Association of Panel Reactive Antibodies (PRA) with Complement Dependent Cytotoxicity (CDC) Cross-Match in Pre-Renal Transplant Recipients

Muhammad Hussain, Muhammad Mukarram Bashir, Hamid Nawaz Tipu, Muhammad Dawood, Noor Afshan, Ayesha Arooj

Armed Forces Institute of Pathology/National University of Medical Sciences (NUMS) Rawalpindi, Pakistan

ABSTRACT

Objective: To determine the association of panel reactive antibodies (PRA) with complement-dependent cytotoxicity (CDC) cross-match in Pakistani pre-renal transplant recipients.

Study Design: Cross-sectional study.

Place and Duration of Study: Immunology Department, Armed Forces Institute of Pathology, Rawalpindi from Oct 2017 to Oct 2018.

Methodology: A total of 162 patients referred to the Department of Immunology for pre-transplant workup for renal transplantation were included. Informed consent was taken, and detailed history was recorded. Frequency and percentages were calculated for cross-match positivity and most frequent anti-HLA antibodies.

Results: Panel reactive antibodies (PRA) were present in 48 patients (30%), while complement-dependent cytotoxicity (CDC) cross-match was positive in 16 patients (10%). Out of 141 male patients, 35 (25%) were positive for PRA, while 10 (7%) had positive CDC cross-match. Out of 21 female recipients, 13 (62%) were positive for PRA, and 6 (28%) had positive CDC cross-match. One male patient positive for CDC cross-match was negative for PRA. Patients positive for both CDC cross-match and PRA have an average mean fluorescent intensity (MFI) of more than 4000. CDC cross-match and PRA were strongly associated, whereas no significant association was found between CDC cross-match and anti-MIC antibodies.

Conclusion: Complement dependent cytotoxicity (CDC) cross-match and panel reactive antibodies (PRA) should be routine in patients undergoing renal transplants as alone CDC cross-match can give false negative or false-positive results. At the same time, CDC cross-match lacks detection of anti MIC antibodies involved in graft rejection.

Keywords: Complement dependent cytotoxicity (CDC) cross-match, Major histocompatibility complex (MHC) class l related chain, Panel reactive antibodies (PRA), Renal transplant.

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INTRODUCTION

Panel reactive antibodies (PRA) are the pre-existing antibodies produced against HLA antigens in the serum of potential allograft recipients, detected by Luminex. The presence of antibodies against human leukocyte antigen (HLA) molecules, which may be directed against HLA class I and class II antigens, is a risk factor for hyper-acute rejection, acute rejection and graft loss.[1] The primary technique for anti-HLA antibody detection was complement-dependent cytotoxicity (CDC), and CDC cross-match has been the gold standard for many years. However, false-positive results can be seen in complement-dependent cytotoxicity related assays.[2] The results of the CDC assay are also related to many limitations such as cell panel used, quality of lymphocytes, rabbit complement, non-HLA complement-fixing antibodies, the low titre of HLA antibodies and low sensitivity when compared with Luminex. As an alternative approach, solid-phase based assays as HLA antigen-coated bead methods have been introduced, which function independently of cell quality and have a higher sensitivity and specificity in detecting anti-HLA antibodies by using Luminex.[3] Each population and its ethnic differences have different demography of HLA antigens, and so the PRA test panel will differ from region to region.[4] Before the transplant, PRA detection is done to identify sensitized patients, which significantly impacts patient mortality and morbidity due to prolonged waiting time and may delay transplantation.[5]

A high PRA means that the individual is primed to react immunologically against a large proportion of the population.[6] These antibodies may develop with previous blood transfusion, pregnancy and transplant, and the degree of sensitization is more substantial and more prolonged when different causes act together.[6] HLA–A, HLA–B, HLA–DR compatibility between recipient and donor does not guarantee rejection free kidney transplant. At this moment, anti–HLA antibodies and non-HLA antibodies detection and identification represent one of the most important points in

Correspondence: Dr Muhammad Hussain, Consultant Immunologist, Armed Forces Institute of Pathology, Rawalpindi Pakistan

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transplant work up,[7] Compared with complement-dependent cytotoxicity cross-match, Luminex assays provide greater sensitivity and specificity in detecting donor-specific antibodies.[8] MIC antigens are not expressed on lymphocytes, so commonly used me-thods such as CDC cross-match or flow cross-match do not detect the antibodies against MIC antigens.[9] In this article, MIC antibodies detected by Luminex are also included under the heading of PRA.

Many studies concluded that the estimated risk of sensitization of patients must be determined by a combination of CDC cross-match and PRA.[10] The subject needs extensive research as there is a paucity of data in the local population to find out and document the panel reactive antibodies, MHC class 1 related chain and association of PRA & CDC cross match. This study will be useful to determine the individual and collective significance of PRA and CDC cross-match in renal pre-transplant compatibility of donors with potential recipients. The development of a transplant bank can be a futuristic approach.

METHODOLOGY

A cross-sectional study was conducted at the Department of Immunology, Armed Forces Instiute of Pathology, Rawalpindi, from October 2017 to October 2018, in which 162 patients with end-stage renal disease (ESRD) were included by using non-probability consecutive sampling technique after approval of the Ethical Review Board, certificate number IRB/17/342. A sample size of 161 patients was calculated with WHO calculator with the confidence level of 95%, the absolute precision required was 7%, and the anticipated population proportion was 29% for PRA.[11]

Inclusion Criteria: Patients of both genders and age range from 14-60 years, waiting for a renal transplant and suffering from ESRD were included.

Exclusion Criteria: Patients other than Pakistani nationality were excluded from the study.

Informed consent was taken from the patients, and detailed history was recorded, including blood transfusion, pregnancy and re-transplant. 3 ml of serum sample was collected for PRA from the patient, and PRA in patient sera was detected by microbead array using Luminex one lambda (USA) kit. For the CDC cross-match, 10 ml heparinized donor blood, and 3 ml patient serum samples were also required. CDC cross-match was performed on fresh blood samples using T and B lymphocytes of the donor.

SPSS-23 was used for the data analysis. Frequency and percentages were calculated for CDC cross-match, PRA positivity, gender distribution, and effect modifiers, and the chi-square test was applied to determine the association. The *p*-value of ≤0.05 was considered statistically significant.

RESULTS

Of 162 patients, 141 (87%) were males, and 21 (13%) were females. PRA was present in 48 recipients (30%). CDC cross-match was observed positive in 16 (10%) recipients and out of these 16 CDC positive, 12 recipients were positive for PRA as shown in Figure-1.

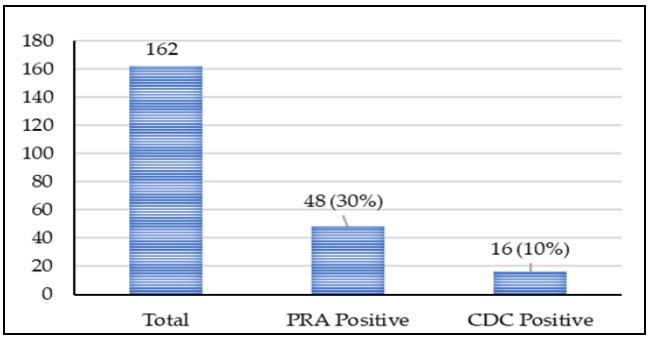

Figure-1: Panel reactive antibodies (PRA) & Complement dependent cytotoxicity crossmatch (CDC) Positive patients waiting for renal transplantPRA Distribution.

In 141 male recipients, 35 (25%) were positive for PRA, and 10 (7%) had positive CDC cross-match. Out of these 35 PRA positive males, six had positive CDC cross-match, six had a transfusion, and one man had a history of a previous kidney transplant. In 21 female recipients, 13 (62%) females were positive for PRA (no female was positive for MIC antibodies), and 6 (28%) had positive CDC cross-match. Of these 13 PRA positive females, all thirteen had multiple pregnancies in their life, six had a blood transfusion, one female had previous kidney transplant history, and six females were positive for CDC cross match.

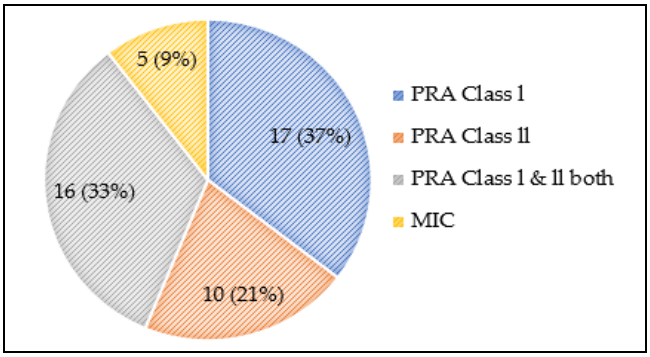

Figure-2: Panel reactive antibodies (PRA) class I, II & Major histocompatibility complex class I related chain (MIC) distribution.

Out of total positive 48 (30%) patients, 17 (35%) were positive for class l, 10 (21%) for class ll, 16 (33%) for both class l and class ll and 5 (9%) were positive for MIC antibodies as shown in Figure-2. One male patient positive for CDC cross-match was negative for PRA, which showed that it was due to non-HLA antibodies in the patients' serum.

PRA positivity in males had less history, suggesting infection as a risk factor for de novo synthesis of PRA. CDC cross-match and PRA were strongly associated with the *p*-value of <0.001, as shown in Table. Whereas no relation between CDC cross-match and anti MIC antibodies was observed with the *p*-value of <0.013 because MIC antigens are absent on lymphocytes.

Table: Association of complement dependent cytotoxicity (CDC) with panel reactive antibodies (PRA).

Parameters	Patients Waiting for Renal Transplant		*p*-value
	Positive	Negative	
PRA	48 (30%)	114 (70%)	0.0001
CDC Crossmatch	16 (10%)	146 (90%)	

Out of the total of 48, PRA positive patients, 12 were positive for CDC cross-match and on the other hand, out of a total of 16 positive CDC cross-match, 12 patients had panel reactive antibodies with the significant *p*-value <0.0001.

DISCUSSION

Data regarding the association of CDC cross-match and PRA are lacking in our region, especially in Pakistan. The presence of antibodies against Human Leukocyte Antigen (HLA) and non HLA molecules, which may be directed against HLA class I, class II and MIC antigens, are risk factors for hyperacute, acute, chronic rejection and graft loss.[11,12]

A study conducted in 2019 analyzed 654 patients (441 males and 213 females). The age of patients ranged from 13-67 years. The study reported that 277 (39.2%) were positive for PRA and 25 (3.8%) were positive for CDC cross-match.[11] Our study showed that PRA were present in 48 (30%) recipients, which was less than the Mishra *et al*, and CDC cross-match was observed positive in 16 (10%) recipients who were higher in comparison with the study conducted by Mishra *et al*.[1]

Another study concluded that CDC cross-match could be negative because of low titter anti HLA antibodies and PRA positive with donor-specific anti-HLA antibodies with MFI less than 1000.[12] Our study also showed four patients with negative CDC cross-match and positive PRA.

Another study conducted by Lieber *et al,* showed that 7.2% positive CDC cross-match occurred among PRA negative patients, and negative CDC cross-match was observed in 6.5% of tests among 100% PRA positive patients.[13] More CDC cross-match positivity in PRA negative patients may be due to IgM antibodies or Non-HLA antibodies. Our study showed that four CDC cross-match positive and PRA negative patients were due to autoimmune and non-HLA antibodies and 36 PRA positive and CDC cross-match negative patients, which may be due to low MFI or the absence of donor-specific antibodies.

In a study conducted at a hemodialysis centre in Brazil in 2014, 70 serum samples of patients waiting for renal transplant were analyzed, 26 (37.5%) were positive for PRA, and 10 (14%) were positive for CDC cross match. Only one of the 70 patients under analysis had a positive CDC cross-match and negative PRA.[14] When these results compared with our study, which has less PRA and CDC cross-match positivity but four CDC positive and PRA negative individual due to the presence of autoantibodies or non HLA antibodies.

A study conducted by Baranwal *et al*, in 2017 analyzed 116 patients' samples waiting for renal transplants. 13 (11%) were positive for CDC cross-match, and 36 (31%) were positive for panel reactive antibodies. CDC cross-match positive samples had PRA MFI >3000.[15] In another study conducted by Lan *et al*, in Canada concluded that the threshold for positive CDC cross-match appears to be 5000 MFI in sera with multiple and 10,000 MFI with single class I donor-specific antibodies.[16] A study conducted by Pandey *et al,* showed CDC cross-match positivity in 14 patients out of 12, with PRA MFI of more than 5000.[17] Our study showed nearly the same result as of Baranwal *et al*, but in our study, PRA MFI was >4000 in CDC positive samples, and CDC assay showed cell death from 20-50%. In comparison with Lan *et al*, our study showed CDC positivity at low MFI maybe because our population was more prone to effects modifiers such as multiparity and blood transfusion.

A study conducted by Chowdhury *et al*, showed anti MIC antibodies in 67 (14.4%) males and 27 (15%) females out of 466 males and 180 females 18, whereas our study showed anti MIC antibodies in 5 (9%) males, but surprisingly no female was positive for anti MIC antibodies which may be due to small sample size of

females available for this study and further need research on a large scale.

PRA against the donor antigens are highly likely to cause transplant rejection even if the cross-match is negative. PRA were more common in recipients, who were more prone to effect modifiers such as pregnancy, blood transfusion and re-transplant. There is a need to convince renal transplant surgeons to use PRA routinely, as PRA is more sensitive and donor-specific antibodies with low titre can be missed in CDC cross match. Many studies concluded that the trusted technique of CDC cross-matching remains crucial and should be coupled with a determination of PRA by Luminex.

CONCLUSION

Complement dependent cytotoxicity (CDC) cross-match and panel reactive antibodies (PRA) should be routine in patients undergoing renal transplants as alone CDC cross-match can give false negative or false-positive results. At the same time, CDC cross-match lacks detection of anti MIC antibodies involved in graft rejection.

Conflict of Interest: None.

Authors' Contribution

MH: Concept, write up, MMB: Design, HNT:, MD: Review, NA: Interpretation of data, AA: Data collection.

REFERENCES

1. Mishra MN, Baliga KV. Significance of panel reactive antibodies in patients requiring kidney transplantation. Saudi J Kidney Dis Transpl 2013; 24(3): 495-499.
2. Mathur A, Thapa S, Jagannathan L. Luminex-based donor-specific antibody crossmatching for renal transplant: A 3-year experience in South India. Glob J Transfus Med 2018; 3(1): 34-38.
3. Moise AM, Nedelcu D, Toader A, Sora M, Tica A. Cytotoxic antibodies - valuable prognostic factor for long term kidney allograft survival. J Med Life 2010; 3(4): 390–395.
4. Keven K, Sengul S, Celebi ZK, Tuzuner A, Yalcin F, Duman T, et al. Kidney transplantation in immunologically high-risk patients. Elsevier. 2013; 45(3): 919-922.
5. Picascia A, Infante T, Napoli C. Luminex and antibody detection in kidney transplantation. Clin Exp Nephrol. 2012; 16(3): 373-381.
6. Böbrek PR, Fonksiyonu YO. The effect of panel reactive antibody results on graft functions of patients with chronic kidney failure. Turk J Immunol 2015; 3(3): 105-110.
7. Peña JR, Fitzpatrick D, Saidman SL. Complement-dependent cytotoxicity crossmatch. Methods Mol Biol. 2013; 1034(1): 257-283.
8. Zou Y, Stastny P, Süsal C, Döhler B, Opelz G. Antibodies against MICA antigens and kidney-transplant rejection. N Engl J Med 2007; 357(13): 1293-1300.
9. Oremo JAA, Xiao Z, Sha Z. MHC Class 1-related chain A and B ligands are differently expressed in cancer cell lines. Immunol Disord Immunother 2016; 1(1): 103.
10. Chowdhry M, Makroo RN, Kakkar B, Thakur Y, Kumar M, Singh M. A positive complement dependent cytotoxicity immunoglobulin G cross match due to auto-antibodies with a negative luminex bead assays in a renal transplant recipient: A Diagnostic dilemma. Asian J Transfus Sci 2018; 12(2): 160.
11. İnal A, Özçelik Ü, Ogan Uyanık E, Külah E, Demirağ A. Analysis of panel reactive antibodies in renal transplant recipients detected by Luminex: a single-center experience. Exp Clin Transplant 2016; 14(4): 401-404.
12. Rees L, Kim JJ. HLA sensitization: can it be prevented. Pediatr Nephrol 2015; 30(4): 577-587.
13. Lieber SR, Perez FV, Tabossi MR, Persoli LB, Marques SB, Mazzali M, et al. Effect of panel-reactive antibody in predicting crossmatch selection of cadaveric kidney recipients. Transplant Proc 2007; 39(2): 429-431.
14. Saito PK, Yamakawa RH, Pereira LC, da Silva WV Jr, Borelli SD. Complement-dependent cytotoxicity (CDC) to detect Anti-HLA antibodies: old but gold. J Clin Lab Anal 2014; 28(4): 275-280.
15. Baranwal AK, Bhat DK, Goswami S, Agarwal SK, Kaur G, Kaur J, et al. Comparative analysis of Luminex-based donor-specific antibody mean fluorescence intensity values with complement-dependent cytotoxicity & flow crossmatch results in live donor renal transplantation. Indian J Med Res 2017; 145(2): 222-228.
16. Lan J, Espino-Hernandez G, Gill J, Keown P. Probability of a Positive AHG-CDC Crossmatch Is Determined by the Number, Strength, and Specificity of Donor-Specific Antibodies [abstract]. Am J Transplant. 2013; 13 (suppl 5). https://atcmeetingabstracts.com/abstract/probability-of-a-positive-ahg-cdc-crossmatch-is-determined-by-the-number-strength-and-specificity-of-donor-specific-antibodies/. (Accessed on October 21, 2018).
17. Pandey P, Pande A, Kumar-Devra A, Kumar Sinha V, Prasad Bhatt A. Comparative analysis of complement-dependent lymphocytotoxicity crossmatch and flow cytometry crossmatch results versus Luminex single-antigen bead-based donor-specific IgG class I antibody MFI values in live related renal transplant cases; a retrospective observation in 102 cases. J Immunoassay Immunochem 2021; 42(3): 300-313.
18. Chowdhry M, Makroo RN, Singh M, Kumar M, Thakur Y, Sharma V. Role of anti-MICA antibodies in graft survival of renal transplant recipients of India. J Immunol Res 2018; 2018(1): 3434050.