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# Clinical Correlations of Peripheral Blood Microchimerism after Liver Transplantation

The aim of this study was to evaluate microchimerism after human liver transplantation (LT). This study included 13 female recipients who received hepatic allograft from male donors at Asan Medical Center. A nested PCR specific for Y-chromosome gene (DYZ3) was used to analyze the small number of male cells in the peripheral blood mononuclear cells of the female recipients. Microchimerism was observed in 6 of 13 recipients and 16 out of 35 samples. Only 3 patients showed microchimerism 3 months after LT. There was no statistical difference between the presence of microchimerism and clinical findings such as type of donor, type of immunosuppression, episode of rejection and age of recipient. This study did not show any clinical relevance of microchimerism and further larger study are needed to confirm the results.

Key Words: *Transplantation Chimera; Liver Transplantation*

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## INTRODUCTION

In the early postoperative period, donor cells accounted transiently for 5 to 15% of circulating mononuclear cells in recipient, generally without any clinical evidence of graft-versus-host disease (1). While these cells usually become undetectable by the cytometry after a few weeks, persistence of donor-derived cells, microchimerism, can still be detected in the blood or various tissues by polymerase chain reaction (PCR) for donor-type DNA sequence (2, 3). Based on the observation of the persistence of donor cells in circulation and other organs in long-term transplant survivors (2, 3), it has been hypothesized that the development and persistence of donor-specific microchimerism may play a crucial role in the induction and maintenance of allograft tolerance (4, 5). In certain animal studies, passenger leukocytes contribute clearly to donor-specific unresponsiveness that develops after liver transplantation (6, 7). However, some studies suggest that microchimerism may be an epiphenomenon without any relation to allograft tolerance (8, 9).

The present study tries to determine the state of microchimerism in liver transplant recipients at varying time intervals after transplantation and whether microchimerism is associated with clinical findings like type of transplantation and immunosuppression, presence of acute rejection and age of recipient.

## MATERIALS AND METHODS

### Patients

Donor leukocyte microchimerism was analyzed in 13 female liver transplant recipients from male donors at Asan Medical Center (Seoul, Korea) from 1993 to 1997. The median age of the recipients was 20 years old (7 months to 44 years). The underlying diseases were biliary atresia in 4, liver cirrhosis due to hepatitis B virus in 3, Wilson disease in 3, fulminant hepatitis in 2, and Caroli disease in 1 recipient. While 7 patients received cadaver donor liver transplantation, 6 patients received living related donor liver transplantation. As an immunosuppression, 9 patients received cyclosporin A and low dose steroid and 4 patients received tacrolimus (FK506). Acute rejection episodes were observed in 6 patients. The diagnostic criteria for acute rejection was discussed elsewhere (10).

### Genomic DNA extraction from peripheral blood mononuclear cells

Ten milliliters of heparinized blood were obtained 35 times from the 13 patients. Peripheral blood mononuclear cells from transplant recipient were isolated by density gradient centrifugation using Ficoll-Hypaque (Pharmacia, Piscataway, NJ). After washing the cells in phosphate buffered saline, the genomic DNA was extracted by the Higuchi method (11). Briefly, cells were lysed in lysis buffer containing 10 mM Tris-HCl (pH 8.0), 400 mM NaCl, and 2 mM ethylenediaminetetraacetic acid (EDTA) with 20 mg/mL protein kinase K (Sigma, St. Louis, MO). The digestion proceeded for 2 hr at 56℃ or overnight at 37℃ followed by 10-15 min at 95℃. The DNA concentration (optical density 260) and purity (optical density 260/280) were determined by spectrophotometer. The DNA obtained had a purity of 1.4-1.6 and was suitable for PCR analysis

### Detection of donor Y-chromosome specific DNA

The Y chromosome specific primers' sequences were: 5'-TGAAAACTACACAGAAGCTG-3' and 5'-ACACATCACAAAGAACTATG-3' for the first reaction and 5'-TGCATTAATTTCCCAGAGTCG-3' and 5'-GCTTCTGCATAGCTTTTAAGG-3' for the second reaction. First PCR was carried out in a volume of 100 μL containing 1 μg of template DNA, 50 mM KCl, 1.5 mM $MgCl_2$, 10 mM Tris-HCl (pH 8.4)/20 μg gelatin, 0.2 mM dNTPs, 2.5 units *Taq* polymerase (Perkin Elmer, Branchburg, NJ), and 2 μmol/L primers. The PCR reaction was carried out in a thermal cyclizer (Perkin Elmer-Cetus, Emeryville, CA). The parameters for thermocycling for the first round were as follows: 30 cycles of denaturation at 95℃ for 30 seconds, annealing at 55℃ for 30 seconds and extension at 72℃ for 1 min. Ten μL of ten fold dilution of the first-round PCR product was used as template DNA for the nested reaction. The second-round reaction was done with half of the first reaction under the same condition. The products were analyzed by electrophoresis in 2% agarose gel, followed by ethidium bromide staining.

### Analysis of microchimerism and correlationship with clinical findings

We analyzed the presence of microchimerism in 13 recipients. Clinical correlations of microchimerism 3 months after liver transplantation were studied. The clinical findings included donor type and type of immunosuppression, acute rejection episode and age of recipient. Statistical analysis was done by Fisher's exact test.

## RESULT

### Detection of microchimerism

The sensitivity of DYZ3 detection by nested PCR was 0.0001% (Fig. 1). Thirty-five samples were studied from 13 patients. Sixteen episodes (48%) of microchimerism were observed in 35 samples. Microchimerism was observed in 6 (46%) of 13 recipients. After 3 months, only 3 episodes of microchimerism were still observed in 3 of 13 recipients (Fig. 2).

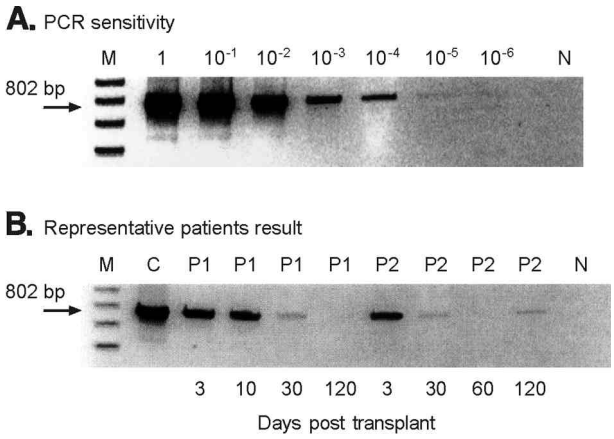


**Fig. 1.** Detection of donor leukocyte microchimerism in female recipient by nested PCR of genomic DNA using short arm of Y-chromosome centromere repetitive sequence (DYZ3). **A:** The sensitivity of this study showed one cell out of $10^5$ recipient cells. **B:** Representative michrochimerism of two patients during follow-up period. Patient 1 showed microchimerisms in early posttransplant period then disappeared 1 month after transplant, and patient 2 showed fluctuation of microchimerisms at variable periods during follow-up time (M, 123 size marker; C, positive control; N, negative control; P1, patient 1; P2, patient 2).

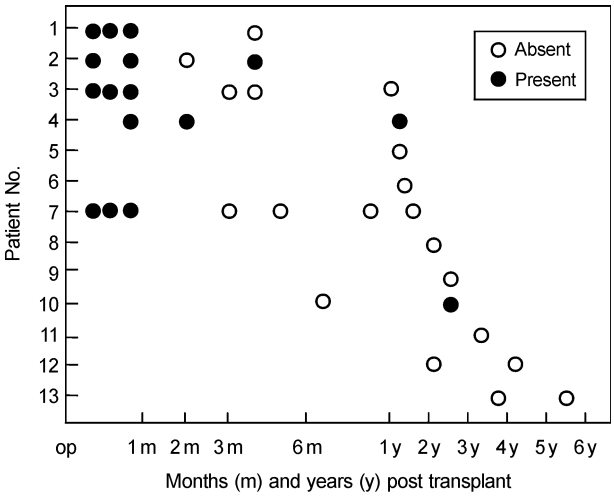


**Fig. 2.** Presence or absence of microchimerism during follow-up period in 13 liver transplant recipients. Most of the microchimerism were noted within 3 months after liver transplantation.

**Table 1.** Correlationship between the presence or absence of microchimerism three months after liver transplantation and the clinical findings in 13 liver transplant recipients

| | Microchimerism | | Statistical significance |
|---|---|---|---|
| | Present | Absent | |
| Episode of rejection | | | 1.000 |
| Present | 1 | 5 | |
| Absent | 2 | 5 | |
| Type of transplant | | | 0.070 |
| Living donor | 3 | 3 | |
| Cadaver donor | 0 | 7 | |
| Age of the patients | | | 0.203 |
| < 2 year | 2 | 2 | |
| ≥ 2 year | 1 | 8 | |
| Type of immunosuppression | | | 1.000 |
| Cyclosporin A | 2 | 7 | |
| FK506 | 1 | 3 | |

### Clinical correlations of microchimerism 3 months after liver transplantation

While none of the 7 recipients with cadaver donor graft showed microchimerism, 3 out of 6 recipients with living related donor graft showed it. As for immunosuppression, microchimerism was observed in 2 of 9 recipients with cyclosporin A and 1 of 4 recipients with FK506. As for acute rejection, while one patient out of 6 with acute rejection showed microchimerism, 2 patients out of 7 without acute rejection showed microchimerism. According to age of recipient, 2 of 4 under the age of 2 yrears showed microchimerism, 1 of 9 over 2 years of age showed microchimerism. There was no statistical difference between the presence of microchimerism 3 months after LT and all clinical findings evaluated such findings as donor type, immunosuppression, presence of acute rejection and age of recipient (Table 1).

## DISCUSSION

Liver transplantation is a definitive, therapeutic modality for end-stage liver disease. After successful transplantation, the primary goal is induction and maintenance of a specific immunological tolerance to the transplanted organ without prolonged administration of immunosuppressive agent.

With respect to immunological relevance of microchimerism in this study, patients with positive microchimerism showed a similar rejection to those who were negative. The lack of evident correlation between the immunological state of the patients in this study seems to be against the hypothesis that systemic microchimerism plays an important role for donor specific hyporeactivity and tolerance (2, 4). Many other data also showed that microchimerism has no clear correlation with the immunological state (8, 12, 13) in suggesting chimerism analysis has no obvious diagnostic value predicting the immunological risk for rejection in individual patients.

The causes of the inconsistent findings may be due at least in part, to the large number of variables affecting organ transplantation. These variables include small sample size, extent of HLA mismatch between donor and recipient, duration and extent of immunosuppression, tissue source, time of sampling, and techniques used for detecting microchimersim (13-15).

In addition, the mechanisms controlling the amount of allogenic cells may differ from those causing acute rejection, since the fluctuation in the intensity of microchimerism seems to be independent of the rejection status (8). Elimination of allogenic hematopoietic cells has been demonstrated to be mainly due to an effect of natural killer cells, whereas the onset of rejection was initially triggered by T lymphocytes (16). The persistence of microchimerism may not contribute to establishing or maintaining specific transplantation tolerance, but may be merely an epiphenomenon representing shedding of immunologically inactive cells (8). Although immunohistochemical staining suggests the multilineage nature of donor leukocytes in case of microchimerism (17), the identity of cells responsible for the positive PCR results cannot be determined in most clinical cases. It has been suggested that antigen-presenting cells (APC), especially dendritic cell populations, are the most prominent migratory cells in long-term stable patients (2, 4). On the other hand, dendritic leukocytes within transplanted tissues are known to be immunogenic components that trigger rejection rather than inducing a tolerance (18, 19), because they generally provide effective costimulatory signals to T cells (20). In contradiction to this, other evidence support that certain APC from transplants could trigger functional deletion or apoptosis of host T and B lymphocytes which causes decline in cytotoxic T cell responses. This contradictory action has been called the "veto effect" (21-23).

With respect to immunosuppression, patients with FK506 did not show any difference from patients with cyclosporin A. Our clinical data showed no difference in survival according to the type of immunosuppression (10). Effective immunosuppression is reported to be necessary for allogenic stem cell to be engrafted initially, then later shift of donor leukocyte chimerism from the lymphoid to the nonlymphoid compartment (24). Without immunosuppression, graft survival was not achieved in allogenic graft and chimerism declined rapidly (25).

With respect to type of graft, patients with living related donor graft showed somewhat higher microchimerism than patients with cadaver non-related donor graft ($p$=0.070), but there was no statistical significance. It may be possible that the acceptance of allogenic stem cell is more feasible in semiallogenic HLA haplo-matched related donor graft (26). It needs further study with a larger sample size.

Since the first description of prenatal tolerance induction by Billingham et al. (27), neonatal tolerance has been induced following transplantation of semiallogenic cells (26). Kim et al. (28) showed fetal and neonatal tolerance induction is associated with presence of peripheral blood microchimerism. Our data revealed that a younger age (<2 year) showed somewhat higher microchimerism, but there was no statistical significance.

In conclusion, this study did not show any clinical relevance between clinical finding and microchimerism but further larger sample studies are needed to confirm the result.

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