

HLA-Matching by DNA Methods: Impact on a Living Related Renal Transplantation Programme

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DNA methods have resulted in improved renal allograft survival rates in cadaveric renal transplantation. This paper describes the impact of DNA typing by PCR-SSP on a living related renal transplant (LRRT) programme. It evaluates error rates in serology, acute rejections, graft function and survival rates between the two typing methods. Serological typing was done on CTS 120 antisera Class 1 and 60 antisera Class 2 and 72 antisera Terasaki Class1 and 72 antisera Class2 Antigens. Low resolution PCR-SSP typing was done by 24 primers for HLA A , 48 for HLA B and 24 for HLA DR. Of the 585 transplants, 159 (Group I) were serology based, 172 serology and PCR-SSP for HLA DR (Group II) and 254 on serology and PCR-SSP for HLA A and B and only PCR-SSP for HLA DR (Group III). Error rates in serology as compared to PCR-SSP were 24% for HLA A, 16% for HLA B and 35% for HLA DR. Acute rejection in Group I were 39%, Group II 30% and Group III 26% (p 0.02). Graft function of serum creatinine < 1.5 mg/dl at 1 year was found in 26% of Group I patients as com-

pared to 48% of Group III (p < 0.0001). One and three year graft survival was 93% and 87% for Group II as compared to 81% and 69% for Group I respectively (p 0.0001). Matching by this combination of serology and PCR-SSP is not only economical for a developing country but also improves graft survival by 12% at 1 and 18% at 3 years.

Keywords: *HLA Matching PCR-SSP Living Related Transplants*

Histocompatibility testing plays an important role in donor selection for kidney transplantation and several studies have shown better kidney graft survival when kidney recipients were given HLA matched organs [1,2]. Advent of DNA based methods for HLA matching has revealed errors rates of up to 25%-30% in serological based methods [3-5]. Better matching based on DNA methods have resulted in improved graft survival rates [6]. This paper describes the impact of DNA typing by polymerase chain reaction (PCR) using sequence specific primers (SSP) in a living related renal transplantation programme in term of error rates in serology, acute rejections, graft function and survival.

Patients and Methods

Five hundred eighty-five patients received live

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related renal transplants between January 1992 and December 2000. All patients received immunosuppression by triple drug regimen. Till 1994 tissue typing was performed by serological methods. Class II HLA-DR typing by PCR using sequence specific primers was introduced in 1994. Class I HLA A and B by PCR-SSP was introduced in 1997.

Serological Testing: Tissue typing was performed using Collaborative Transplant Study (CTS, Heidelberg) plates using 120 antisera for HLA-A and B and 60 antisera for HLA-DR. One Lambda (USA) 72 antisera class I and 72 antisera class II trays were used for confirmation in difficult cases with low reactivity or cross reaction. T and B lymphocytes were purified using magnetic beads from Dynal (Norway).

DNA Typing: Sequence specific primers were obtained from CTS (Heidelberg). Twenty four primers for HLA-A, 48 for HLA-B and 24 for HLA-DRB1. Taq polymerase PCR buffer, dNTPs were obtained from Perkin Elmer (USA) and Proteinase K (Roche). DNA was extracted by Olerup method [7]. PCR cycling was preceded by a hot start of 2 minutes at 94°C for HLA A and B and 30 seconds for HLA DR. After electrophoresis

of 20 minutes gels were visualized on 1 VILBER LOORMAT video camera and Pharmacia gel illumination. HLA B alleles were analysed by a computer programme CTS typer. Statistical analysis included Chi-square, Kaplan Meier survival curves and Log rank test.

Results

Of the 585 transplants, 159 transplants were based on serology alone till 1994 (Group I). Thereafter for 172 transplants class I HLA, A and B and class II HLA-DR were tested by serology and HLA-DR was also done by PCR-SSP till 1997 (Group II). From 1997 for 254 transplants Class I were tested on serology however all homozygous, those with HLA A-19 and those showing weak or cross reaction were confirmed by PCR-SSP for HLA-A and B while Class II HLA-DR were tested by PCR-SSP only (Group III). Of the 172 in Group II who were tested for serology and PCR-SSP for HLA-DR the discrepancy rate for HLA-DR 1-10 was 30% and HLA-DR 1-18 was 35% (Figure 1). The alleles most effected were DR 1, 16, 4, 8, 9, 10 and DR13 and DR14. In most instances DR10 was assigned as DR1 by serology. In the missed Group DR6 comprised 51% of the cases. In group III, 110 were con-

Table 1. Graft function grades at 1 year and rejection episodes

	A	B	C	D	Failed	Acute Rejections
Group I n = 159	42 (26%)	44 (28%)	38 (24%)	4 (3%)	31 (19%)	62 (39%)
Group II n = 172	67 (39%)	72 (42%)	10 (6%)	5 (3%)	18 (11%)	51 (30%)
Group III n = 254	121* (48%)	88 (35%)	18 (7%)	10 (4%)	17 (7%)	66** (26%)
A=<1.5 * p = 0.0001	B=<2.0	C=<3.0 ** p = 0.02	D=>3.0 mg/dl			

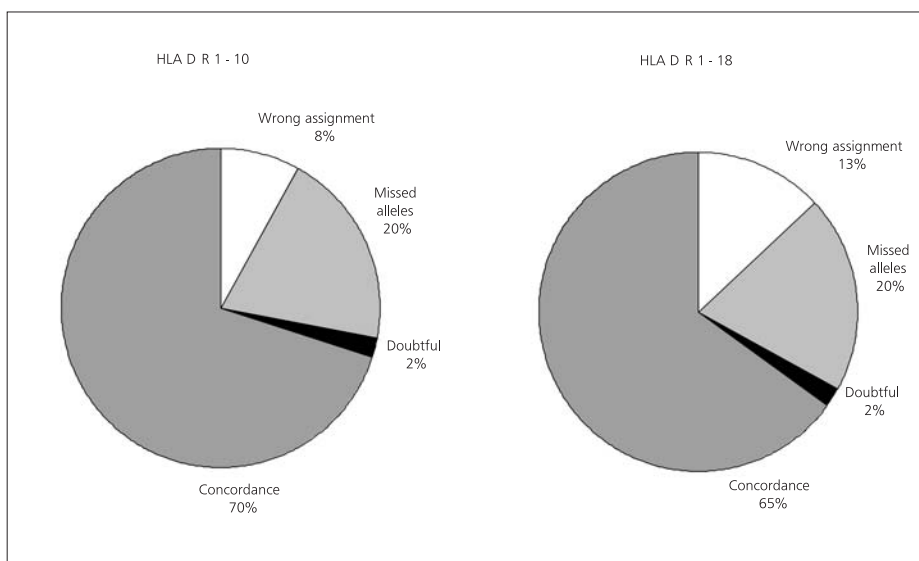


Figure 1. Analysis of HLA-DR results by serology compared with PCR-SSP

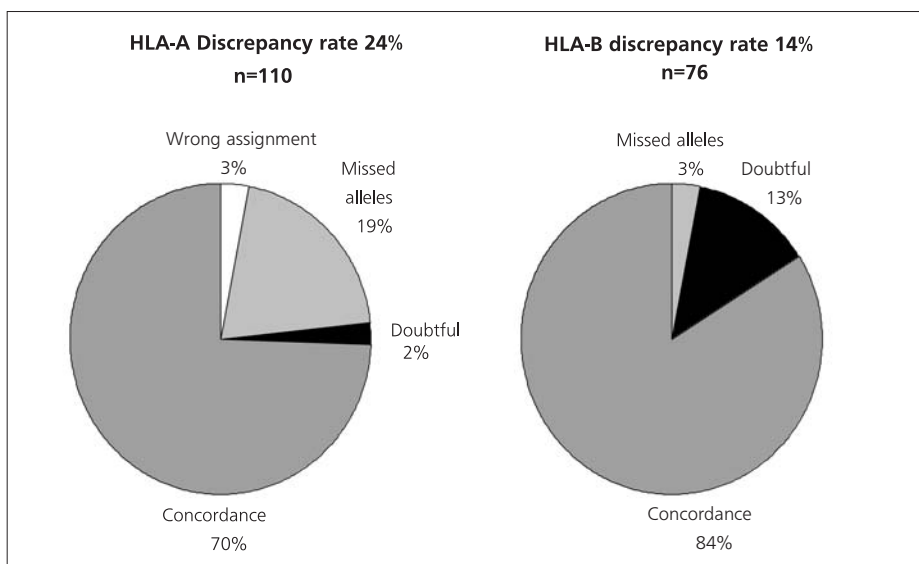


Figure 2. Comparison of HLA-A and B results by serology and PCR-SSP

firmed by PCR-SSP for HLA A and the discrepancy rate was 24% while 76 were confirmed for HLA-B with a discrepancy rate of 16% (Figure 2). At the A locus the most effected allele was A19 and its splits, A24, A68 and A74. In the missed group A19 comprised 88% of the cases. At B locus cross reactions were the main problem specially

B5 and B21

HLA Matching: The percentage of HLA identical in the Groups, I, II and III were 58 (36%), 55 (32%) and 76 (30%) respectively. The one haplotype matched (3 antigen) were 63 (40%), 75 (44%) and 129 (51%) respectively. The rest in Group I and II were more than one haplotype (4 or 5 antigen

match) while in group III 29 (11%) were more than one haplotype and 20% less than one haplotype (2 antigen match with one match at HLA-DR)

Acute Rejections and graft function: The acute rejection rates in the first three months was 62 (39%) in Group I, 51 (30%) in Group II and 66 (26%) in Group III ($p < 0.05$) and a graft function of serum creatinine of $< 1.5\text{mg/dl}$ was found in 42 (28%) Group I, 67 (39%) Group II and 121 (48%) of Group III respectively (Table 1). One and three year graft survival was 93% and 87% in Group III as compared to 81% and 69% in Group I (Figure 3).

Discussion

The role of HLA matching on graft outcome in kidney transplantation has long been established. Advent of DNA based typing methods has shown discrepancy rates and errors in serological typing for both classes I HLA-A and B and class II HLA-DR [3-5]. In this study we have observed similar errors in our serological methods using high quality plates from CTS and One Lambda. Working in a developing country the combination of serology for class I and confirmation by PCR-SSP and class II HLA-DR by PCR-SSP is both economical as DNA methods for HLA-DR are cheaper than serological methods when one considers the cost of magnetic beads and has impacted on both graft function and survival rates. It has increased 1 year survival by 12% and 3 year survival by 18%. Better matching has allowed us to utilize the refined method of CREG matching where now poorly matched kidneys on conventional matching can be transplanted on CREG basis with good results, thus increasing our donor pool [8]. This is of special significance since living donors offer a once in a lifetime chance of transplantation.

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