Hepatitis B and C Virus Infection Among Hemodialysis Patients in Yogyakarta, Indonesia: Prevalence and Molecular Evidence for Nosocomial Transmission

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学位論文の内容要旨

Hepatitis B and C Virus Infection Among Hemodialysis Patients in Yogyakarta, Indonesia: Prevalence and Molecular Evidence for Nosocomial Transmission

インドネシア、ジョグジャカルタの血液透析患者におけるB型およびC型肝炎ウイルス感染：院内感染による流行と分子疫学的根拠

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INTRODUCTION

Hemodialysis patients are at increased risk of acquiring hepatitis B virus (HBV) and hepatitis C virus (HCV) infection. Consequently, the prevalence of both infections in hemodialysis patients is very high. It varies among countries and hemodialysis units within the same country. Unfortunately, few data are available for Indonesia.

The implementation of blood product screening in blood banks and the use of erythropoietin treatment have decreased the prevalence of infection with these viruses in hemodialysis units. However, outbreaks of both viruses still intermittently occur. Nosocomial transmission might play an important role in such outbreaks.

HBV and HCV genotypes show marked geographic distributions. Indonesia is a multiethnic country with as many as 24 HBV subgenotypes, of which HBV subgenotype B3 (HBV/B3) is dominant. At least 15 subgenotypes of HCV have been identified throughout Indonesia, of which HCV/1b is the most prevalent. Nevertheless, few data exist for hemodialysis patients.

The objectives of this study were to investigate the prevalence and genotype distribution of HBV and HCV among hemodialysis patients in Yogyakarta, Indonesia. The possibility of nosocomial transmission was also assessed using molecular analysis.

MATERIALS AND METHODS

Patients dan Staff Members: A total of 161 patients with end-stage renal disease who were undergoing hemodialysis and 35 staff members at one of the hemodialysis unit in Yogyakarta, Indonesia, were enrolled in this study. The demographic and risk factors associated with HBV and HCV infection data were collected using a standardized questionnaire. Blood was collected in plain tubes, allowed to clot, and centrifuged at room temperature. The sera were separated and stored at -80°C until used.

Liver Enzymes and Serologic Markers: ALT and GGT levels were determined using methods recommended by the Japan Society of Clinical Chemistry. All samples were screened and confirmed for HBsAg status using reverse passive hemagglutination assays and chemiluminescent immunoassays. Patients with HBsAg seropositivity were then tested for HBeAg. Only patients with HBeAg negativity were further tested for anti-HBe antibody using enzyme immunoassays. Testing for anti-HBs and anti-HBc was conducted using the passive hemagglutination method for all samples. Anti-HCV antibodies were detected using the particle agglutination method.

Detection of Hepatitis Viruses: HBV DNA was extracted from 200 ml of sera using a DNA extraction kit. The HBV genome was amplified by nested PCR. The amplified products were visualized on a 2% agarose gel stained with ethidium bromide. HBV DNA was quantified by real-time detection PCR with Taq Man chemistry using an Applied Biosystems 7500 RT-PCR System. HCV RNA was extracted from 140 ml of sera using an RNA extraction kit. HCV genome was amplified by nested RT-PCR using a SuperScript III One-Step RT-PCR System with specific primers.

Sequencing and Phylogenetic Analysis: The amplified fragments were directly sequenced using a BigDye Terminator v3.1. Cycle Sequencing Kit and an ABI PRISM 3100-Avant Genetic Analyzer. Sequences were aligned and analyzed using Clustal X software version 2.0.12 and MEGA software version 4.0.2. The phylogenetic trees were constructed using the neighbor-joining method based on Kimura two-parameter distance estimation. Genotypes and subgenotypes were determined based on the Pre-S2/ S region for HBV and the NS5B region for HCV, through comparisons with confirmed reference sequences.

RESULTS

Distribution of Hepatitis Viral Markers: Eighteen of 161 hemodialysis patients (11.2%) and 2 of 35 staff members (5.7%) were HBsAg positive. HBV DNA was detected in all of the patients and staff members who were positive for HBsAg. Twenty-one of 143 (14.7%) patients had occult infection. In contrast, none of the staff members had occult infection. Thus, the overall rates of HBV infection among patients and staff members were 24.2% and 5.7%, respectively. Overall, 134 (83.2%) patients had HCV infection. In contrast, none of the staff members were positive for anti-HCV or HCV RNA. HCV RNA was detected in 96 of 130 seropositive patients (73.8%) and 4 of 31 seronegative patients (12.9%) were classified as having occult infection.

Risk Factors Associated With Infection: Hemodialysis duration and the number of blood transfusions were independently associated with HCV infection.

HBV and HCV Genotypes: Twenty three of 39 strains (59.0%) isolated from patients belonged to HBV/B. Surprisingly, HBV/C and HBV/A were identified in 11 (28.2%) and 5 (12.8%) strains, respectively. All of the HBV/B strains were classified as HBV/B3. Ten (25.6%) HBV/C strains were classified as C2 and the remaining 1 strain (2.6%) as C7. All of the HBV/A strains were classified as HBV/A2. HBV/C2 was detected mainly in patients with occult infection. HBV/A2 was only detected in patients with occult infection. Some of the strains were identical. HCV genotype 1 (HCV/1) was the most common genotype (98%), followed by HCV/3 (2%). HCV/1a was dominant (95%), while HCV/1b, HCV/1c, and HCV/3a were identified in 1 (1%), 2 (2%), and 2 (2%) patients, respectively. The HCV/1a strains showed high similarity. Twenty of them were identical.

DISCUSSION

The prevalence of HBV and HCV infection among hemodialysis patients in Yogyakarta was consistently higher than that among local healthcare workers and blood donors, suggesting that hemodialysis patients are at greater risk of infection. The prevalence rates were also similar to those in developing countries, and were higher than those in developed ones. It might depend on the differences in the prevalence rates among the general population.

In Yogyakarta, the prevalence rates of HBsAg and anti-HCV were reported to be 7% and 81%, respectively, in a study performed in 1994. Our study revealed a higher prevalence of HBsAg but a similar prevalence of anti-HCV than these reported in study. It indicates that the current infection-control procedures did not decrease the prevalence of HBV and HCV infection. In these circumstances, nosocomial transmission might play an important role.

Multiple blood transfusions and duration of hemodialysis were independently associated with the prevalence of HCV infection among hemodialysis patients. In Indonesia, HCV screening was introduced at blood banks in 1995. However, HCV screening was only started in 2001 in Yogyakarta. Thus, hemodialysis patients who received blood transfusions before 2001 were at increased risk of acquiring HCV infection. Unfortunately, blood transfusion, rather than erythropoietin treatment, is still being used to correct renal anemia in the hemodialysis unit. The duration of hemodialysis was independently associated with the prevalence of HCV infection, supporting the hypothesis that nosocomial transmission was responsible for HCV infection within the hemodialysis unit.

Occult HBV infection was detected in 14.7% of hemodialysis patients in Yogyakarta, higher than that in general population. These results suggest that hemodialysis patients are

more susceptible to occult HBV infection compared with the general population. Low viral load and nucleotide mutations might explain the high prevalence of occult HBV infection.

Our study revealed a high prevalence of occult HCV infection (12.9%). The differences in the definitions, methods, hemodialysis unit conditions, and geographical areas resulted in marked differences in the prevalence rates of occult HCV infection.

This study showed significant differences in genotype distribution. The increased prevalence of HBV/C and HBV/A suggests that cross-infection occurs among patients. HBV/C2 and HBV/A2 were particularly common in patients with occult infection, and the partial HBV genome of several strains was identical. Thus, occult hepatitis B cases might play an important role in HBV transmission in our hemodialysis unit.

The most prevalent HCV genotype was 1a, differing from that observed in HCV-infected blood donors or patients with liver diseases, where genotype 1b prevailed. This predominance might reflect an outbreak of HCV infection from a common source. Phylogenetic tree analysis of strains isolated from patients and unrelated strains isolated from the same geographic region, and other countries supported this finding. The present findings provide convincing evidence for nosocomial transmission of HCV.

Several mechanisms may explain the predominance of HCV/1a. This genotype might be adapted for patients with impaired immunity, and may be easily transmitted between patients. Furthermore, frequent exposure to HCV-contaminated blood, supplies, or surfaces, may favor the emergence of mixed genotype infection. When mixed-genotype infection involving HCV/1a occurs, this subtype tends to prevail and persists as the only subtype during the course of the disease.

There are many possible routes of viral transmission that may be responsible for nosocomial infection. HBV and HCV cannot pass through the dialyzer membrane to cause

cross-infection between patients. The reuse of dialyzers is unlikely to provide a route of nosocomial transmission. It is suspected that the most likely cause of cross-infection between patients treated in the same hemodialysis unit is cross-contamination of supplies and surfaces through a failure to follow established infection-control procedures.

In conclusion, the prevalence of HBV and HCV infection among hemodialysis patients in Yogyakarta, Indonesia, remains high. Nosocomial transmission might play an important role in infection through a failure to follow infection-control procedures within the hemodialysis unit. Hemodialysis units should implement and adhere to strict infection-control procedures designed to prevent the transmission of HBV and HCV.

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

受付番号	甲 第 2390 号	氏 名	Hanggoro Tri Rinonce
論文題目 Title of Dissertation	Hepatitis B and C Virus Infection Among Hemodialysis Patients in Yogyakarta, Indonesia: Prevalence and Molecular Evidence for Nosocomial Transmission インドネシア、ジョグジャカルタの血液透析患者における B 型および C 型肝炎ウイルス感染: 院内感染による流行と分子疫学的根拠		
審査委員 Examiner	主 査 Chief Examiner 西 慎一 副 査 Vice-examiner 副 査 Vice-examiner 白川 利朗		

（要旨は１，０００字～２，０００字程度）

インドネシアにおいては透析患者の肝炎罹患率が高いことが知られているが、インドネシアの血液透析患者における B 型および C 型肝炎ウイルス感染の正確な罹患率とウイルス遺伝子型(genotype)に関するデータがなく、疫学的調査を行った。これらのデータを基に特に院内感染の可能性がないか検討した。対象患者は、インドネシアのジョグジャカルタの 1 透析施設における 161 例の血液透析患者と 35 人の透析施設スタッフである。調査期間は 2010 年の 1 月から 2 月までである。

方法としては、B 型肝炎に関しては、HBs 抗原, HBe 抗原, HBe 抗体, HBc 抗体, HBs 抗体、HBV DNA を調査した。C 型肝炎に関しては、HCV 抗体、HCV RNA を調査した。また、性別、透析期間、輸血回数、肝機能なども調査した。HBs 抗原陰性であるが、2 カ所以上の領域で HBV DNA が陽性である場合は HBV 潜在感染とした。また、HCV 抗体陰性であるが、HCV RNA 陽性であれば HCV 潜在感染とした。更に、ウイルス感染の系統発生学的調査も行った。HBV 感染に関しては、Pre-S2 と S 領域の解析と専用ソフトならびに Kimura two-parameter distance estimation 法を用いて系統図を作成した。HCV 感染の系統発生学的調査に関しては、NS5B 領域を用いて確定参照配列との比較で行った。

透析患者の調査結果からは、多くの症例が透析期間 1 年は超えており、透析器は再利用され、一般に輸血を頻回に受けていることがわかった。HBs 抗原陽性症例は透析患者で 11.2%、スタッフで 5.7%であった。潜在感染率は 14.7%であった。リスク因子と各肝炎感染率との関連では、単変量解析では HBV 感染に関して有意寄与因子はなかったが、HCV 感染に関しては透析期間と輸血回数が有意寄与因子であった。また、年齢と性で補正した多変量解析を行ってもこの二つの因子は HCV 感染に関して有意な寄与因子であった。系統発生学的調査の結果では、HBV 感染では B3 を中心とする遺伝子型 B が 59%、C2 を中心とする遺伝子型 C が 28.2%、A2 を中心とする遺伝子型 A が 12.8%であった。HCV 感染では遺伝子型 1a が 95%を占め、これらの比率は一般人口の比率と大きく異なっていた。

インドネシアの一般的罹患率は、HBV 感染、HCV 感染ともに 3.9%程度である。透析患者における高い HBV 罹患率の原因に関しては、透析患者が HBV 感染に対する予防接種が受けられていない状況であることが関与している。透析患者の HBV 潜在感染罹患率も一般人口のそれより高く、これは透析患者が B 型肝炎に関して易感染性を有しいているためとも考えられた。HCV 感染に関しては、輸血との関連性が考えられた。インドネシアでは 2001 年から輸血に対する C 型肝炎チェック体制が確立したが、この年以前に輸血を受けた透析患者が HCV に罹患しているのではないかと考えられた。

また、系統発生学的調査の結果からは、HBV 遺伝子型 B が 80%を超えるインドネシア一般人口と比較して、透析患者では HBV 遺伝子型 B と C に偏りがあった。また、HCV 感染関して HCV 遺伝子型 1a に大きく偏がみられ、これも HCV 遺伝子型 1b が多いインドネシア一般人口と異なる傾向であった。透析患者では特定の遺伝子型が集中してみられることから、HBV 感染、HCV 感染ともに院内感染であると考えられた。ただし、どのようなルートで院内感染を起こすのか、その詳細は不明であった。更なる調査研究が必要と考えられた。本研究は、インドネシアの透析患者の肝炎罹患率、肝炎遺伝子型に関する疫学調査を実施し、院内感染の可能性を検討した研究である。同国におけるこのような研究は従来ほとんどなく、重要な知見を得たものとして価値ある集積であると認める。よって、本研究者は、博士(医学)の学位を得る資格があると認める。