Virtual Crossmatching in Kidney Transplantation, Shiraz Experience in Development of a Web-Based Program

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ABSTRACT

Background: Kidney transplantation can increase survival and quality of life in patients with end-stage renal disease. In any allocation system, the crossmatch test plays an essential role in donor-recipient compatibility.

Objective: In this study, we aim to test the benefits of a web-based program that captures HLA antibody analyses and provides a report to allow fast and accurate virtual crossmatches.

Methods: One hundred potential recipients in the waiting list of renal transplants were selected. The included patients all had a complete HLA antibody profile. Also, 10 potential donors from previous kidney transplants (2020), with available HLA typing results for A, B, and DR locus, were also selected. A comparison was made between 100 recipients against ten potential donors, and virtual crossmatching (VXM) was performed by the web-based program and manually by an experienced immunologist.

Results: The average time for a manual VXM was 30 minutes per patient, while the virtual cross web-based program took 5 minutes per patient. In 12% of the manual VXM cases, a secondary review of data improved final results. In two manual virtual crossmatches, the VXM results had errors in matching recipient antibodies with the donor HLA typing that could affect the final decision for transplantation.

Conclusion: In conclusion, a web-based VXM program that assesses HLA data can accurately perform a VXM with fewer human errors. It is especially true for highly sensitized candidates.

KEYWORDS: Kidney transplantation; HLA; Virtual crossmatch

INTRODUCTION

Anti-human leukocyte antigen (HLA) antibodies in recipients of renal transplants are associated with an increased risk of graft loss and rejection. These antibodies can be discovered by testing for donor-recipient crossmatch prior to transplantation. Donor specific antibodies (DSA) are commonly generated by blood transfusions, pregnancies, or previously rejected transplants [1]. Patients with anti-HLA antibodies are at risk for hyper acute rejection and delayed graft function [2]. Therefore, Kidney transplantation in these patients remains challenging in any allocation system, and it is difficult to find a proper donor. These patients may spend prolonged periods on the waiting list, which increases their morbidity and mortality [3, 4].

The compatibility of donor and recipient is assessed by physical crossmatching in most allocation programs just before the transplantation. Physical crossmatch is being done by

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Table 1: Demographic data of 100 recipients.

Sex - no. (%)	
Femele	40 (40)
Male	60 (60)
Blood group - no. (%)	
A	26 (26)
B	10 (10)
AB	12 (12)
O	52 (52)
Age - yrs (mean)	
Femele	42.3
Male	45.2

cytotoxic assays or by flow cytometric methods. Physical crossmatching is costly, needs expert personnel, and is time-consuming [5, 6]. With solid-phase immunoassays such as single antigen beads (SAB) in Luminex assay, compatibility between donor and recipients can be assessed without the need for physical crossmatching. In this method, the recipient's HLA antibody and donor HLA typing are evaluated, and the technique is called virtual cross-matching (VXM) [6].

In VXM, the unacceptable HLA antibodies of the recipient will be compared with the donor's HLA typing to find the most compatible donor [7]. The presence of donor-specific antibodies indicates a positive VXM, which is a contraindication for transplantation [8]. Nowadays, the number of centers using VXM as the primary method for crossmatching is increasing [7-12]. For example, a single center experience by Puri, *et al*, revealed that VXM could eliminate the physical crossmatch test, minimize cold-ischemia time (CIT), and accurately predict the results of flow cytometric crossmatch [11].

Our center started using solid-phase immunoassays for the detection of unacceptable HLA antibodies in 2018. Our unpublished experience showed that Luminex immunoassay was more sensitive than complement cytotoxicity or flow cytometry tests to detect DSA. So, since January 2020, we started using the VXM as the first test for assessing the compatibility of donors and recipients. A considerable number of our patients on the waiting list are sensitized. Anti-HLA antibodies in these patients are a mixture of HLA class 1 and class 2 antibodies. The number of these antibodies is sometimes more than fifty in each panel, which is hard to match with donor HLA to find unacceptable antigens. In this situation, human error may affect the result. This gap in VXM encouraged us to build a program for VXM. So, we have designed a web application to match each candidate's unacceptable antigens to the donor HLA typing.

In this study, we aim to evaluate the benefits of web-based software compared to the manual VXM in the analysis of DSA and other risk factors to allow fast and accurate VXM for kidney transplantation.

MATERIALS AND METHODS

Our web-based program used anti-HLA antibody data acquired from SAB panels and matches each unacceptable antigen to the donor HLA typing.

Recipients' sera were evaluated for HLA class I and class II antibody using SAB Luminex technology (Lab Screen Single Antigen, USA) against HLA-A, B, C, DRB1, DQB1, and DPB1.

We used two independent reviewers for HLA antibody assignments in the web-based program, and the intensity of antibodies was based on the median fluorescent intensity (MFI) threshold. HLA antibody against HLA class I and class II were reported in high resolution (4-digit level) format. According to the MFI of each SAB, the strength of each antibody was classified as strong (>1000) and moderate (500–1000). Beads with MFIs of <500 were considered as negative. For VXM purposes, the highest MFI for each HLA antibody was considered.

We normalized the MFI signal for each SAB against the negative control to correct the nonspecific binding. HLA typing for the lo

Table 2: Demographic and HLA data of ten potential donors.

Name	Blood Groups	Sex	Age	HLA A	HLA A	HLA B	HLA B	HLA DRB1	HLA DRB1
Donor 1	O	Male	35	A*02	A*32	B*51	B*51	DRB1*03	DRB1*11
Donor 2	A	Male	36	A*02	A*24	B*39	B*58	DRB1*03	DRB1*15
Donor 3	AB	Female	31	A*24	A*68	B*38	B*55	DRB1*11	DRB1*14
Donor 4	O	Female	26	A*02	A*68	B*35	B*50	DRB1*03	DRB1*13
Donor 5	O	Male	60	A*02	A*24	B*35	B*50	DRB1*07	DRB1*10
Donor 6	B	Male	34	A*02	A*31	B*08	B*40	DRB1*11	DRB1*16
Donor 7	AB	Female	54	A*02	A*68	B*18	B*52	DRB1*11	DRB1*16
Donor 8	O	Female	29	A*02	A*29	B*35	B*41	DRB1*07	DRB1*11
Donor 9	O	Male	43	A*03	A*11	B*18	B*35	DRB1*01	DRB1*11
Donor 10	A	Male	51	A*02	A*32	B*07	B*35	DRB1*01	DRB1*11

ci-A, -B, -DRB1 for each potential donor was recorded for each program. In the presence of DSA, the recipient was highlighted in the application based on the strength of DSA (as strong or moderate).

We also considered HLA typing results for each candidate report HLA mismatches between donor and recipient. Additional alerts in application included ABO matching, the illustration of sensitized patients based on calculated panel reactive antibody (cPRA), and the United Nation of Organ Sharing (UNOS) score of the recipient [13]. Donor HLA-typing for the loci-A, -B, -DRB1, was performed by the immunology lab of Abu Ali hospital affiliated to Shiraz University of Medical Sciences, using the polymerase chain reaction (PCR) method by Qiagen kit. HLA-alleles of each locus were recorded in the web-based program at low resolution (the 2-digit) level.

One hundred potential recipients with a complete HLA profile on the waiting list were randomly selected. Ten potential donors with available HLA typing for A, B, and DR loci from previous kidney transplants in 2020, were also selected randomly for the study. Informed written consent was obtained from all participants at time of enrolment. The study protocol was approved by the Ethics Committee of Shiraz University of Medical Sciences. The data of ten donors is listed in Table 1.

Our web-based program was using structured query language (SQL) server 2016, which is a relational database management system and is made up of a collection of tables that matches each donor's against the recipients by a two-step process. First, compatible ABO blood groups of each donor and each recipient are found, and in the second step, HLA-typing of each ABO compatible donor is checked with the DSAs of recipients. A comparison was made between all compatible recipients against all potential donors, and VXM was performed using the web-based program and manually by an experienced immunologist. The average time for a manual VXM was 30 minutes per patient, while the virtual cross web-based program took 5 min per patient. In manual VXM of highly sensitized patients, it took more time and was done more repeatedly due to more anti-HLA antibodies in these patients. In the web-based VXM, there was no difference in time between sensitized and non-sensitized patients.

Patients were included in this study if they were adults (>18 years of age). Patients were excluded if their panels of antibodies or their HLA typing were incomplete. All statistical analyses were conducted using SPSS v24.0.

RESULTS

Among 100 selected recipients, 60 (60%) were males, and 40 (40%) were females. The average

Table 3: The result of the virtual cross-match (according to MFI) was compared between Donor 1 and Recipients.

Name	Blood Groups	Sex	Age	Match level in HLA	CPRA	UNOS Score	Virtual cross match Result
Recipient 1	A	51	F	3/6	52	2296	Positive
Recipient 2	B	49	M	3/6	0	1	Negative
Recipient 3	AB	37	M	3/6	91	2637	Positive
Recipient 4	A	30	F	3/6	63	868	Negative
Recipient 5	B	62	F	3/6	0	612	Negative
Recipient 6	O	46	M	3/6	90	964	Positive
Recipient 7	O	21	F	2/6	76	1325	Positive
Recipient 8	O	64	M	2/6	0	498	Negative
...	...	...	...	...	...	...	...
Recipient 99	O	40	M	2/6	0	655	Negative
Recipient 100	O	63	F	2/6	34	4522	Positive

CPRAl: calculated panel reactive antibody; UNOS: united nation of organ sharing

age of men was 45.2 (range: 29-61), and the average age of females was 42.3 (range: 38-55). The majority (62%) of these candidates were sensitized with cPRA>20%, and 12 candidates had cPRA of 99-100%. Other recipient's data, including blood group, age, the panel of HLA typing, cPRA, and the panel of single bead antibodies, were recorded in the recipient's profile in a web-based program. The type of blood groups of recipients is listed in Table 1.

Among 10 selected potential donors, 6 were males, and 4 were females (shown in Table 2). The average age of men and women among donors were 46.5 (range: 34-60) and 35 (range: 26-54) years old, respectively. Demographic data such as blood group, age, and the panel of HLA typing were recorded in the donor profile in the web-based program.

A comparison was made between all compatible recipients against all potential donors. The average time for a manual VXM was 30 minutes per patient, while the virtual cross web-based program took 5 minutes per patient.

Nevertheless, in most cases, manual VXM analysis was performed repeatedly for the same patient. In 12% of manual VXM cases, a secondary review of data improved final results. In two manual VXM, the VXM results had errors in matching recipient antibodies with the donor HLA typing that could affect the final decision for transplantation.

In the web-based program, with regards to transplant characteristics, recipient cPRA, HLA mismatches, age of donor and recipient, UNOS score of the recipient, and the result of the VXM in two levels (depends on MFI) were compared. Table 3 shows the result of the VXM (depends on MFI) between donor 1 and recipients. The web-based virtual application also records all former data to help decide whether to accept or reject the current offer.

DISCUSSION

Physical crossmatching has been the usual way for preventing hyper acute rejection since the introduction of the cytotoxic crossmatches by Patel and Terasaki in 1969 [14]. In order to perform physical crossmatch, access to donor lymphocyte cells is still necessary [15].

The introduction of the SAB was the primary cause of using the VXM before transplant and not performing physical crossmatch [16, 17]. With VXM and SAB testing, we were able to safely transplant patients efficiently, irrespective of cPRA [18, 19]. The results of previous studies demonstrated that VXM results could be used for renal transplantations even for

highly sensitized patients. These results can predict the final crossmatch and have acceptable concordance with physical crossmatch [20, 21].

For VXM, we created a web-based program to show the benefits of having a digital program in the kidney allocation system. In transplant centers that are using VXM in their allocation system, it is necessary to create such a program to facilitate the process of crossmatching [22]. The importance of these programs is mostly illustrated in the VXM of highly sensitized patients (with cPRA>80 %) because these patients have high levels of antibodies, and performing manual VXM for them can be time-consuming with a higher chance of mistake in VXM [23]. Two errors in manual VXM occurred in our study among highly sensitized patients with cPRA>99%. These false-positive results in manual VXM were due to mistakes in matching recipient HLA antibodies and donor HLA typing by an immunologist. Our work was the same as that of the study by Vega, *et al*, which designed the program for VXM and compared it to manual crossmatch. Their result showed the VXM program is beneficial, especially for highly sensitized patients, because it increases efficiency, reduces possible errors, and helps fast and accurate VXM [24]. Highly sensitized patients have priority to other candidates for kidney transplantation, and error in VXM in these patients could cause major issues [25, 26]. Candidates with high cPRA (>98%) have been given higher priority over the local ones with lower cPRA in the new update of the Kidney allocation system (KAS) (27, 28).

Many patients on the waiting list perform SAB testing every six months or after any sensitizing event. Many of these antibodies may change over time, but they are essential in graft survival and should be included in the VXM [29, 30]. By using software, it is easier to add them in the recipient profile of the antibody and then to perform a VXM in comparison to checking them manually [31, 32].

The advantage of our web-based program is that it can sort the candidate based on HLA matching, CPRA, or the UNOS score. Such a program can help our coordinators to select the best candidate for kidney transplantation, and can especially be helpful for highly sensitized patients.

One limitation of our VXM program in our center is that HLA typing of each locus was entered in the computer program at the 2-digit level. Still, our Luminex device reported HLA antibodies for HLA class I and class II at the 4-digit level format. So, the program compares unacceptable antigens in 2 digits to calculate probabilities of all possible low-resolution donor HLA alleles.

In conclusion, our data demonstrate that virtual crossmatch can be a time-consuming challenge for centers that do not record all HLA antibodies as unacceptable, especially for sensitized waitlist candidates. In these centers, the result of the virtual crossmatch for potential donors must be reviewed, and the result may take about one hour of the initial offer to prepare. A web-based virtual crossmatch program that assesses HLA data can increase accuracy and reduce human errors in evaluating risk for highly sensitized candidates.

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REFERENCES

1. de Klerk M, Witvliet MD, Haase-Kromwijk BJ, *et al*. Hurdles, barriers, and successes of a national living donor kidney exchange program. *Transplantation* 2008;**86**:1749-53.
2. Ferrari P, Woodroffe C, Christiansen FT. Paired kidney donations to expand the living donor pool: The Western Australian experience. *Med J Aust* 2009;**190**:700-3.
3. Ferrari P, De Klerk M. Paired kidney donations to expand the living donor pool. *J Nephrol* 2009;**22**:699-707.
4. Keizer KM, de Klerk M, Haase-Kromwijk BJ, Weimar W. The Dutch algorithm for allocation in living donor kidney exchange. *Transplant Proc*

2005;**37**:589-91.

5. Sayer D, Whidborne R, Brestovac B, *et al*. HLA-DRB1 DNA sequencing based typing: An approach suitable for high throughput typing including unrelated bone marrow registry donors. *Tissue Antigens* 2001;**57**:46-54.
6. de Klerk M, Witvliet MD, Haase-Kromwijk BJ, *et al*. A highly efficient living donor kidney exchange program for both blood type and crossmatch incompatible donor-recipient combinations. T*ransplantation* 2006;**82**:1616-20.
7. Johnson RJ, Allen JE, Fuggle SV, *et al*. Early experience of paired living kidney donation in the United kingdom. *Transplantation* 2008;**86**:1672-7.
8. Saidman SL, Roth AE, Sonmez T, *et al*. Increasing the opportunity of live kidney donation by matching for two- and three-way exchanges. *Transplantation* 2006;**81**:773-82.
9. Tait BD. Solid phase assays for HLA antibody detection in clinical transplantation. *Curr Opin Immunol* 2009;**21**:573-7.
10. Tambur AR, Bray RA, Takemoto SK, *et al*. flow cytometric detection of HLA-specific antibodies as a predictor of heart allograft rejection. *Transplantation* 2000;**70**:1055-9.
11. Puri S, Kopchaliiska D, Roberts J, Tomlanovich S. Successful Use of Virtual Crossmatch (VXM) in Deceased-Donor Renal Transplantation (DDRT): A Single Center Experience. *Transplantation* 2014;**98**:600.
12. Eby BC, Redfield RR, Ellis TM, *et al*. Virtual HLA crossmatching as a means to safely expedite transplantation of imported pancreata. *Transplantation* 2016;**100**:1103.
13. Scandling JD, Norman DJ. United network for organ sharing (UNOS) organ allocation policy and kidney utilization. American Journal of Kidney Diseases. 2010 Jul 1;56(1):7-9.
14. Patel R, Terasaki PI. Significance of the positive crossmatch test in kidney transplantation. *N Engl J Med* 1969;**280**:735-9.
15. Gibney EM, Cagle LR, Freed B, *et al*. Detection of donor-specific antibodies using HLA-coated microspheres: Another tool for kidney transplant risk stratification. *Nephrol Dial Transplant* 2006;**21**:2625-9.
16. Bielmann D, Honger G, Lutz D, *et al*. Pretransplant risk assessment in renal allograft recipients using virtual crossmatching. *Am J Transplant* 2007;**7**:626-32.
17. Duquesnoy RJ. Antibody-reactive epitope determination with HLA Matchmaker and its clinical applications. *Tissue Antigens* 2011;**77**(6):525-34.
18. Milius RP, Heuer M, Valiga D, *et al*. Histoimmunogenetics Markup Language 1.0: Reporting next generation sequencing-based HLA and KIR genotyping. *Hum Immunol* 2015;**76**:963-74.
19. Paunić V, Gragert L, Schneider J, *et al*. Charting improvements in US registry HLA typing ambiguity using a typing resolution score. *Hum Immunol* 2016;**77**(7):542-549.
20. Bielmann D, Honger G, Lutz D, *et al*. Pretransplant risk assessment in renal allograft recipients using virtual crossmatching. *Am J Transplant* 2007;**7**(3):626-32.
21. Worsley CM, Mayne ES, Suchard MS. Luminex-based virtual crossmatching for renal transplantation in South Africa. *S Afr Med J* 2011;**102**:40-3.
22. Peng B, Zhuang Q, Yu M, *et al*. Comparison of Physical Crossmatch and Virtual Crossmatch to Identify Preexisting Donor-Specific Human Leukocyte Antigen (HLA) Antibodies and Outcome Following Kidney Transplantation. *Med Sci Monit* 2019;**25**:952.
23. Shrestha S, Bradbury L, Boal M, *et al*. Logistical Factors Influencing Cold Ischemia Times in Deceased Donor Kidney Transplants. *Transplantation* 2016;**100**(2):422-8.
24. Vega RM, Kaplan I, Jackson AM. P060 Next generation virtual crossmatch: A program that captures data and provides rapid and accurate virtual crossmatch assessments. *Hum Immunol* 2017;**78**:98.
25. Lunz J, Hinsdale L, King C, *et al*. The coordination of allocation: Logistics of kidney organ allocation to highly sensitized patients. *Hum Immunol* 2017;**78**:16-8.
26. Sullivan HC, Dean CL, Liwski RS, *et al*. Utility of the physical crossmatch for living donor evaluations in the age of the virtual crossmatch. *Hum Immunol* 2018;**79**:711-5.
27. Eby BC, Redfield RR, Ellis TM, *et al*. Virtual HLA Crossmatching as a Means to Safely Expedite Transplantation of Imported Pancreata. *Transplantation* 2016;**100**(5):1103-10.
28. Morris GP, Phelan DL, Jendrisak MD, Mohanakumar T. Virtual crossmatch by identification of donor-specific anti-human leukocyte antigen antibodies by solid-phase immunoassay: a 30-month analysis in living donor kidney transplantation. *Hum Immunol* 2010;**71**:268-73.
29. Paramesh AS, Neidlinger N, Salvatore M, *et al*. OPO Strategies to Prevent Unintended Use of Kidneys Exported for High PRA (>98% cPRA) Recipients. *Am J Transplant* 2017;**17**(8):2139-43.
30. Piazza A, Ozzella G, Poggi E, *et al*. Virtual crossmatch in kidney transplantation. *Transplant Proc* 2014;**46**:2195-8.
31. Jaramillo A, Reddy KS, Heilman RL. Using the virtual crossmatch to allow for safer and more efficient kidney transplantation of highly sensitized patients. *Transplantation* 2020;**104**:1121-2.
32. Morris AB, Sullivan HC, Krummey SM, Gebel HM, Bray RA. Out with the old, in with the new: Virtual versus physical crossmatching in the modern era. *HLA* 2019;**94**:471-81.