Clinical and Virological Characteristics of Hepatitis B or C Virus Co-Infection With HIV in Indonesian Patients

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学位論文の内容要旨

Clinical and Virological Characteristics of Hepatitis B or C Virus Co-Infection With HIV in Indonesian Patients

インドネシア国 HIV 患者における B 型および C 型肝炎ウイルス重複感染の臨床的およびウイルス学的特徴

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INTRODUCTION

Human immunodeficiency virus (HIV), hepatitis B virus (HBV), and hepatitis C virus (HCV) are ranked in the top 10 of all infectious diseases in terms of mortality rates. Worldwide, approximately 370 million people are thought to have chronic HBV infection while HCV and HIV are thought to affect 130 and 40 million people, respectively. Because of their shared routes of transmission, superinfection with HBV or HCV among patients infected with HIV is not uncommon. Although transmission of HBV and HCV is similar, there are some differences; HCV is transmitted more effectively by percutaneous routes; whereas HBV is transmitted principally sexually and perinatally. Since the introduction of highly active antiretroviral therapy (HAART) for HIV-, HBV-, and HCV-related end-stage liver disease has become the second leading cause of death after AIDS-related mortality in many countries, a trend that is more apparent in developed countries. Co-infection with HBV or HCV worsens substantially the prognosis of HIV, and vice versa, and interacts with antiretroviral therapy (ART). Some studies have reported that HIV accelerates the progression of fibrosis in patients co-infected with HCV, and that ART-related hepatotoxicity is increased in these patients. Indonesia is one of several countries in Asia experiencing a massive growth in the prevalence of HIV infection. HIV is mainly transmitted among injecting drug users, and then spreads to sex workers, the sex workers' clients, the clients' female partners, and ultimately their children via vertical transmission. This study was conducted to determine the prevalence of HBV and HCV co-infection in patients infected with HIV in Yogyakarta Province, Indonesia. The study also investigated the associations of co-infection with demographic characteristics, risk factors, $CD4^+$ T cell count, liver disease, and biochemical markers of liver fibrosis.

MATERIALS AND METHODS

Serum samples were obtained from 126 patients with HIV at Dr. Sardjito Hospital, Yogyakarta, Indonesia, between April and July 2010. The patients were aged 21–60 years (median, 31 years), 78 (61.9%) were males, 35 (27.8%) were females, and 13 (10.3%) were transvestites. ALT, AST, platelet counts, APRI, HBsAg, anti-HBc, anti-HCV antibody, molecular HBV, and molecular HCV was determined. Patient interviews and medical record reviews were also conducted for some patients to confirm the mode of HIV transmission, demographic characteristics, $CD4^+$ T cell (CD4) count, and clinical state, including therapeutic status. The study was reviewed and approved by the Ethics Committees of Kobe University, Japan, and by Gadjah Mada University, Indonesia.

Reversed passive hemaglutination methods were used to determine HBsAg and anti-HBc. HBV-DNA was detected by quantitative and nested qualitative PCR assays. The HBV viral load was measured by realtime PCR using an ABI Prism 7300 Real-Time PCR. Briefly, HBV-DNA was extracted from 200 ml of serum using a DNA extractor kit.

A passive serological assay kit was used to detect anti-HCV antibodies. In addition, HCV-RNA was extracted using QIAamp UltraSense Virus Kit or QIAamp Viral Mini Kit and reverse-transcribed into cDNA using specific primers and SuperscriptTM III One-Step RT-PCR System with Platinum Taq DNA Polymerase.

HBV genotyping was determined using the phylogenetic tree of the S region, while HCV genotyping was based on NS5B. The analyses were conducted using Molecular Evolutionary Genetics Analysis (MEGA) software. Statistical analyses were performed using SPSS software version 17.0.

RESULTS

Of 126 patients infected with HIV, 6 (4.8%), 43 (34.1%), 4 (3.2%), and 73 (57.9%) patients had HIV/HCV/HBV triple infection, HIV/HCV co-infection, HIV/HBV co-infection, and HIV mono-infection, respectively. Of 49 (38.9%) patients positive for anti-HCV antibody, serum samples from 48 patients were also tested for HCV RNA; of these, 44 (92%) were positive for HCV-RNA. Among 10 HBsAg-positive patients, 2 (20%) HAART-naive patients were HBV DNA positive, with viral loads of 3.4 and 4.6log copies/ml (Fig. 1b). The other eight patients were HBV-DNA-negative, of which seven (87.5%) were receiving HAART consisting of lamivudine or tenofovir. None of those patients were undergoing HCV therapy. All of the patients were tested for anti-HBc. Overall, 38 (30.2%) of the patients were positive for anti-HBc, including 5 (83.3%) with triple infection, 13 (30.2%) with HIV/HCV, 4 (100%) with HIV/HBV, and 16 (21.9%) with HIV mono-infection. Among 29 (23%) patients with the HBsAg-negative and anti-HBc positive, two had a single Q129R mutation within the "a" determinant region.

Phylogenetic analysis showed that two cases with HBsAg and HBV-DNA-positive were classified into genotype B3. Various genotypes of HCV were found, including genotypes 1a, 1b, 1c, 3a, 3k, 4a, and 6n in 23 (52%), 1 (2%), 4 (9%), 5 (11%), 7 (16%), 3 (6%), and 1 (2%) patients, respectively. There were no significant differences in age, $CD4^+$ count, ALT, or APRI among HCV genotypes, although $CD4^+$ count was lower and APRI was higher in patients with genotype 3 than in those with genotype 1. All of the sequence data were registered to GenBank under the accession numbers AB661784–AB661827 for HCV and the accession numbers AB661463 and AB661464 for HBV.

The APRI was significantly different between the HIV mono-infection group and the HIV/HCV coinfection group ($P = 0.04$). By contrast, ALT was not significantly between two groups even though it tended to be higher in the co-infection groups than in the HIV mono-infection group.

There were no significant differences in age, race, marital state, HAART, CD4 count, HBsAg, or anti-HBc between patients with HIV without or with HCV co-infection. In univariate analyses, male sex, higher education level, injection drug use, sexual contact, ALT $\geq$ 40 IU/L, and APRI > 0.5 were significantly associated with HCV co-infection. However, in multivariate analysis, only injection drug use [odds ratio (OR): 26.52; 95% confidence interval (CI): 3.52–199.54] and ALT $\geq$ 40 IU/L (OR: 6.36; 95% CI: 1.23–32.89) were associated independently with HCV co-infection.

DISCUSSION

In this study, 10 patients (8%) were co-infected with HBV and HIV, and HBV-DNA was detected in just 2 HAART-naive patients. The other eight patients without HBV-DNA were mostly treated by HAART containing lamivudine or tenofovir, which suggests that HB viremia was controlled by lamivudine or tenofovir. However, HBV monotherapy in patients infected with HIV in Indonesia is concerning because it could facilitate the appearance of drug-resistant and vaccine escape mutants.

Anti-HBc-positive/HBsAg-negative status may appear in several conditions, including past HBV infection and chronic infection of HBV with HB escape mutants. In the present study, the Q129R single mutation within the "a" determinant region was detected in two anti-HBcpositive/HBsAg-negative patients. It was previously reported that this mutation is frequently encountered in patients with genotype B HBV.

Several HCV genotypes, including 1a, 1b, 1c, 3a, 3k, 4a, and 6n, were found in this study, and reflect various transmission networks. HCV genotypes are not only associated with marked geographic distribution, but also the clinical efficacy of interferon and ribavirin therapy. Genotypes 1 and 4 tend to show a lower sustained virological response rate (SVR) compared with genotypes 2 and 3. In the present study, patients with genotype 3 had lower $CD4^+$ counts and higher APRI compared with patients with genotype 1, although these differences were not statistically significant. Further studies in a larger number of patients may provide further insight into the present findings.

In univariate analyses, male sex, higher educational level, and APRI were associated with HCV coinfection. In fact, an estimated 80% of drug users in Indonesia inject their drugs, particularly those in urban areas and males aged 18–24 years. In Cambodia, this was also apparent in school-age children. In the present study, HCV co-infection was independently

associated with injection drug use and elevated ALT levels, similar to that reported in several other studies. The putative sexual transmission in relation to HCV co-infection was investigated in a previous study, but, for this sexual or permucosal transmission of HCV to emerge, there must be mucosal disruption as a result of traumatic sexual practices, with exposure to infected body fluids.

CONCLUSION

HIV patients with a history of or current injection drug use should be treated timely and their HCV status should be examined. Illicit drug trafficking between countries and the increase in injection drug use seem to be the main causes of this epidemic, and should be eradicated to reduce the burden caused by these diseases. The possibility of vaccine-related mutants associated with the use of HBV-related drugs, such as lamivudine and tenofovir, should also be investigated in future studies.

神戸大学大学院医学(系)研究科（博士課程）

論文審査の結果の要旨			
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（要旨は１，０００字～２，０００字程度）

背景： ヒト免疫不全症候群ウイルス(HIV), B 型肝炎ウイルス(HBV), C 型肝炎ウイルス(HCV)は、いずれも高い致死率を示す感染症である。HIV 感染患者においては、共通する感染経路から、肝炎ウイルスとの重複感染が高頻度に認められる。HAART 治療の導入により、HIV 感染患者においては AIDS 関連死が減少する一方で肝疾患関連死が増加しつつあり、HIV 感染患者における肝炎ウイルスの把握は重要である。

対象と方法： インドネシア国ジョグジャカルタ市の HIV 感染患者 126 人を対象とし、HIV については感染経路や感染様式を確認した。B 型 C 型肝炎ウイルス感染については血清学的に診断した。血清学的に肝炎ウイルス陽性であった患者からは HBV-DNA, HCV-RNA についても検出を行った。HBV 遺伝子型は S 領域、HCV 遺伝子型は NS5B 領域について塩基配列を決定し、系統樹解析法により決定した。

結果： 126 名の HIV 感染患者の内訳は、HCV/HBV との重複感染 6 例(4.8%)、HCV との重複感染 43 例(34%)、HBV との重複感染 4 例(3.2%)、HIV 単独感染例 73 例(58%)であった。HCV 抗体陽性者 48 例のうち、44 例(92%)は HCV-RNA 陽性であった。

HBs 抗原陽性は 10 例認められ、HBV-DNA 陽性は 2 例(20%)であった。この 2 例はいずれも遺伝子型 B3 であった。HBc 抗体陽性は 38 例(30%)であり、その内訳は HCV/HBV 重複感染 5 例、HCV 重複感染 13 例、HBV 重複感染 4 例、HIV 単独感染 16 例であった。HBs 抗原陰性かつ HBc 抗体陽性の 29 例のうち、2 例に抗原決定基である α 領域内に Q129R 変異が認められた。HCV 遺伝子型については、1a 23 例(52%)、1b 1 例(2%)、1c 4 例(9%)、3a 5 例 (11%)、3k 7 例(16%)、4a 3 例(6%)、6n 1 例(2%)であった。

HIV 単独感染例と HIV/HCV 重複感染例を比較すると、APRI 値が HCV 重複感染例で有意に高かった。年齢、人種、結婚歴、HAART 治療歴、CD4 陽性細胞数、HBs 抗原・HBc 抗体陽性率に有意差は見られなかった。単変量解析では、男性、高学齢、注射薬物使用者、性交渉があること、ALT 値≥40 IU/L、APRI 値>0.5 は、HCV 重複感染例と有意な相関が認められた。多変量解析では注射薬物使用者であることのみ有意な相関があった。

考察： 本研究では、10 名(8%)が HBV/HIV 重複感染であった。HBV-DNA は HAART 治療を行っていない 2 名に検出されたが、他の 8 名の患者のほとんどは、抗ウイルス剤であるラミブジンやテノフォビルを含む HAART 治療を受けていた。このことは、HBV のウイルス血症がラミブジンやテノフォビルで制御されていたことを意味していた。また、2 名の HBs 抗原陰性かつ HBc 抗体陽性患者において Q129R 変異が認められた。この変異は、遺伝子型 B の HBV でしばしば認められる変異であると以前に報告されている。HIV/HCV 重複感染例においては、HCV の遺伝子型は 1a、1b、1c、3a、3k、4a、6n が認められ、多様な感染経路があることが推測された。

インドネシアでは、約 80%の注射薬物使用者が薬物を自己注射しており、特に都市部の 18 歳から 24 歳の若年男性に高頻度であるとされている。HIV 単独感染例と HIV/HCV 重複感染例の比較結果から考察すると、不法薬の国家間での密売や薬物注射の増加が主な原因であると推察される。HIV/HCV の共通する感染経路である薬物注射を早期に検挙し、蔓延を未然に防ぐことが、HIV/HCV 感染阻止に大きな役割を持つものと考えられた。

結　論：　インドネシア国ジョクジャカルタ市においても HIV 患者の肝炎重複感染は比較的高頻度に認められた。HARRT 治療により HBV については良好に制御されていたが、潜在的な HBs エスケープ変異も存在することから、HBc 抗体、HBV-DNA によるスクリーニングも必要であると考えられた。また、薬物注射使用の経験を持つ HIV 患者は HCV 重複感染が高頻度であり、両疾患を減らしていくためにも不法薬の国家間の密売や薬物注射を阻止し、感染防止に努めていく必要があると考えられた。