effect of XCHT was consistent in sensitivity analyses that included other potential confounders. When the data were stratified according to age, sex, and severity of cirrhosis, the effects of XCHT did not remain significant in every subgroup analysis. However, the effect of XCHT remained significant for a cumulative dosage higher than 140 g per year for all subgroups, except for the

doi:10.6342/NTU201701710

subgroup of patients younger than 50 years.

Discussion

According to our review of the literature, the present study is the first to document a dose–response relationship between the use of XCHT in patients with HBV-related cirrhosis and the risk of HCC, after controlling for confounders including age, sex, income, urbanisation, diabetes, and other medications that might reduce the risk of HCC. This study has several strengths. First, the study population was mainly obtained from a computerised database that is population based and highly representative. Because the cirrhosis patients were selected from all HBV-infected patients by using admission and outpatient visit records, we can rule out the possibility of selection bias. Second, because the data on XCHT, anti-HBV treatment, and other chemopreventive medicine use were obtained from a historical database that collects all available prescription information between the diagnosis dates of cirrhosis and HCC, we can rule out the possibility of recall bias. Third, we used a Cox proportional hazards regression with time-dependent covariates to estimate the relationship between XCHT use and HCC risk and to avoid the effect of time-varying prescription changes. We also used a Cox proportional hazards regression without time-dependent covariates to confirm that the results were consistent. Last, we

doi:10.6342/NTU201701710

conducted sensitivity analyses and subgroup analyses to clarify the effects of potential confounders. Anti-HBV drugs and other chemopreventive medications did not alter the effect of XCHT in sensitivity analyses; XCHT remained significant at a cumulative dosage of more than 140 g per year for each subgroup analysis, except for the patients younger than 50 years.

Unlike hepatitis C, development of HCC in HBV-infected patients does not require preceding cirrhosis; HCC can induce by carcinogenic effect of HBV as well. The pathogenesis of HCC differs between HBV patients with and those without cirrhosis;[22] thus, we excluded patients who had received diagnoses of HCC before or within 6 months of receiving diagnoses of cirrhosis.

Shimizu and colleagues reported that XCHT suppresses the induction of hepatic fibrosis through its antioxidant activity,[12] suggesting that XCHT may have beneficial effects on cirrhosis and HCC development in patients with chronic liver disease. The main mechanisms by which XCHT protects users against HCC and cirrhosis include its immunomodulatory effect, antifibrotic effect, and antineoplastic activity. XCHT can downregulate the mRNA of TGF-α, TGF-β1, and platelet-derived growth factor.[15] In addition, XCHT can suppress the proliferation of IL-1 and stimulate the production of TNF-α to inhibit Ito cell proliferation and collagen formation. XCHT can significantly reduce the formation of 8-hydroxy-2-deoxyguanosine (8-OHdG),[14]

doi:10.6342/NTU201701710

which is a known genetic risk factor for hepatocarcinogenesis. XCHT also can inhibit proliferation of cancer cell lines or hepatic stellate cells by causing cells to arrest at the G0/G1 phase, and inducing apoptosis. [10]

The potential limitations of this study should be noted. First, several unmeasured confounders, including body mass index, smoking status, alcohol intake, and over-the-counter drug use, which are associated with HCC, are not included in our database. Second, this study did not include FHPs purchased directly from traditional Chinese medicine pharmacies, and there is no way to verify the exact dosages that the study participants actually ingested. We presumed that all medications were taken by the patients as prescribed and thus may have overestimated the actual ingested dosages because some degree of noncompliance is always expected. Finally, because the data on drug prescriptions were not complete in 1996, we examined only medication use after 1997; the use of these drugs before 1997 could not be included in our analysis.

In conclusion, among HBV-infected cirrhosis patients in Taiwan, this study showed that the risk of HCC was decreased in patients who received XCHT compared with those who did not receive XCHT. Further mechanistic and clinical research is required.

doi:10.6342/NTU201701710

Acknowledgments

The author's would like to thank Center of Excellence for Chang Gung Research Datalink (CORPG6D0161) for the comments and assistance in data analysis. This study was supported by a grant from Chang Gung Memorial Hospital, Chia-yi Branch, and based on the National Health Insurance Research Database provided by the Central Bureau of National Health Insurance, the Department of Health, and managed by the National Health Research Institutes. The interpretation and conclusions contained herein do not represent those of Bureau of National Health Insurance, Department of Health, or National Health Research Institutes.

doi:10.6342/NTU201701710

References:

1. Chen CJ, Wang LY, Yu MW: Epidemiology of hepatitis B virus infection in the Asia-Pacific region. J Gastroenterol Hepatol 15 Suppl:E3-6, 2000

2. El-Serag HB: Epidemiology of viral hepatitis and hepatocellular carcinoma. Gastroenterology 142:1264-1273 e1, 2012

3. Pan CQ, Zhang JX: Natural History and Clinical Consequences of Hepatitis B Virus Infection. Int J Med Sci 2:36-40, 2005

4. Fattovich G, Stroffolini T, Zagni I, et al: Hepatocellular carcinoma in cirrhosis: Incidence and risk factors. Gastroenterology 127:S35-S50, 2004

5. Torresi J, Locarnini S: Antiviral chemotherapy for the treatment of hepatitis B virus infections. Gastroenterology 118:S83-103, 2000

6. Oka H, Yamamoto S, Kuroki T, et al: Prospective study of chemoprevention of hepatocellular carcinoma with Sho-saiko-to (TJ-9). Cancer 76:743-9, 1995

7. Okuno M, Kojima S, Moriwaki H: Chemoprevention of hepatocellular carcinoma: concept, progress and perspectives. J Gastroenterol Hepatol 16:1329-35, 2001

8. Singh S, Singh PP, Roberts LR, et al: Chemopreventive strategies in hepatocellular carcinoma. Nat Rev Gastroenterol Hepatol 11:45-54, 2014

9. Zheng N, Dai J, Cao H, et al: Current understanding on

doi:10.6342/NTU201701710

antihepatocarcinoma effects of xiao chai hu tang and its constituents. Evid Based Complement Alternat Med 2013:529458, 2013

10. Yano H, Mizoguchi A, Fukuda K, et al: The herbal medicine sho-saiko-to inhibits proliferation of cancer cell lines by inducing apoptosis and arrest at the G0/G1 phase. Cancer Res 54:448-54, 1994

11. Kayano K, Sakaida I, Uchida K, et al: Inhibitory effects of the herbal medicine Sho-saiko-to (TJ-9) on cell proliferation and procollagen gene expressions in cultured rat hepatic stellate cells. J Hepatol 29:642-9, 1998

12. Shimizu I, Ma YR, Mizobuchi Y, et al: Effects of Sho-saiko-to, a Japanese herbal medicine, on hepatic fibrosis in rats. Hepatology 29:149-60, 1999

13. Watanabe S, Kitade Y, Masaki T, et al: Effects of lycopene and Sho-saiko-to on hepatocarcinogenesis in a rat model of spontaneous liver cancer. Nutr Cancer 39:96-101, 2001

14. Shiota G, Maeta Y, Mukoyama T, et al: Effects of Sho-Saiko-to on hepatocarcinogenesis and 8-hydroxy-2'-deoxyguanosine formation. Hepatology 35:1125-33, 2002

15. Chen MH, Chen JC, Tsai CC, et al: The role of TGF-beta 1 and cytokines in the modulation of liver fibrosis by Sho-saiko-to in rat's bile duct ligated model. J Ethnopharmacol 97:7-13, 2005

doi:10.6342/NTU201701710

16. Ohtake N, Yamamoto M, Takeda S, et al: The herbal medicine Sho-saiko-to selectively inhibits CD8+ T-cell proliferation. Eur J Pharmacol 507:301-10, 2005

17. Tsan YT, Lee CH, Wang JD, et al: Statins and the risk of hepatocellular carcinoma in patients with hepatitis B virus infection. J Clin Oncol 30:623-30, 2012

18. Tsan YT, Lee CH, Ho WC, et al: Statins and the risk of hepatocellular carcinoma in patients with hepatitis C virus infection. J Clin Oncol 31:1514-21, 2013

19. Yang YH, Chen WC, Tsan YT, et al: Statin use and the risk of cirrhosis development in patients with hepatitis C virus infection. J Hepatol 63:1111-7, 2015

20. Wang C, Wang X, Gong G, et al: Increased risk of hepatocellular carcinoma in patients with diabetes mellitus: a systematic review and meta-analysis of cohort studies. Int J Cancer 130:1639-48, 2012

21. Lai SW, Chen PC, Liao KF, et al: Risk of hepatocellular carcinoma in diabetic patients and risk reduction associated with anti-diabetic therapy: a population-based cohort study. Am J Gastroenterol 107:46-52, 2012

22. Brechot C, Kremsdorf D, Soussan P, et al: Hepatitis B virus (HBV)-related hepatocellular carcinoma (HCC): molecular mechanisms and novel paradigms. Pathol Biol (Paris) 58:278-87, 2010

doi:10.6342/NTU201701710

Table1 Patient demographics and clinical characteristics of study cohort.

	XCHT use		
	Non-user	User	P value
Total follow-up person-year	523,391.6	26,007.5	
No. of HCC	13,652 (15.8%)	418 (12.7%)	<.0001
Incidence	2,608.4	1,607.2	
95% CI	2,565.0-2,652.5	1460.3-1768.9	
Gender			
Male	63,401(73.6%)	2,437(74.2%)	0.3797
Female	22,783(26.4%)	845(25.8%)	
Age			
18-29	3,529 (4.1%)	109 (3.3%)	<.0001
30-39	13,584 (15.8%)	594 (18.1%)	
40-49	23,506 (27.3%)	1,031 (31.4%)	
50-59	21,080 (24.5%)	899 (27.4%)	
>=60	24,485 (28.4%)	649 (19.8%)	
Comorbidity			
decompensated liver cirrhosis	12,062 (14.0%)	403 (12.3%)	0.0053
Diabetes	25,296 (29.4%)	802 (24.4%)	<.0001
Hypertension	39,166 (45.4%)	1,354 (41.3%)	<.0001
alcohol-related disease*	25,094 (29.1%)	806 (24.6%)	<.0001
Chronic kidney disease	8,771 (10.2%)	296 (9.0%)	0.0309
Hyperlipidemia	36,785 (42.7%)	1,627 (49.6%)	<.0001
Biliary stones	20,353 (23.6%)	890 (27.1%)	<.0001
Interferon and nucleoside analogues			
No	75,178 (87.2%)	2,822 (86.0%)	0.0362
Yes	11,006 (12.8%)	460 (14.0%)	
Statin, cDDDs			
0-27 cDDD	77,580 (90.0%)	2,985 (91.0%)	0.0794
≧28 cDDD	8,604 (10.0%)	297 (9.0%)	
Aspirin, cDDDs			
0-27 cDDD	72,227 (83.8%)	2,756 (84.0%)	0.7981
≧28 cDDD	13,957 (16.2%)	526 (16.0%)	
NSAID, cDDDs			
0-27 cDDD	34,222 (39.7%)	928 (28.3%)	<.0001
≧28 cDDD	51,962 (60.3%)	2,354 (71.7%)	

doi:10.6342/NTU201701710

ACEI, cDDDs			
0-27 cDDD	72,721 (84.4%)	2,798 (85.2%)	0.1754
≧28 cDDD	13,463 (15.6%)	484 (14.8%)	
Metformin, cDDDs			
0-27 cDDD	73,686 (85.5%)	2,921 (89.0%)	<.0001
≧28 cDDD	12,498 (14.5%)	361 (11.0%)	

* alcohol-related disease: ICD-9 codes 291, 303.0, 303.9, 305.0, 571.0, 571.1, 571.2, or 571.3.

doi:10.6342/NTU201701710

Table2 Adjusted hazard ratios (HRs) of Xiao-chai-hu-tang use in reduction of HCC risk in HBV-related cirrhosis cohort by cox proportional hazards models with time-dependent covariates for drug exposure.

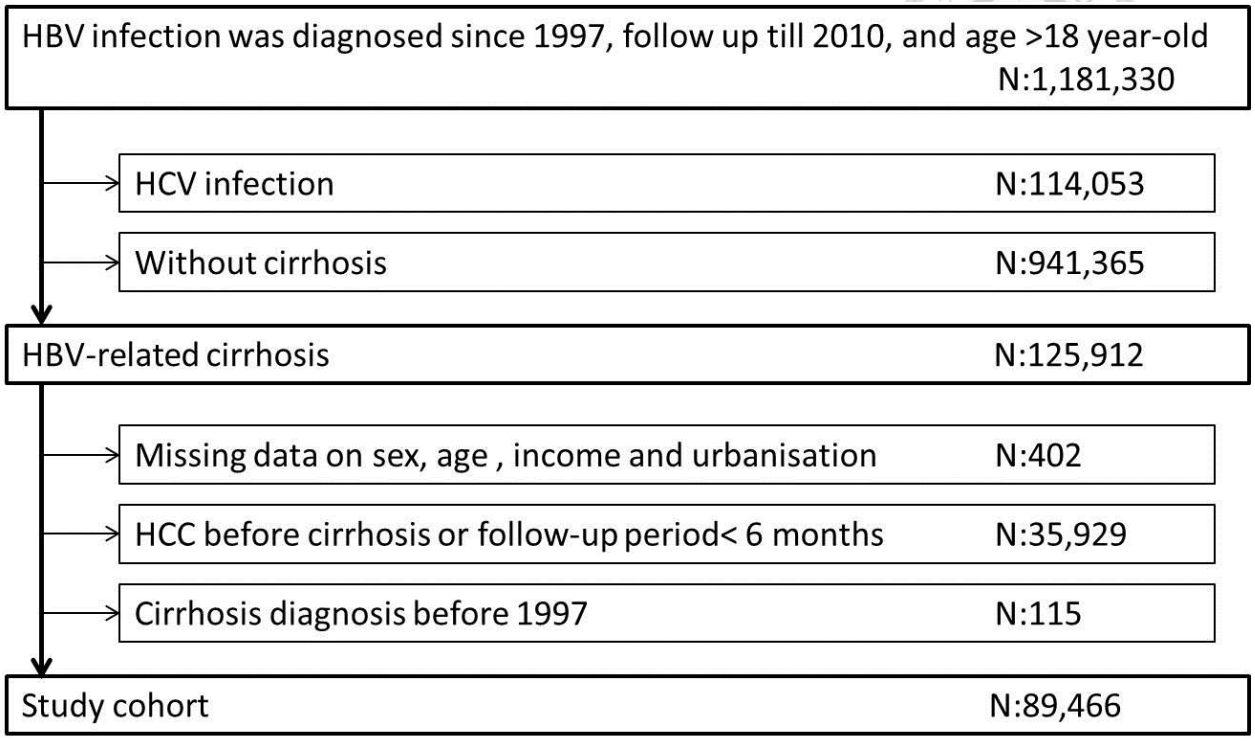
	Xiao-chai-hu-tang use per year					
	35 to 69g		70 to 139g		≥140g	
	HR	**95% CI**	**HR**	**95% CI**	**HR**	**95% CI**
Main model*	0.79	0.65-0.97	0.73	0.57-0.94	0.67	0.52-0.86
Additional covariates†						
Main model+statin	0.79	0.64-0.96	0.73	0.56-0.93	0.66	0.52-0.85
Main model+acei	0.79	0.65-0.97	0.73	0.57-0.94	0.66	0.52-0.85
Main model+aspirin	0.80	0.65-0.97	0.73	0.57-0.94	0.66	0.52-0.85
Main model+nsaid	0.81	0.66-0.99	0.73	0.57-0.94	0.66	0.51-0.85
Main model+metformin	0.81	0.66-0.99	0.73	0.57-0.94	0.66	0.51-0.85
Main model+anti-HBV treatment	0.80	0.65-0.97	0.73	0.57-0.94	0.68	0.53-0.87
Subgroup effects						
Gender						
Male	0.85	0.68-1.06	0.80	0.61-1.05	0.71	0.54-0.92
Female	0.61	0.37-0.99	0.46	0.23-0.93	0.43	0.19-0.95
Age, years						
≧50	0.84	0.67-1.06	0.69	0.51-0.94	0.63	0.46-0.85
<50	0.58	0.38-0.88	0.76	0.48-1.19	0.73	0.47-1.12
Severity of LC						
Decompensated LC	0.77	0.49-1.21	0.64	0.37-1.13	0.46	0.25-0.85
Compensated LC	0.77	0.61-0.96	0.74	0.56-0.98	0.74	0.56-0.97

*Main model is adjusted for age, sex, urbanization, income, diabetes, and severity of liver cirrhosis.

†The models were adjusted for covariates in the main model as well as each additional listed covariate.

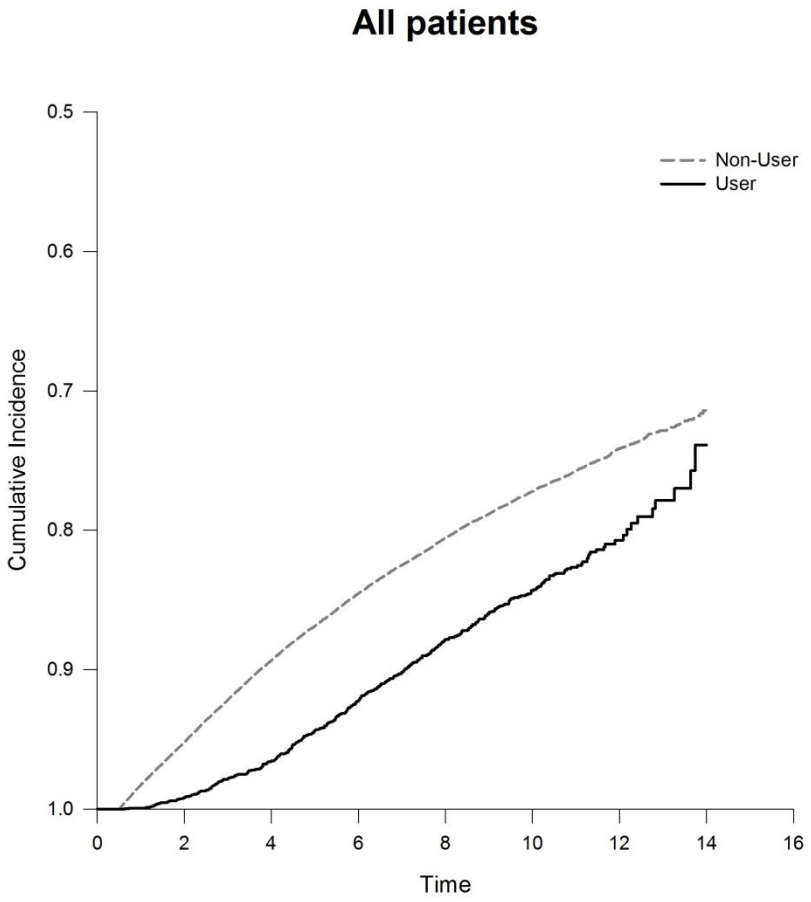
doi:10.6342/NTU201701710

Figure1 Flowchart of the patient enrollment process of study cohort.

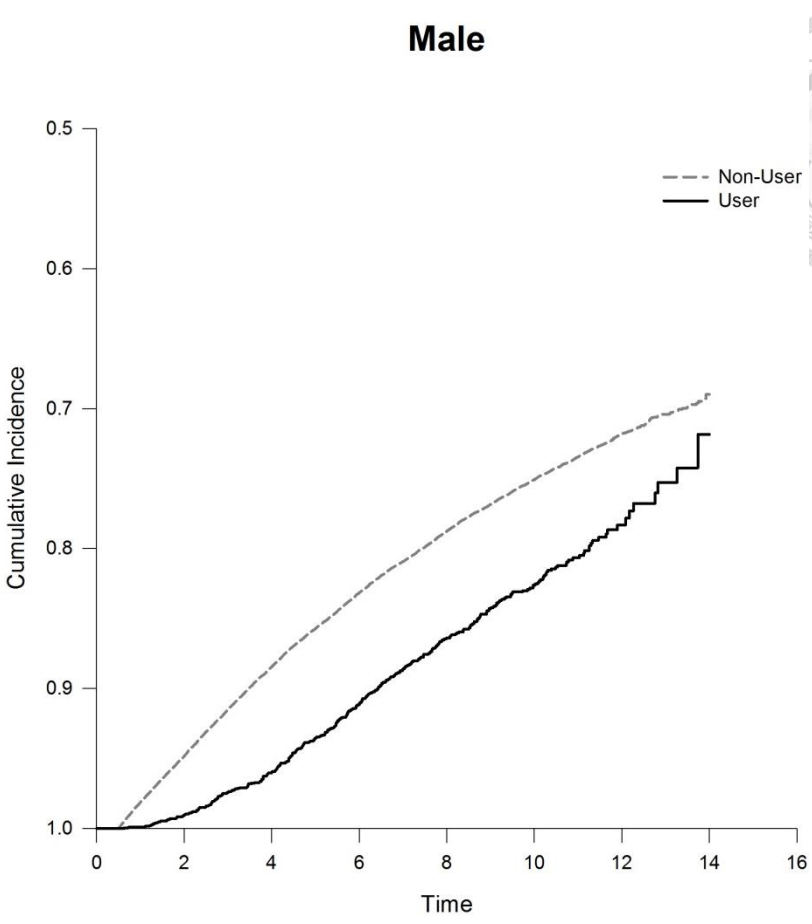


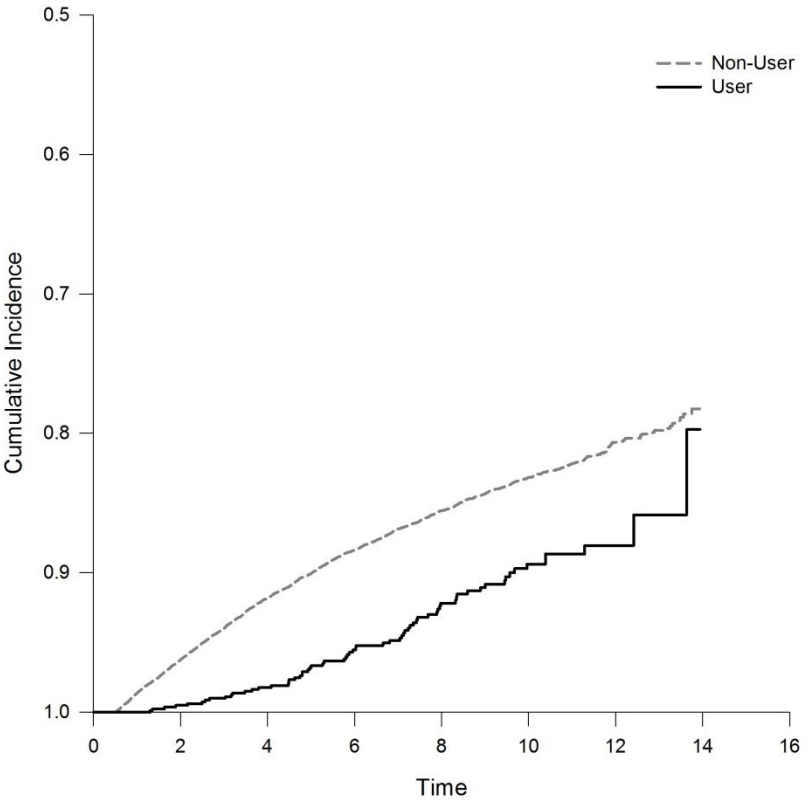
doi:10.6342/NTU201701710

Figure 2 Cumulative incidence of hepatocellular carcinoma development by Xiao-chai-hu-tang use during the follow-up period of all patients (a) and subgroup analysis of sex (b,c), age (d,e), and severity of cirrhosis (f,g).

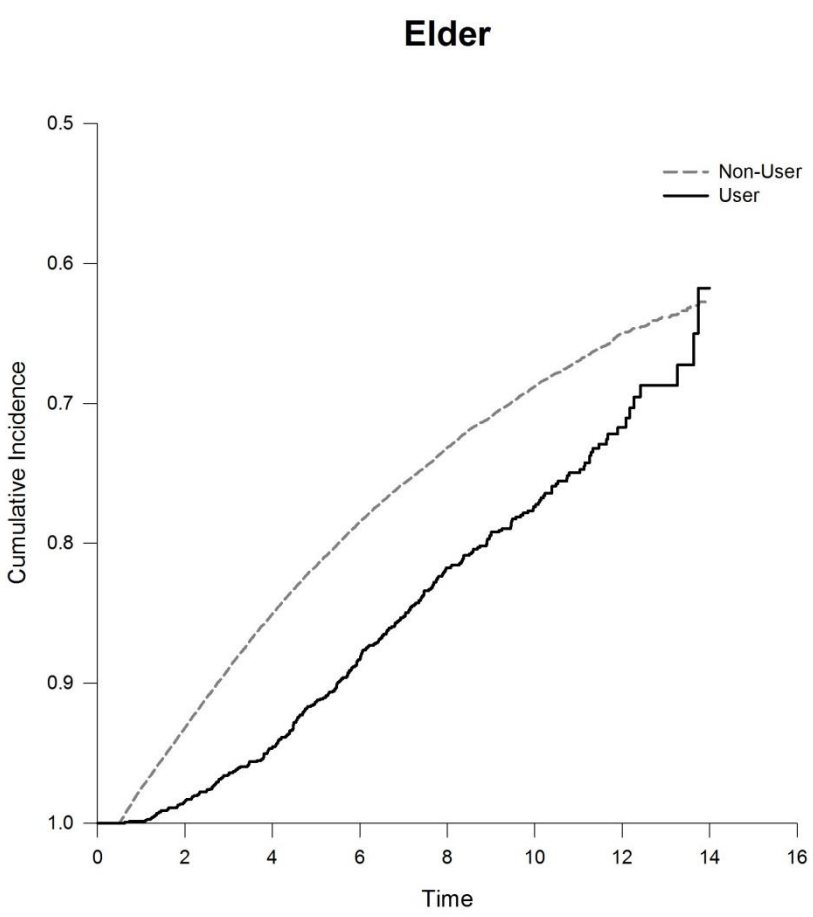


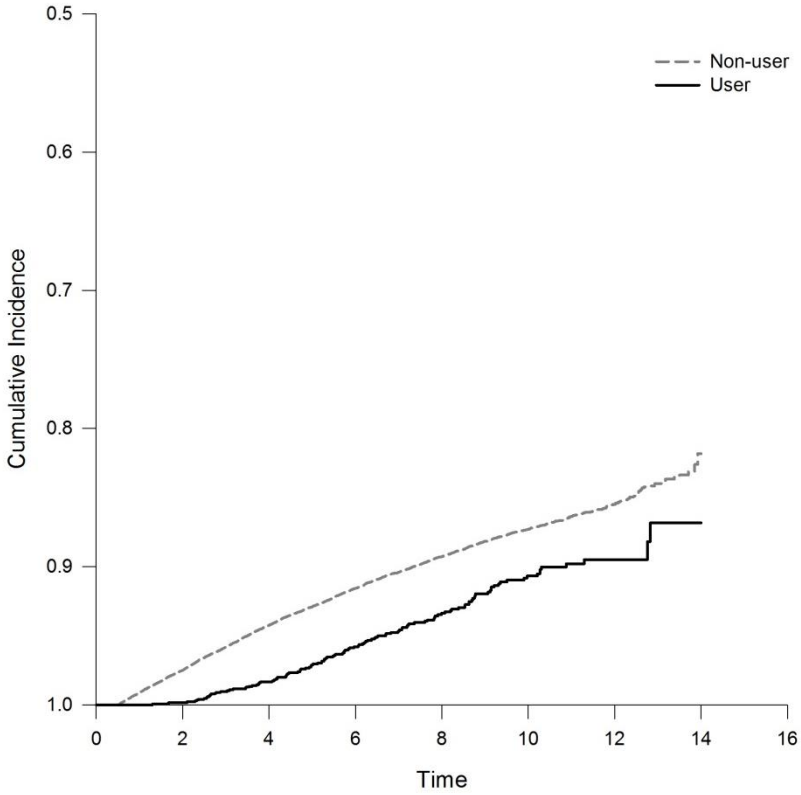
doi:10.6342/NTU201701710



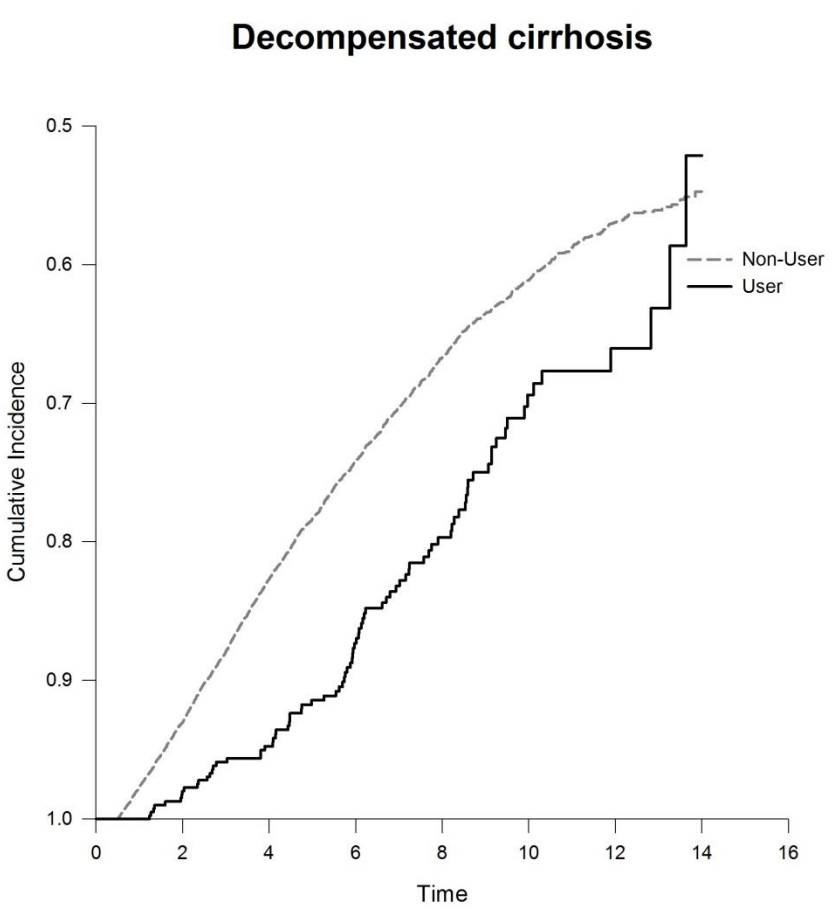


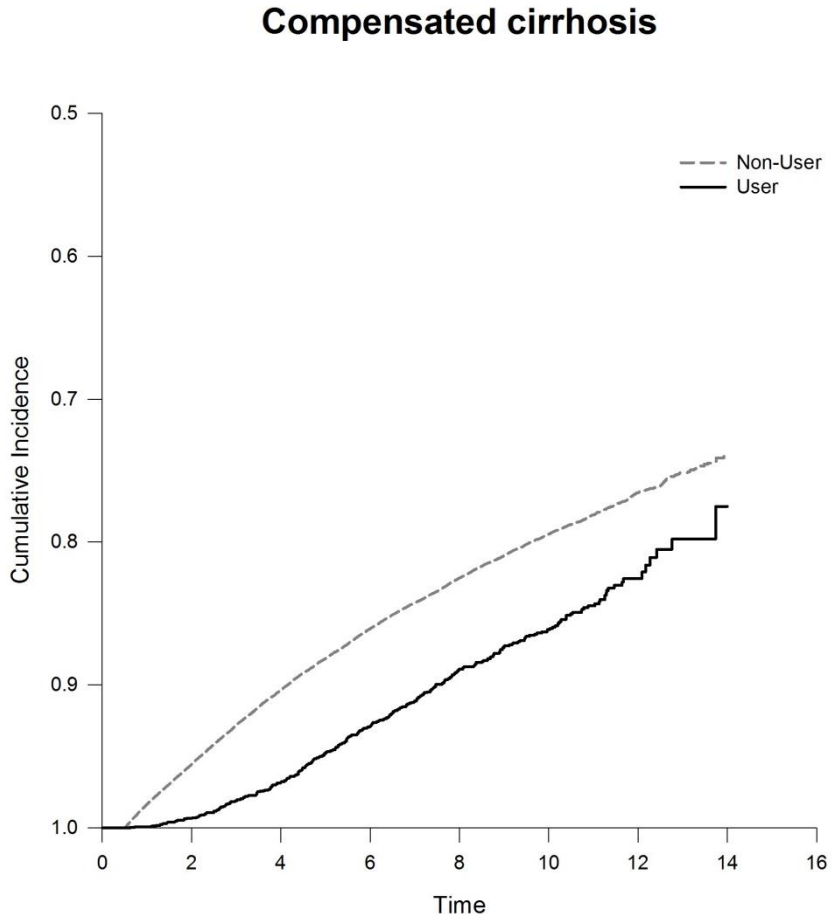
doi:10.6342/NTU201701710





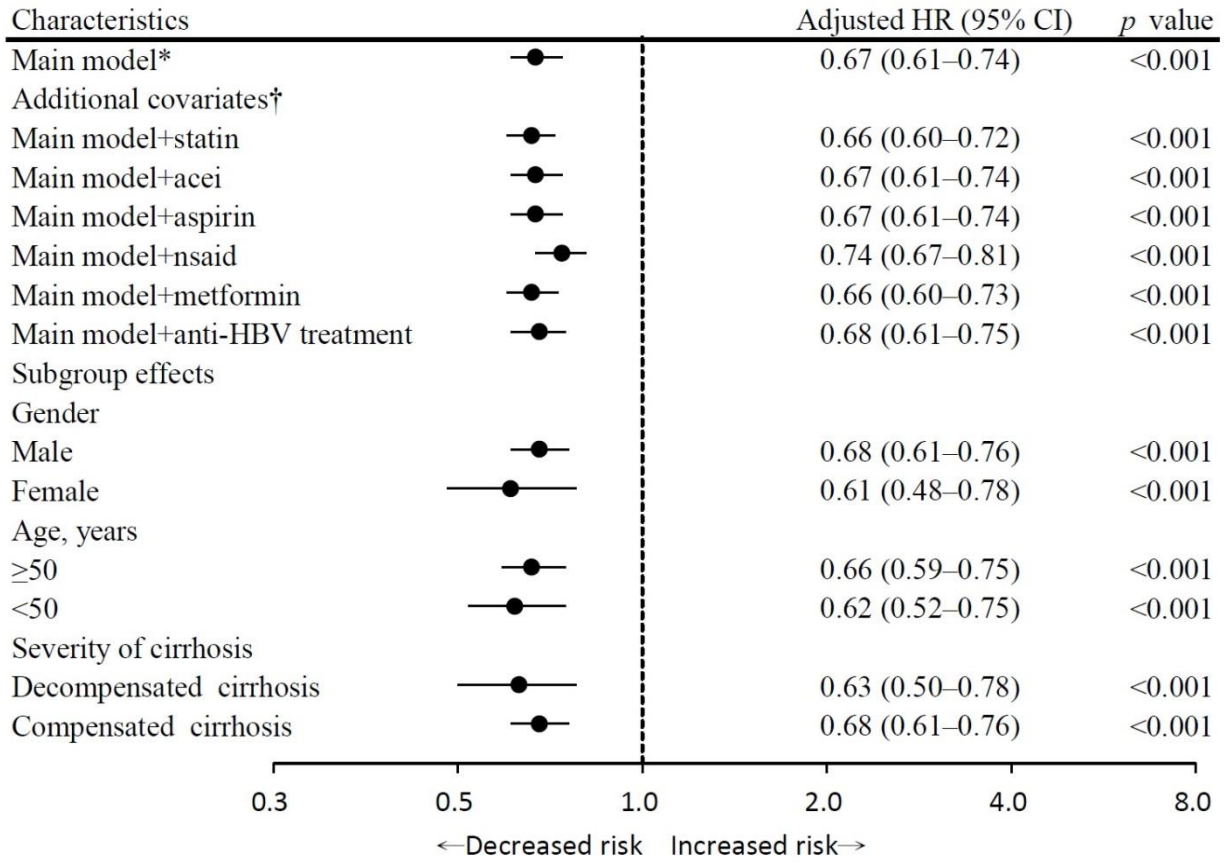
doi:10.6342/NTU201701710





doi:10.6342/NTU201701710

Figure 3 Sensitivity analyses and subgroup analyses for Xiao-chai-hu-tang use and the risk of hepatocellular carcinoma by cox proportional hazards models without time-dependent covariates for drug exposure.



*Main model is adjusted for age, sex, urbanization, income, diabetes, and severity of liver cirrhosis.

†The models were adjusted for covariates in the main model as well as each additional listed covariate.

doi:10.6342/NTU201701710