iJRASET
International Journal For Research in Applied Science and Engineering Technology



INTERNATIONAL JOURNAL FOR RESEARCH

IN APPLIED SCIENCE & ENGINEERING TECHNOLOGY

Volume: 11 **Issue:** IV **Month of publication:** April 2023

DOI: https://doi.org/10.22214/ijraset.2023.50016

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International Journal for Research in Applied Science & Engineering Technology (IJRASET)
ISSN: 2321-9653; IC Value: 45.98; SJ Impact Factor: 7.538
Volume 11 Issue IV Apr 2023- Available at www.ijraset.com

Biostatistical Analysis of Relation between HLA-A/B and HLA-DR Eplet Mismatches and Renal Graft Outcome

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Abstract: ***Despite all the clinical interventions that happened during the last 2 decades, the unavailability of a potent donor for renal transplantation remains a concern in the field of transplantation immunology. Recent studies promise the establishment of an effective clinical method to find a potent renal transplant donor, in the near future. Combining the use of molecular techniques with biostatistical methods can be a great tool for clinicians in choosing a potent donor. The present study was conducted from a cohort of 1144 (n=572 pair) renal transplant recipients and their donors to analyse eplet matches and mismatches. Initial observation of increased serum creatinine level which ranged from 2.0 to 21.0 mg/dL in patients treated for end-stage renal disease implied a single event of graft rejection. The statistical correlation between eplet mismatches and graft survival was observed to be significant with P value 0.003. A value of -0.215 at 0.01 significance showed the positive linear correlation between the increased numbers of HLA DR eplet mismatches towards chronic renal graft rejection. The correlation coefficient of -0.888 observed between the number of HLA DR eplet mismatches and days of graft survival clearly implies the effect of increased HLA DR eplet mismatch delayed graft rejection. The study gives an idea on the minimization of class II HLA DR eplet mismatches to decreased frequency of graft rejection as indicated by increased survival rate.***
Keywords: graft rejection, eplets mismatch, HLA DR, transplantation, HLA match maker.

I. INTRODUCTION

Renal transplantation, being the therapy of choice for patients with end-stage renal disease, is highly concerned with graft rejection as there may be potential mismatches between donor and recipient's HLA allele. An eplet, set of triplet amino acids, that aids in the 3-dimensional structural conformation of an HLA allele decides the graft outcome[1]. The identification of the presence of eplets in HLA alleles and HLA eplet matching has tremendously changed the way how clinicians viewed organ transplantation and has further led to the better understanding of its relation in determining the survival of a transplanted graft[2]. After the introduction of HLA Matchmaker, an algorithm for calculating eplet mismatches, more investigations at transplant centres worldwide have suggested that HLA eplet matching is statistically associated with better transplant outcome[3,4]. There are literatures which expresses the correlation existing between low class I and class II eplet mismatches with lower antibody response induction and high eplet loads to increase risk of rejection and graft loss[5]. Several reports within the past decade have showed that minimization of mismatches between the eplets present in HLA ABC (class I) and HLA DPDQDR (class II) is better for ensuring graft survival than choosing a patient-donor allelic pair from the existing donor pool or organ library in case of renal transplantation[6–14]. Multiple researches in the past decade have demonstrated the fact that reduction of HLA-DR and HLA-DQ mismatching at the eplet level can reduces allograft rejection and can improve transplant outcome[4]. Nonetheless, not all eplet mismatches elicits an immune response, indicative of a difference in immunogenicity of individual eplets. As the allelic variations among DP & DQ are very low, HLA DR allele is mostly studied in class II. While HLA DQ allelic expression have been related with various disease conditions like the autoimmune Vogt-Koyanagi-Harada disease (VKH), celiac disease and inflammatory bowel disease (IBD), which are chronic intestinal disorders. HLA DQB1 DSA is also involved in direct promotion of inflammation via endothelial activation and indirect limitation of Treg cell expansion. HLA DR has been known to be involved commonly in lung, heart and renal transplantation[6,15–19]. Various other studies have attempted to address the effect of eplet load in renal transplant patient in case of specific kidney diseases[7,20,21].

The role of computational statistics methodologies in developing superior data analysis tools has improved the quality of 'evidence-based computational statistics'[22].

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The same has been mostly used in this study, which enables us to get a better understanding between the relation of HLA eplet mismatches and graft loss. The HLA Matchmaker algorithm is different from conventional methods of counting the numbers of mismatched HLA alleles, rather helps in identifying the presence of shared amino acid eplet patterns among dissimilar alleles, called eplet matching. In 2007, Duquesnoy and Askar demonstrated that the eplet version of HLA Matchmaker has provided itself to be clinically useful[23] and in 2019, Duquesnoy and Marrari, added that the database of the HLA Matchmaker and eplet registry needed much updating as it provides an incomplete description of the structural HLA epitope repertoire[24]. Since analysis of eplet mismatches is clinically relevant than that of identifying the presence of broad antigen mismatches HLA Matchmaker can become a strong tool in transplantation immunology.

The present study involves the biostatistical analysis between the presence of increased number of HLA DR eplet mismatches and its relation to renal graft outcome in selected cohort of south Indian population. The study was performed using SPSS V16, to carry out correlation and regression analysis which led to the present results. It was statistically observed that the increased HLA DR eplet mismatch is responsible factor for chronic renal graft rejection.

II. DESIGN AND METHODS

A. Study Population and Data Collection

The study was conducted within a cohort of renal transplant donors and recipients, between January 2016 and Feb 2020, from Transplantation Immunology and Molecular Diagnostic (TIMD) Laboratory, MIMS Hospital, Calicut, Kerala, India. The laboratory keeps details of pre- and post-transplant patients which include histocompatibility data, serum creatinine levels, biopsy details and other comprehensive clinical diagnostic details. HLA allelic data of all the patients was collected from the hospital. HLA-A/B/DR alleles of patients and donors were identified via molecular typing method of the sequence-specific primer-PCR (BD™ HLA-B27 Kit, Qume Drive, San Jose, CA). HISTO MATCH HISTO SPOT® HLA AB and HLA DR (BAG Diagnostics GmbH) SSP module has been used for the interpretation SSP-PCR results and has been used for analysis and identification of 4-digit HLA ABDR alleles of both patient and donor. All HLA-A/B/DR 4-digit allelic data were used for the eplet analysis. Comparison of recipient HLA-A/B/DR alleles and donor HLA-A/B/DR alleles were performed prior to transplantation.

B. HLA A/B/DR Eplet Analysis Using HLA Matchmaker

The allelic data obtained using molecular typing was used to find the presence and number of eplets as well as eplet mismatches between the donors and recipients using the ABC Eplet Matching Program V4.0 and DRDQDP Eplet Matching Program V3.1. The algorithm of HLA Matchmaker allows its user to easily find the number of antibody-verified mismatched eplets in each HLA allele. After typing in the alleles of both patient and donor are entered to the Matchmaker data table and the algorithm directly provides the number of eplets present and those which are mismatched. The mismatched eplets are also classified as those which are Ab-verified and those which are not. The Ab-verified eplets are those which cause a potential immunological response.

C. Statistical Analysis

Statistical analysis was performed using SPSS (Version 16). The statistical data were analysed for finding the association between HLA ABDR eplet mismatches and chance of rejection of the transplanted graft. The patients were grouped according to the higher number of eplet mismatches for HLA AB and HLA DR alleles. The distribution of donor-recipient allelic incompatibility via eplet mismatch was found using regression analysis method: HLA-A/B and HLA-DR as well as patients' clinical outcomes were evaluated as two independent variables. The association between HLA-A/B and HLA-DR eplet mismatches and graft rejection was performed using Pearson correlations. A Pearson's correlation coefficient of 0.3 showed a strong correlation between the variables, HLA AB & DR. Correlations were presented with P value and 95% confidence interval (95% CI). Comparison of Wald statistics was also performed.

III. RESULTS AND DISCUSSIONS

Of the total of 650 transplant recipients, complete HLA A/B/DR typing was available for 572 patients, with a follow up of 50 months (Fig. 1). 25 patients faced at least one episode of graft rejection during the follow up period, based on serum creatinine level and/or biopsy result. The study cohort included patients and donors age category ranging from 19 to 65 years. All the samples were tested for crossmatch negativity. Analysis of HLA ABDR alleles via HLA Matchmaker revealed the number of eplet mismatches. Compared to the recipients who did not face any kind of rejection episode, the recipients who faced rejection is more likely to have greater number of HLA ABDR eplet mismatches rather than allele mismatches.

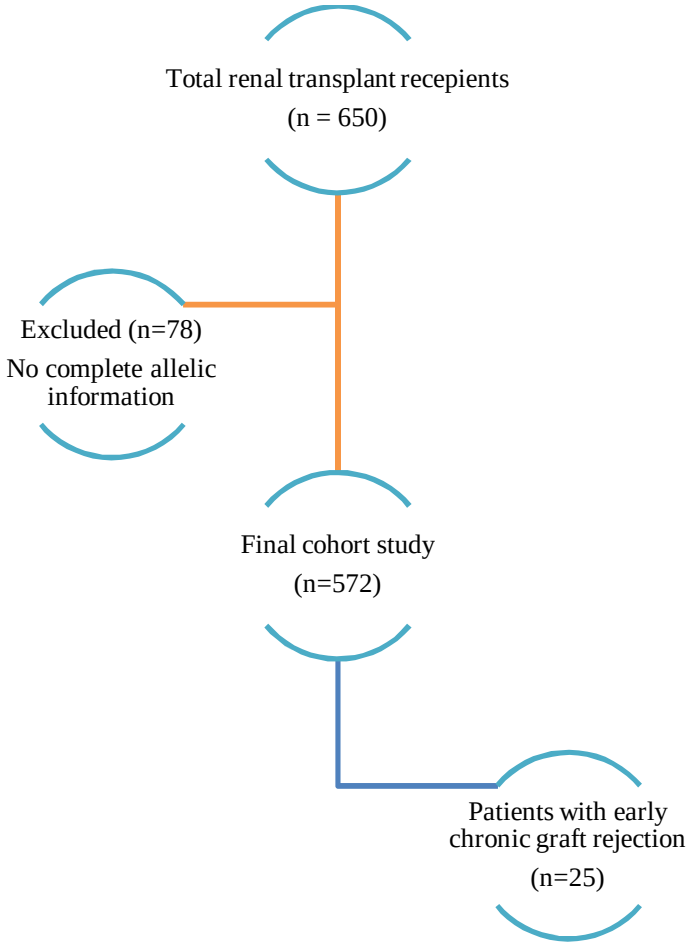


Fig 1: Study flow diagram

A. Analysis oBf HLA ABDR Allele and Eplet Mismatch

Comparison of eplets in case of the allele HLA A*02:01/26:01, A*02:11/33:01, A*11:01/24:02, B*07:02/57:01 & B*37:01/58:01 showed zero mismatch and HLA DRB1*01:01/08:01, DRB1*01:01/15:01, DRB1*01:01/16:01, DRB1*10:01/13:01, DRB1*13:01/14:01 & DRB1*14:01/16:01 showed eplet mismatch below 10, between the patient (p) and donor (d); the patients showed a mean survival period of 640 days from the date of transplantation. This clearly indicates the presence of a sharing pattern amino acid sequence in both alleles which might have reduced the chance of graft rejection. In another case, having 10 mismatches between A*02:01/11:01 (p) and A*07:02/57:01 (d), B*11:02/33:03 (p) and B*37:01/58:01 (d) and 16 mismatches between DRB1*07:01/04:01 (p) and DRB1*07:01/08:01 (d), the patient suffered chronic rejection on the 124th day, from the date of transplant (table 1). This points to the fact that increase eplet mismatch between HLA DRB1 allele and the chance of graft rejection is highly likely. Combining the matching effects of HLA AB with HLA DR, HLA AB antigen matching did not seem to affect the graft survival, while HLA DR antigen matching did show an influence in the survival of the transplanted graft. The comparison of eplet mismatches between HLA AB alleles A*11:01/32:01 (p), A*31:01/33:01 (d) & B*35:01/51:01 (p), B*44:03/51:01 (d) and HLA DR alleles DRB1*04:03/07:01 (p) & DRB1*13:02/14:04 (d) was 8 and 20 mismatches respectively; the survival period was 93 days. Similarly, the patient and donor having A*02:11/11:01 (p), A*24:02/24:02 (d), B*07:05/51:01 (p), B*07:05/08:01 (d) and DRB1*04:03/14:04 (p), DRB1*03:01/15:01 (d) allele, and patient and donor having the allele HLA A*02:01/32:01 (p), A*33:03/33:03 (d), B*07:02/48:01 (p), B*40:06/44:03 (d) and DRB1*12:02/15:02 (p), DRB1*07:01/07:01 (d), in both case the graft survived for a few months only. The eplet analysis on these HLA DR alleles directly correlates the increased number of HLA DR eplet mismatch to the early chronic graft rejection. List of total HLA ABDR eplet mismatch and total graft survival days has been provided in table 3. The median survival period of the patients with HLA DR eplet mismatch was found to be 118 days.

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ISSN: 2321-9653; IC Value: 45.98; SJ Impact Factor: 7.538
Volume 11 Issue IV Apr 2023- Available at www.ijraset.com

TABLE 1
NUMBER OF TOTAL EPLET MISMATCH OF HLA AB AND HLA DR BETWEEN PATIENT AND DONOR HAVING MISMATCHED HLA ABDR ALLELE PAIR WHICH RESULTED IN GRAFT REJECTION

Sl. no.	Total eplets		Rejected after
	AB	DR	
1	10	32	74
2	17	8	269
3	30	10	186
4	12	9	246
5	11	8	252
6	10	16	124
7	12	22	91
8	22	16	122
9	17	7	305
10	13	18	201
11	24	7	321
12	20	19	117
13	8	20	93
14	20	19	103
15	20	13	133
16	18	9	244
17	23	11	159
18	11	25	81
19	27	19	95
20	21	18	118
21	14	28	65
22	16	27	71
23	21	26	77
24	25	26	77
25	18	28	64

TABLE 2
NUMBER OF TOTAL EPLET MISMATCH OF HLA DR AND MEAN GRAFT SURVIVAL DAYS

Number of DR eplet mismatches	Mean graft survival days
Below 10	263
10-20	125
Above 20	75

The relation between increased HLA DR eplet mismatch and total days of graft survival was carried out via Pearson correlation coefficient analysis. A value of -0.888 showed strong inverse correlation between the variables.

B. Relation between HLA-A/B and HLA-DR Eplet Mismatch and Chronic Graft Rejection

The coefficient of correlation between variables HLA AB eplet mismatch and present condition of the graft (surviving or rejected) for the total sample (n = 572) is 0.076. It shows that there is a poor correlation between the two variables since the value is near to zero. The *P*-value is 0.003 which is lower than 0.05 hence there is a significant correlation between the two variables. When comparing HLA DR eplet mismatch with present condition for the total sample, the regression coefficient is found to be -.215 which is significant at 0.01. This indicates a strong negative correlation existing between the two variables.

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When comparing the Wald statistics, used for comparing statistical models for best fit criteria in logistic regression, obtained for HLA-A/B and HLA-DR eplet mismatch there was a high difference in the values. The value for HLA-A/B, 8.641, was near to zero which showed low probability of change to the existing model and the value for HLA-DR, 74.264, showed strong correlation between the independent variable and clinical outcome. The results are summarized in table 3.

The results show an increased positive and linear correlation between the numbers of HLA DR eplet mismatches towards the graft rejection. The statistical analysis reveals that the increased number of HLA DR eplet mismatches over HLA AB eplet mismatch number can cause delayed graft rejection. HLA DR eplet mismatches are highly influential in causing graft rejection. Other variables like sex and age were taken for statistical analysis but no significant results were obtained.

TABLE 3
LOGISTIC REGRESSION ANALYSIS OF HLA AB/DR EPLET MISMATCHES

		B	S.E.	Wald	df	Sig.	Exp(B)
Step 1[a]	HLA AB	.076	.026	8.641	1	.003	1.079
	HLA DR	-.215	.025	74.264	1	.000	.086
	Constant	3.472	.344	101.871	1	.000	32.190

a-Variable(s) entered on step 1: HLA AB and HLA DR
Data presented as correlation coefficient.
P-value is <0.01.

IV. CONCLUSION

The present work is a collaborative study which was conducted within the south-Indian population. The overall analysis of relation between the increased number of HLA ABDR eplet mismatches between donor and recipient showed a clinically relevant statistical significance. In the present study the presence of alleles like, class I HLA A*02:01, A*24:02, B*15:02 and HLA DRB1*07:02, DRB1*15:01 was found to be frequently occurring in the population. With the knowledge from earlier and recent reports, analysis of HLA eplet mismatches via matchmaker algorithm and biostatistical analysis, here we have discussed the existence of correlation between the increased number of eplet mismatches of HLA AB and HLA DR allele to the outcome of renal transplantation, within a local population. HLA DR is already known to be involved in chronic renal rejections in various other populations. The correlation coefficient of -0.888 observed between the number of HLA DR eplet mismatches and days of graft survival directly implies the effect of increased HLA DR eplet mismatch delayed graft rejection. This study statistically signifies the importance of eplet matching between donor and recipient for both related and unrelated donors. Furthermore, in early studies involving transplant patients who underwent renal transplantation having allelic mismatches exhibited a higher occurrence of postoperative complications, such as, late acute rejection, infections, etc. The analysis of both donor and recipient HLA ABDR allele through the matchmaker algorithm can be used as a prediction method to find the possibility of existence of a chronic rejection, mostly for patients opted with unrelated donors.

V. ACKNOWLEDGMENT

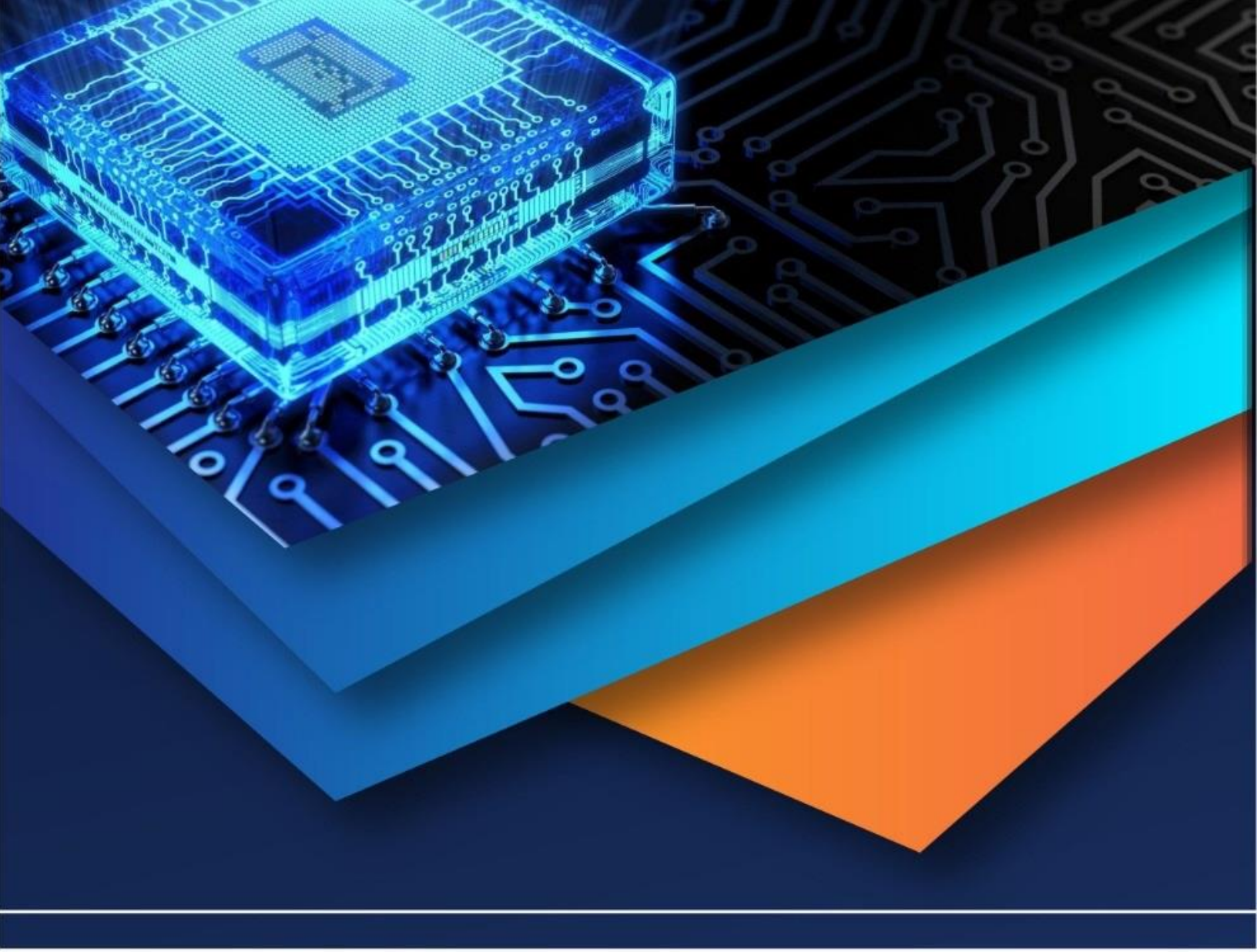
The authors acknowledge the generous contributions provided by Ms. Drishya, Mr. Ginil Benny from the Transplantation Immunology and Molecular Diagnostic Laboratory, MIMS Hospital, Calicut, India. The help offered by Mr. Praveen, Department of Statistics, University of Calicut is greatly appreciated.

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