Modified urine typing to enhance clinical management in kidney transplant patients with unknown donor human leukocyte antigen typing: abridged secondary publication

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KEY MESSAGES

1. Availability of donor human leukocyte antigen (HLA) typing is crucial for early diagnosis of antibody-mediated rejection (AMR) and prompt medical intervention to salvage the graft from failure. Recipients' urine samples are valuable for deduction of donor HLA typing.
2. In 727 urine samples collected from recipients of kidney transplantations, donor mismatched HLA antigens were successfully deduced from 79.0% of the samples.
3. Anti-HLA IgG antibodies against HLA Class I and Class II antigens were detected in 27.9% of the patients. Presence of donor-specific antibody (DSA) was found in 11.1% of the patients. The DSA correlation rate was comparable to that in patients who received transplantations in Hong Kong with known donor typing.
4. With early detection of DSA, AMR were under control in 88.5% patients. Allograft failure with histologic AMR was found in 11.5% of patients before the commencement of this study.
5. This protocol can complement earlier transplant recipients with incomplete donor typing of the newly defined antigens such as HLA-C and -DQ.

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Introduction

Kidney transplantation is the most cost-effective treatment for end-stage kidney diseases. Its survival outcomes is superior to other treatment modalities. However, there is a severe shortage of kidney donors in Hong Kong. Many patients have their transplantations performed outside Hong Kong because of the better accessibility to kidney transplantation. These patients eventually return to Hong Kong healthcare system for subsequent clinical management, including immunosuppressive therapy and follow-up care. In case of graft failure, these patients will get back on the waiting list for renal replacement and pose a burden on the healthcare system.

New immunosuppressive regimens and advancements in drug efficacy in controlling T-cell alloimmunity have reduced episodes of T-cell-mediated acute rejection considerably. Nonetheless, antibody-mediated rejection (AMR) still plays a major role in acute and chronic allograft rejection. Development of de novo donor-specific antibody (DSA) also leads to chronic allograft rejection. With the improvement in both sensitivity and specificity of antibody identification, DSA could be detected in its early stage in patients who received kidney transplantations locally. The test results facilitate early clinical intervention by adjusting the immunosuppressant dosage and minimising expensive treatments.

Currently, more than two-thirds of the transplant patients received their kidney transplantations outside Hong Kong, with unknown donor human leukocyte antigen (HLA) typing and specificities of the anti-HLA antibodies. There is an urgent need to determine the donor HLA typing and DSA for local clinical management. We developed a protocol using a convention HLA typing

method to deduce donor mismatched HLA typing from recipient allograft biopsy.[1] To circumvent the requirement of biopsy, we developed a novel protocol using urine sample to facilitate correlation of DSA (Fig 1).[2]

We evaluated the significance of using urine sample to determine donor HLA typing in DSA correlation. We aimed to achieve a comparable DSA correlation rate for patients with unknown donor typing as that of patients who received transplantations locally. Early medical intervention improves the graft survival rate and reduces the cost of treating acute and chronic humoral rejection.

Methods

A total of 727 patients who received kidney transplantations outside Hong Kong with unknown donor HLA information were recruited. Recipient HLA typing was determined using LIFECODES Luminex-PCR-SSO typing kit (Immucor, Stanford, CT, USA). Allograft donor HLA typing was deduced from DNA extracted from fresh kidney allograft biopsies using our previously established protocol[1] with CTS-PCR-SSP tray kit (University of Heidelberg, Germany). Urine HLA typing was deduced from DNA extracted from fresh early morning urine. HLA typing was performed according to our established protocol.[2]

Sera were collected from post-transplanted patients. For the control cohort of local transplant patients, prospective sera samples were collected according to the protocol of Guarding Recipient Against Failed Transplant programme of the Hospital Authority.

The Luminex bead-based solid-phase immunoassay was used to detect and characterise IgG antibodies against HLA class I and II antigens. LABScreen Mixed kits, Single Antigen kits (One Lambda, CA, USA) and LIFECODES LSA Kits (Immucor, CA, USA) were used where appropriate.

Donor mismatched HLA antigens from urine or biopsy sample's DNA were analysed by subtracting the recipient's own HLA typing from the PCR-SSP banding pattern as described.[1,2]

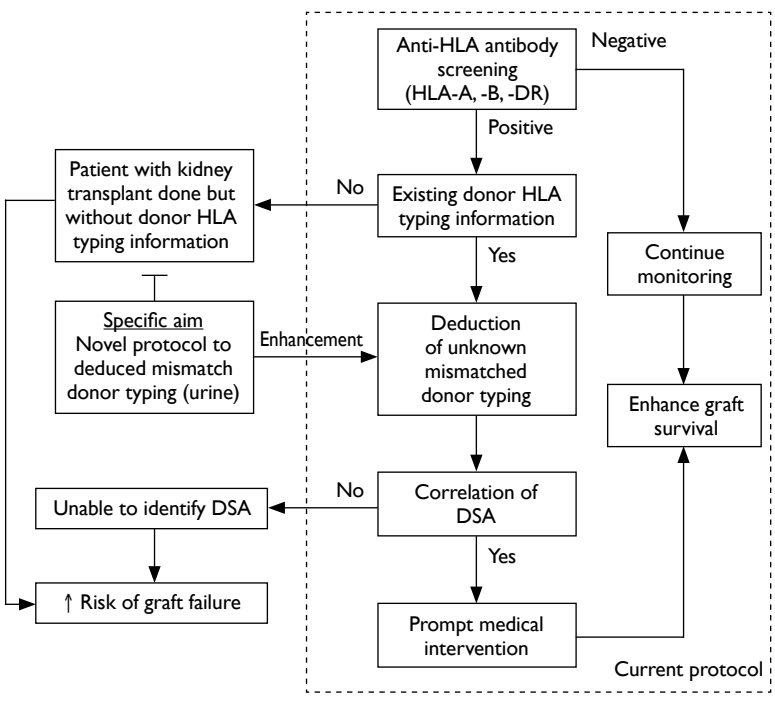

FIG 1. Clinical management protocol for kidney transplant recipients with unknown donor human leukocyte antigen (HLA) typing to facilitate the correlation of donor-specific antibodies (DSA), the diagnosis of antibody-mediated rejection, and early medical intervention.

Results

The 727 patients received kidney transplantations from China (98.6%) and other Asian countries (1.4%) including Taiwan, Cambodia, and Pakistan between 1 April 1989 and 31 March 2018. 80.5% of them received their transplantation <10 years. DNA extracted from the urine samples showed that most had acceptable purity. CTS-PCR-SSP typing was performed for all samples, except for 10.9% with low DNA yield or poor A260/280 ratio (DNA issue). The urine samples were typed for HLA-A, -B, -DR, and -DQ. Additional HLA-C or -DQA1was added if indicated.

Donor mismatched HLA typing was found in 79.0% of the urine samples, whereas no mismatched HLA typing was found in 7.3% of the urine samples, which could be due to absence of HLA mismatched between donors and recipients or total chimerism in the urine samples. 100% concordance rate in the deduced donor typing between both urine and allograft biopsy samples was observed in the same 20 recipients. This suggests that our method was accurate in deducing matched donor HLA antigens.

Anti-HLA IgG antibodies were detected in the serum samples of 203 (27.9%) of 727 recipients (Fig 2). With the availability of deduced donor mismatched HLA antigen information, the presence of DSA was detected in 81 (39.9%) of 203 antibody-positive recipients (11.1% of all recruited recipients). Of the 81 antibody-positive DSA recipients, 72 (88.9%) were against HLA Class II antigens, 16 (19.8%) were against HLA Class I antigens, and 7 (8.6%) were against both HLA Class I and II antigens. 66.7% (54/81) of the antibodies were against newly defined HLA-DQ antigens, consistent with a recent report.[3] Nonetheless, the presence of DSA was unable to confirm in 4.7% of the recipients, owing to poor quality of urine DNA or presence of ambiguities of PCR-SSP.

Recipients who received kidney transplantations in Hong Kong were prospectively monitored. They were compared with those who had transplantation outside Hong Kong from 2013

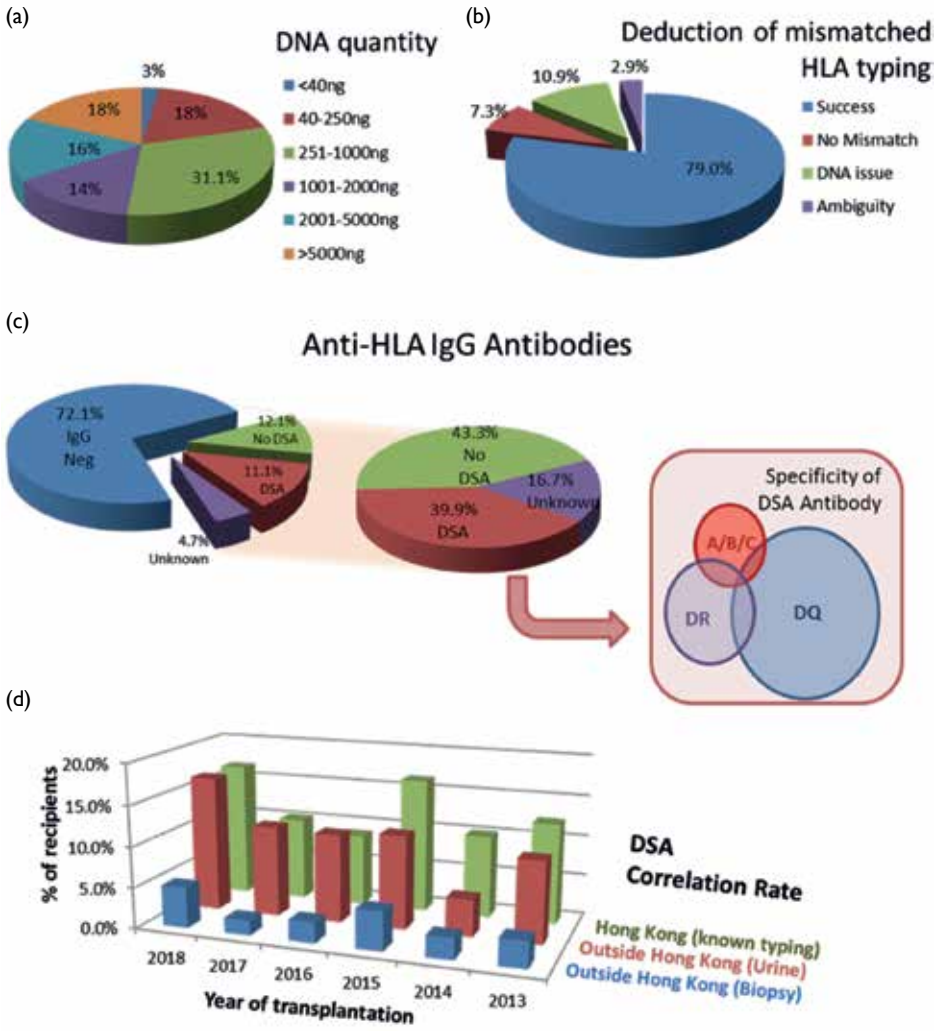

FIG 2. (a) DNA quantity of urine samples, (b) donor human leukocyte antigen (HLA) typing of urine samples, (c) anti-HLA IgG antibody of serum samples in 727 recipients. Of 203 recipients with positive anti-HLA IgG antibodies, 81 were detected to have donor-specific antibodies (DSA). (d) The correlation rate was comparable between the local cohort with known HLA typing and the outside Hong Kong cohort with unknown HLA typing who underwent urine HLA typing (P=0.852) but was significantly lower in those with unknown donor typing who relied on typing deduction from allograft biopsy (P<0.001).

to 31 March 2018. The two groups were comparable in terms of sex and follow-up period but differed significantly in terms of patient age and donor type (Table). The two groups were comparable in the detection rate of anti-HLA IgG antibodies (24.4% vs 25.6%) and average DSA correlation rate (9.6% vs 11.8%). However, for those with unknown donor typing relying only on typing deduction from allograft biopsy, the DSA correlation rate significantly dropped from 9.6% to 3.3% (P<0.001). This indicated that urine DNA could be a feasible alternative to facilitate the detection of DSA and aid clinical diagnosis.

Of 26 recipients with DSA detected, 23 (88.5%) had stable graft function with active clinical interventions such as close monitoring, adjustment of immunosuppressant regimen, biopsy diagnosis, and intravenous immunoglobulin treatment. In three (11.5%) of the 26 recipients with DSA detected, antibodies already existed before the commencement of this study while no HLA information was available when the antibody first appeared. Plasmapheresis and intravenous immunoglobulin treatment were given to the patients at a late stage; however, the damage could not be reverted and resulted in allograft lost with histologic AMR. Although DSA were found in 50 (11.8%) of 422 recipients in the local cohort, prompt clinical intervention was initiated and no AMR-related allograft failures were reported. These results suggested that prompt DSA correlation is important to guide clinical management in preventing AMR-related allograft failure.

Discussion

We used a non-invasive method to deduce

TABLE. Comparison of recipients who underwent transplantation between 2013 and 2018 outside Hong Kong or within Hong Kong

	Outside Hong Kong	Hong Kong	P value
Age, y			<0.0001
Range	23-80	6-77	
Mean	54.53	45.9	
Median	56	48	
No. (%) of patients			0.155
Male	168	237	
Female	103	183	
Donor type			<0.0001
Living	6 (2.2%)	83 (19.8%)	
Deceased	251 (92.6%)	337 (80.2%)	
Unknown	14 (5.2%)	0	
Transplantation year			0.068
2018 as of 31/3/2018	12	24	
2017	36	78	
2016	46	79	
2015	61	79	
2014	66	79	
2013	50	81	
Total	**271**	**420**	
Follow-up duration, months			
Range	10-73	10-73	
Mean	44.1	41.5	
Median	46	43	
Antibody status			0.788
IgG negative	204 (75.0%)	314 (74.4%)	
IgG positive	66 (24.4%)	108 (25.6%)	
Donor-specific antibody	26 (9.6%)	50 (11.8%)	0.748
No donor-specific antibody	34 (12.5%)	58 (13.7%)	
Unknown	7 (2.6%)	-	
Outcome			
Antibody-mediated rejection related graft failure	3	0	

mismatched donor HLA typing from the recipient urine sample using conventional PCR-SSP method. Donor mismatched HLA typing was successfully deduced in 79% of the recruited subjects. The accuracy of using urine to deduce donor mismatched HLA typing was confirmed by allograft biopsies. Around 10% DSA correlation rate was achieved and was comparable with that for the locally transplant recipients with known donor typing.

In the past, DSA correlation could only be made when impaired renal function was indicated. At the time of the diagnosis of presence of DSA or histologic AMR, the allograft damage cannot be reverted and may result in allograft loss. Therefore, availability of donor HLA information is important in clinical diagnosis and management.

In the present study, only recipients who received transplantations between 2013 and 2018 were included in the outcome analysis, because prospective anti-HLA antibody screening was introduced in 2013. Therefore, the sample size is limited, and the incidence of AMR within 5 years is relatively low. Long-term studies to review allograft survival are needed.

Deduction of donor mismatched HLA typing was hampered by technical limitations, including DNA quantity requirement and resolving power of the conventional PCR-SSP method for HLA typing. Newer techniques with higher resolution and sensitivity are warranted. Next-generation sequencing–based HLA typing has superior resolving power and higher sensitivity. It enables detection of additional loci such as HLA-C, -DQ, or even -DP, with increasing clinical significance in antibody-mediated rejection.[4]

Conclusion

Recipient's urine sample is accurate in deducing matched donor HLA antigens. It circumvents the need for renal biopsy and facilitates the detection of DSA and aid clinical management of kidney transplant patients. Our protocol has been implemented since October 2018.

Funding

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