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# Comparative Study of HLA-DR Typing by Serology and Sequence-Specific Primer Analysis in a Genetically Highly Diverse Population of Kidney Transplant Recipients

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IN 1987, 24 BRAZILIAN dialysis and kidney transplant centers in the state of São Paulo, located in 17 cities, joined efforts to participate in a single society named São Paulo Interior Transplant (SPIT). The main objective of SPIT is the allocation of cadaver kidneys according to HLA matching.[1] Accordingly, all recipient candidates and cadaver donors were typed for HLA antigens in the city of Ribeirão Preto, and the allocation, scheme used the criteria proposed by The United Network for Organ Sharing (UNOS). Since its foundation, SPIT has performed over 1000 kidney transplants.

The Brazilian population was formed by the contribution of genes of individuals from several ethnic backgrounds, including Caucasian immigrants from many regions of Europe, African American individuals predominantly from the Niger-Congo area of Equatorial Africa, and native Amerindian populations.[2] DNA typing of HLA alleles for kidney transplantation has been considered to be much more accurate than serologic identification, leading to an increased allograft survival, according to the data published by several large multicenter studies.[3,4] Since the Brazilian population is genetically highly diverse and since HLA molecular typing has been correlated with better allograft survival, all kidney recipients on the waiting list of SPIT were retyped for HLA-DRB1 alleles by DNA technology using sequence-specific primers (PCR-SSP) in polymerase chain reactions. In this study, we compared the results of previous HLA-DR typing using conventional serology with HLA-DRB1 typing using the PCR-SSP technique.

## PATIENTS AND METHODS

### Study Population

A total of 1160 patients with end-stage renal disease were enrolled in SPIT from 1986 to 2000, including 839 (72.3%) white; 111 (9.5%) black; 186 (16%) mulattoes; and 22 (1.9%) orientals.

### Serologic Typing

HLA-DR typing was carried out by a standard complement-dependent microlymphocytotoxicity assay.[5] Peripheral blood lymphocytes were enriched for B lymphocytes by passage through nylon wool columns. A panel of commercially available HLA antisera (France Transplant, France; Biotest, Germany; Fred Hutchinson Cancer Research Center, Pel Freez, Gen Track, and One-Lambda, USA) was used.

### PCR-SSP Typing

DNA was extracted from peripheral blood leukocytes using a salting-out procedure.[6] Sequence-specific primer analysis was performed using a previously described method.[7,8] The mixture of nucleotides and the dNTPs were prepared by the Collaborative Transplant Study (Ruprecht-Karls-Universität, Heidelberg, Germany). All amplifications were performed in a thermocycler (Perkin Elmer 9600, Norwalk, Conn, USA) using the following conditions: initial denaturation 94°C for 2 minutes, denaturation 94°C for 10 seconds, annealing + extension 65°C for 1 minute, 10 cycles, followed by 94°C for 10 seconds, 61°C for 50 seconds 72°C for 30 seconds for 20 cycles, and a final extension of 72°C for 10 minute.

### Statistical Analysis

Statistical analysis was performed using the multiple chi-square test.

## RESULTS

The rates of concordant versus discordant results between serology and SSP analysis are shown in Table 1. Considering the group as a whole, 32.6% of the patients had at least one HLA-DR discrepancy when the results of the two methods were compared. Different results were observed among patients stratified according to ethnic background 32.7% of whites; 32.8% of blacks; 31.5% of mulattoes; and 27.3% of orientals. When the assignment of a particular antigen by serologic tests was different from that by DNA typing, it was denoted as an antigen/antigen discrepancy, a finding that was observed in 24.8% for the group as a whole, in 24.7% for whites and blacks, 26.1% for mulattoes, and 18.2% for orientals (Table 1).

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**Table 1. Analysis of Comparisons Between HLA-DR Typing by Serology and PCR-SSP Molecular Typing of a Series of Brazilian Individuals with End-Stage Renal Disease**

| | Individuals | | | | | |
|---|---|---|---|---|---|---|
| | Total Group ($n$ = 1160) | White ($n$ = 839) | Black ($n$ = 186) | Mulatto ($n$ = 111) | Oriental ($n$ = 22) | Black + Mulatto + Oriental ($n$ = 319) |
| No discrepancy | 67.4% | 67.3% | 67.2% | 68.5% | 72.7% | 68.0% |
| Discrepancy | 32.6% | 32.7% | 32.8% | 31.5% | 27.3% | 32.0% |
| Antigen/Antigen* | 24.8% | 24.7% | 24.7% | 26.1% | 18.2% | 24.8% |
| Serology blank/DNA antigen | 9.7% | 9.8% | 11.3% | 7.2% | 9.1% | 9.7% |
| Serology antigen/DNA blank | 3.2% | 3.2% | 3.8% | 1.8% | 4.6% | 3.1% |

*Antigen/antigen: A certain antigen was assigned by serology but a different antigen could be determined by SSP.

When a blank was assigned by serologic testing and a second antigen was determined by DNA analysis, it was referred to as "Serology Blank/DNA Discrepancy," and when the opposite occurred as "Serology Antigen/DNA Blank" (Table 1). Serologic testing failed more frequently (9.7%) than molecular analysis (3.2%) to assign HLA-DR specificities. Stratification according to ethnic background showed similar results for each racial group.

The comparison between the two methods when individual HLA-DRB1 alleles were considered is shown in Table 2. The percentage of discrepancies decreased from 32.6% to 24.1% in the group as a whole. Similar results were observed for individuals of all ethnic backgrounds.

## DISCUSSION

Histocompatibility testing is of pivotal importance in the selection of kidney donors for transplantation, particularly HLA-DRB1 alleles, which code for molecules with a central role in the immune response. The limitations in HLA class II typing by serology involve the quality and specificity of antisera reactive with certain HLA-DR types as well as dependence on the presence of viable B lymphocytes and on adequate cell surface expression of histocompatibility molecules.[9] Particularly, HLA-DR typing by serologic techniques fails more frequently in transplant candidates than in organ donors or in healthy individuals, probably due to the inadequate quality of the lymphocytes.[10] Long-term hemodialysis may alter peripheral blood lymphocytes, influencing the serological reaction patterns.[11] Thus, methods that improve HLA typing of recipients may be expected to contribute to a better HLA assignment and to better HLA matching.

Considering the group of patients as a whole, discrepancies between serology and SSP analysis reached 32.6%, and when this analysis was performed in terms of individual allelic discrepancies, the corresponding figure was 24.1%. In a large study conducted on 1522 individuals, including healthy subjects, organ donors, kidney recipients, and leukemia patients, HLA-DRB1 typing by serology versus restriction fragment length polymorphism (RFLP) analysis revealed discrepancies from 20% to 25%, with the highest rate being observed in kidney recipients.[10] The Leuven Collaborative Group for Transplantation also reported a large discrepancy rate (19%) for HLA class II assignment using serologic versus RFLP typings.[12] The comparisons of HLA-DR typing between serology and oligotyping have revealed heterogeneous results. Discrepancy rates ranged from 26.4% for the analysis of 110 kidney transplant patients[13] to 9.2% for the analysis of 1052 cadaveric organ donors from the Eurotransplant centers.[14] One reason that might contribute to the high discrepancy rate observed in our study may be the long study period (14 years), involving the utilization of several different panels of HLA antisera.

Overall, serologic methods failed to assign HLA-DR specificities more often than oligotyping. In 9.71% of the individuals a serological "blank" turned out to be a definable allele by SSP, while in 3.2% of the individuals a second antigen revealed by serology turned out to be a DNA blank. In the latter instances, the serological reactions might have been incorrect due to cross-reactivity of typing reagents, or conversely poor-quality DNA might have accounted for the failures in SSP typing. Both serologic and SSP typing used in this study are considered to be low-resolution methods; the combined frequency of DR blanks in this study was relatively high, suggesting a high rate of homozygosity. Recently, we established the HLA class II profile of a

**Table 2. Comparative Analysis of HLA-DR Alleles/Antigens Using Serology and PCR-SSP**

| | Alleles | | | | | |
|---|---|---|---|---|---|---|
| | Total Group ($n$ = 2320) | White ($n$ = 1678) | Black ($n$ = 372) | Mulatto ($n$ = 222) | Oriental ($n$ = 44) | Black + Mulatto + Oriental ($n$ = 638) |
| No discrepancy | 75.9% | 75.8% | 75.5% | 77.5% | 79.6% | 76.5% |
| Discrepancy | 24.1% | 24.2% | 24.5% | 22.5% | 20.4% | 23.5% |
| Antigen/antigen | 16.5% | 16.5% | 16.4% | 16.7% | 9.1% | 16.0% |
| Serology blank/DNA antigen | 6.1% | 6.1% | 6.2% | 5.0% | 9.1% | 6.0% |
| Serology antigen/DNA blank | 1.6% | 1.6% | 1.9% | 0.9% | 2.3% | 1.6% |

Brazilian population and several alleles of certain allele groups were observed frequently, particularly the DRB1*04, *08, *11, and *13 groups.[15] These data suggest that an individual typed as homozygous for one of these broader groups may be heterozygous if analyzed at the high-resolution allelic level. In other studies conducted in less heterogenic populations, the investigation of DR "blank" revealed that in most instances blanks result from true homozygosity.[16]

Although the Brazilian population is highly miscegenated, the percentage of discordance between serologic and molecular analysis seen in this study was similar to that reported for other populations. The present study indicates that HLA typing using molecular techniques is more accurate than serological typing and that this method should be preferred for HLA matching, since many transplants that were previously thought to be HLA matched were in fact mismatched, and this finding may account for previously unexplained graft failures.

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