Investigation of Anti-HLA Antibodies of Highly Sensitized Patients by Single Antigen Bead and C1q Tests

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ABSTRACT

Purpose. The single antigen bead (SAB) test contributes to conventional cellular and solid phase crossmatch tests in renal transplantation. However, the determination of anti-HLA antibodies of the patients may not reflect the pathologic features of these antibodies. Highly sensitized patients produce antibodies against a number of HLAs; therefore, their transplantation chance decreases. In this study, we aimed to evaluate SAB and C1q test results of highly sensitized patients.

Method. In this study, 33 end-stage renal failure patients with >80% panel reactive antibody were included. Of the patients, 58% (n = 19) were female, and 42% (n = 14) were male. The mean age was 46.2 ± 12.4. All of the serum samples were inactivated by heat before use. SAB and C1q tests were performed according to the manufacturer's instructions.

Results. We obtained statistically significant results between the positive bead counts and raw mean fluorescence intensity (MFI) values of 2 tests ($P < .01$ for class I and II). There was a statistically significant difference between the 2 tests in terms HLA-A, -C, -DR, and -DP MFI values, whereas HLA-B and -DQ MFI values were similar for the 2 tests.

Conclusion. The difference of raw MFI values between the 2 tests may be due to the fact that the C1q test detects only IgG1 and IgG3 antibodies, whereas the SAB test can detect all IgG subtypes. We considered that anti-HLA-B and -DQ antibodies have high complement-fixing features; these antibodies should be investigated selectively due to the similarity of anti-HLA-B and -DQ antibody MFI values in the 2 tests.

ALLOSENSITIZATION is an important problem for kidney transplant candidates. Approximately 10% to 30% of these patients are sensitized on the waiting list, and this sensitization leads to more waiting time. Under this situation, transplantation has a greater risk of rejection and graft failure [1]. Development of the single antigen bead (SAB) test based on Luminex became a milestone for anti-HLA antibody testing. It increases the sensitivity and can detect low levels of anti-HLA antibodies. Unacceptable HLAs can be identified by the SAB test. This test is called virtual crossmatch, which is especially significant for hypersensitized patients because the waiting time for organ transplantation for these patients can be reduced by using specific programs that were developed for the analysis of SAB test results. For this reason, unacceptable HLA profiles should be defined [2]. On the other hand, this technique has a very high sensitivity, and clinical significance of the donor specific antibodies detected by only the solid phase technique is contradictive. There are a number of recipients with pretransplantation donor specific antibodies detected only by the SAB test who did not have an antibody-mediated rejection episode and had long-term graft survival. Thus, the pathogenicity of detected antibodies is important.

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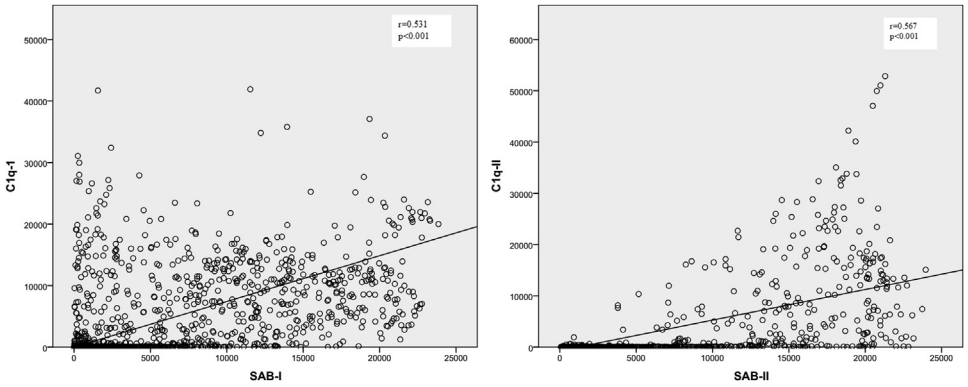

Fig 1. Correlation of raw MFI values after SAB and C1q tests.

Various factors such as antibody class, isotype specificity, strength, and the capacity of complement fixation (CF) may affect the pathogenicity. The evaluation of the CF ability of the antibodies may be significant for the characterization of the antibody because complement activation determines the cytotoxic potential of the antibody [3]. The C1q test determines CF anti-HLA antibodies with high sensitivity and specificity. C1q, which is the complement of the first step, activates the classical complement cascade. In this study, it was aimed to determine the correlation between SAB and C1q tests in hypersensitized patients [4].

MATERIALS AND METHODS

In this study, 33 end-stage renal patients with > 80% Luminex panel-reactive antibody (PRA) results who applied to our laboratory between March 2017 and June 2018 were included. The mean age was 46.2 ± 12.4. Of the patients, 58% were female, and 42% were male; 90%, 75%, and 65% had blood transfusion, transplantation, and pregnancy, respectively.

This study was performed according to the principles expressed by the Declaration of Helsinki and approved by the ethics committee of our institution (İzmir Katip Celebi University).

Of these patients, 45.4% (n = 15), 27.3% (n = 9), and 27.3% (n = 9) had > 80% class I and II PRA, only class I PRA, and only class II PRA, respectively. Twenty-four patient serum samples were tested by SAB-I and C1q-I, and 24 patient serum samples were tested by SAB-II and C1q-II. All of the serum samples were inactivated by heat before the tests. SAB (Immucor, Lifecodes Transplant Diagnostics, Stamford, Conn, United States) and C1q (C1qScreen, One Lambda, Canoga Park, Calif, United States) tests were performed and analyzed in parallel according to the manufacturer's instructions. The mean fluorescence intensity (MFI) value of 500 was accepted as the cutoff value.

Statistical Analyses

The test results were analyzed using the IBM SPSS version 22.0 (IBM, Armonk, NY, United States). The correlation of raw MFI values between SAB and C1q was assessed by the Spearman test. The number of positive beads was compared using the Wilcoxon test, and $P < .05$ was considered as significant. Mann-Whitney U test was used to evaluate the mean and total MFI values.

RESULTS

A total number of 2016 class I and 1488 class II antigens on beads were evaluated after the SAB and C1q tests. There was a positive correlation between SAB-I and C1q-I raw MFI values (r = 0.531; $P < .001$), whereas the correlation between raw MFI values of SAB-II and C1q-II was better (r = 0.567; $P < .001$) (Fig 1).

The number of positive reacted beads was counted in each condition (SAB and C1q). In the SAB-I and C1q-I tests, 50.04% (n = 1009) and 31.94% (n = 644) of the beads were positive, whereas in the SAB-II and C1q-II tests, 44.95% (n = 669) and 16.12% (n = 240) of the beads were positive, respectively. When the test results were assessed in terms of the total bead positivity, there was a statistically significant difference between the class I ($P < .001$) and II ($P < .001$) results of the 2 tests according to Wilcoxon analysis. It was determined that the mean and total MFI values of anti-HLA-B and -DQ beads obtained by the 2 tests were similar, whereas the others were significantly different (Table 1).

Table 1. The Mean and Total MFI Values of SAB and C1q Tests

	Mean MFI	*P*	Total MFI	*P*
Total SAB-I	4134	.013	73,868	.016
Total C1q-I	3755		40,040	
SAB-A	4692	.010	140,786	.020
C1q-A	1919		59,500	
SAB-B	2599	.650	129,957	.650
C1q-B	2766		138,328	
SAB-C	650	.013	11,700	.11
C1q-C	85		1363	
Total SAB-II	4231	<.001	144,202	<.001
Total C1q-II	2330		69,481	
SAB-DR	3197	<.001	124,706	<.001
C1q-DR	574		20,675	
SAB-DQ	7477	.058	231,817	.030
C1q-DQ	4222		118,228	
SAB-DP	495	<.001	13,390	<.001

Abbreviations: MFI, mean fluorescence intensity; SAB, single antigen bead.

DISCUSSION

Tissue typing laboratories should assess anti-HLA antibodies accurately [2]. The SAB technique allows the characterization of complex antibody mixtures and provides the determination of HLA specificity. Today, this test is essential for the pretransplant evaluation of sensitized patients [5]. However, the SAB technique has some problems that have not yet been solved. The prozone effect is one of the technical issues. In recent research, it has been suggested that the C3 component of complement leads to this effect by binding to the beads and preventing the binding of IgG antibodies. Preheating can solve this problem by damaging the complement activity. We also removed the prozone effect by preheating our serum samples. The SAB test detects both CF and non-CF (NCF) antibodies. Because of this, a number of transplants are denied for many patients due to donor-specific NCF antibodies, and the clinical significance of these antibodies has not been established. Most commonly, the C1q test is used by laboratories to distinguish CF from NCF HLA antibodies [5]. In our study, it was determined that positive bead count and total bead raw MFI values of SAB were significantly higher than the C1q test. We considered that the difference between the 2 tests was due to the fact that the SAB test can detect all of the IgG subtypes, whereas the C1q test can detect CF antibodies such as the IgG1 and IgG3 subtypes. When we classified the anti-HLA antibodies according to antigen groups, it was interesting that the mean and total SAB MFI values of anti-HLA-A, -C, -DR, and -DP were significantly higher, whereas the SAB and C1q MFI values of anti-HLA-B and -DQ were similar due to their high pathogenicity. This result showed that the specialists may pay more attention to HLA-B and -DQ mismatches. Today, the most common post-transplant class II anti-HLA antibody is anti-HLA-DQ antibodies, and the effects of these antibodies on graft survival has been discussed [6].

CONCLUSION

In conclusion, the SAB and C1q tests provide the assessment of the differential clinical impact of these antibodies on allograft.

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