

Comparative Study between Cytotoxicity and Flowcytometry Crossmatches before and after Renal Transplantation

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Renal transplantation, in most countries, is based on human leukocyte antigen (HLA) matching of the donor kidney with the recipient. Traditional human leukocyte antigen matching is based on defining human leukocyte antigen specificities by antibodies utilizing cytotoxicity crossmatch techniques. Newer techniques have emerged, which challenge the accuracy of serological typing and crossmatching. We compared the results of the standard complement-dependent cytotoxicity crossmatch (CDCXM) with the anti-human globulin augmented cytotoxicity (AHG-CDC), and Flowcytometry crossmatch (FCXM) for the detection of anti-HLA antibodies in 150 pre-transplant patients. The development of post-transplantation sensitization was screened utilizing these three techniques within two weeks post-operative and correlated with rejection episodes. Comparison between the results of CDCXM and AHG-CDC in 150 recipients, revealed no significant correlation ($P>0.05$). When comparing these results with that of FCXM in 50 recipients a significant correlation was shown ($P<0.05$). Relative to CDCXM, the sensitivity of AHG-CDC was 100%, specificity 97.4%, positive predictive value 92.3%, and negative predictive value 100%. On the other hand, the sensitivity of FCXM was 100%, specificity 76.3%, positive predictive value 57.1%, and negative predictive value 100%. According to the results of CDCXM, AHG-CDC, and FCXM, no difference was detected between pre- and posttransplant anti-HLA sensitization within two weeks after the operation. Patients with negative cytotoxicity crossmatch (CDCXM and AHG-CDC) and positive FCXM may have an increased risk of early graft loss and may represent a relative contraindication to transplantation. Given the important theoretical advantages of FCXM over the CDC XM, further testing of the clinical relevance is warranted.

The clinical significance of a positive crossmatch in kidney transplantation was established in the 1960s (Patel and Terasaki, 1969) by showing a high incidence of immediate failure of kidneys that were transplanted across a positive lymphocytotoxic crossmatch. The mechanism of graft failure is that preformed HLA antibodies react immediately with HLA antigens in the donor kidney. Those antibodies then bind complement, which leads to tissue destruction in the allograft, hyperacute rejection, and prompt graft loss (Scornik et al., 1992).

Crossmatching is carried out immediately before transplantation to detect antibodies directed against the donor organ. The traditional method used for the detection of donor-reactive antibodies involves incubating donor lymphocytes with sera from the

potential recipient, adding complement and determining the degree of cell killing or cytotoxicity that occurs. It is good at detecting high levels of HLA-specific antibodies directed against the donor organ. Hyperacute rejection, caused by preformed antibodies against donor HLA is now rare because of pre-transplant crossmatching. However, this method has been criticized because it does not detect low levels of anti-HLA antibodies nor does it detect the presence of non-complement-fixing donor-specific antibody. Early acute rejection and graft loss have been associated with low levels of antibody directed against HLA (Karuppan et al., 1992). To accomplish an increase in sensitivity, other crossmatch techniques have been introduced. Variants of the CDC technique have been added by modifying the incubation time or

temperature, the number of wash steps, and the addition of antiglobulin reagent. New crossmatch techniques have also been used, one of these being the flow cytometry crossmatch (FCXM). The FCXM technique is used predominantly in the United States, but an increasing number of European laboratories are in favor of this technique. The main advantages mentioned are increased sensitivity (Talbot et al., 1988), detection of non-complement-fixing antibodies (Garovoy, 1988), and correlation with transplant outcome (Kerman et al., 1990). The main disadvantages are the large number of cells needed to perform a test, the amount of serum required, and the initial cost of the apparatus. Patients who had received first transplants with a positive FC XM and a negative CDCXM were shown to have decreased graft survival (Ogura et al., 1993). Also, the number of rejection episodes was correlated with positive FC crossmatch. There was not much difference in clinical outcome when the FC crossmatch results were compared with the anti-human immunoglobulin test (Kerman et al., 1990).

The aim of this study is to evaluate the standard complement- dependen cytotoxicity crossmatch (CDCXM) utilizing dithiothriitol method for antibody screening in pre-transplant renal allograft recipients, in comparison with the anti-human globulin augmented cytotoxicity (AHG-CDC) and the Flowcytometry (FCXM) crossmatches. The development of humoral alloreactivity during the first two weeks post-operative was screened by these techniques and its association with acute rejections was evaluated.

Subjects and Methods

Subjects

Between May 2003 and December 2004, 150 adult pre-transplant-kidney recipients (93 male and 57 female) with ages ranged from 19-51 years (mean \pm SD 38.4 \pm 1.6) were undergoing dialysis and waiting for

transplantation. The 150 donors were selected on the base of ABO group and on the best donor-recipient HLA matching. Patients were selected from Ain Shams University Specialized Hospital and El Matareyya Hospital. They were monitored for anti-HLA antibodies utilizing crossmatching techniques.

They were classified into two groups

Group I: included 150 patients who were monitored by the standard complement-dependent cytotoxicity crossmatch (CDCXM) using dithiothriitol method as described by El-Masry et al, (1990); and anti-human globulin augmented cytotoxicity technique (AHG-CDC) as described by Hopkins, (1990). Fifty patients out of this group were additionally monitored with flow cytometry crossmatch (FCXM) technique as described by Robert et al, (1989).

Group II: included 15 negative CDCXM and AHG-CDC recipients out of the 50 renal transplant recipients of group I. Donor cells and recipient sera were tested for post-transplant sensitization by CDCXM, AHG - CDC, and FCXM within two weeks after the operation in order to evaluate the impact of positive FCXM on the graft rejection (0 - 8 months post- transplant).

All cases were initially subjected to the following:

1- Detailed history taking including:

- History of repeated blood transfusion.
- History of previous transplantation.
- History of previous rejection

2- Clinical examination.

3- Laboratory investigations including:

- Blood grouping for both donors and patients (according to ABO. RH. System).
- Complete blood count [CBC].
- Blood chemistry:
 - Random serum glucose
 - Renal function tests [serum urea, creatinine, and uric acid].
 - Liver function tests [Total serum protein, Serum albumin, Alanine transaminase (ALT), Aspartate transaminase (AST), and Alkaline phosphatase (ALP)].
- Complete urine analysis by urine strips.
- Serological tests for detection of viruses [Anti-HCV, HBs Ag and anti-HIV (1 & 2)].
- Detection of Cytomegalo virus antibodies (CMV Abs; IgG & IgM) in the serum of recipients and donors.

4- Tissue typing:

- Standard lymphocytotoxicity technique for HLA class I and II typing.

Samples

Blood samples from recipient and donor were collected during the period of preparation before transplant.

- 20 ml blood were collected on preservative-free heparin (200 U), from the donor and patients, for the preparation of the peripheral lymphocytes suspension necessary for performing the donor-specific and the autologous cross matches tests.
- 5 ml clotted blood were collected from the patients in clean dry tubes for obtaining sera necessary for cross matching.

Methods

Isolation of peripheral blood lymphocytes

Diluted anticoagulated blood was layered over Ficoll-Hypaque and centrifuged. The blood lymphocyte layer band over the gradient solution was collected using a fine pasture pipette and washed twice in Hank's balanced salt solution (HBSS).

Standard complement - dependent cytotoxicity crossmatch (CDCXM) with dithiothriitol method

The test was performed by adding 1 μ l of donor lymphocytes (2.0×10^6 /ml) to triplicate samples of patient serum at various concentrations i.e., 1:2 (diluted), 1:1 (undiluted) 2:1 (concentrated). Sera were pretreated with dithiothriitol (DTT) resulting in inactivation of IgM. After incubating 60 min at 25°C, five μ l of rabbit complement was added to each well of the trays and incubated for another 2 hours at room temperature. Three μ l of eosin stain was then added to each well of the trays, and 2 minutes later, 8 μ l of formaldehyde was added to stop the reaction. The plates were read under the phase-contrast inverted microscope.

For all donor crossmatches, lymphocytes were incubated with (1) the recipient's serum, (2) a positive control serum, and (3) a negative control serum. The positive control was an alloantiserum routinely used in our laboratory with 100% panel reactive antibodies (PRA). The negative control was serum from unsensitized, healthy male donors of blood group AB. Occasionally the recipient's serum is cytotoxic to the recipient's cells as well as to the donor's and identification of these sera requires that they be tested against the recipient's cells (auto control).

Anti-human globulin augmented cytotoxicity crossmatch (AHG-CDC)

AHG-CDC crossmatch was performed by incubating 1 μ l of donor cell-enriched preparation (2.0×10^6 T cells/ml) with 1 μ l of serum from the recipient for 1 hr at 25°C. The criteria for choosing the battery of serum used in the AHG crossmatch were the same as those described above for the standard crossmatch. The trays were washed by addition of saline to the wells and then the trays were "flicked" to remove the wash solution. After four washes, 1 μ l of AHG at the appropriate dilution was added, followed by 2-min incubation at 25°C, and then the addition of 5 μ l of complement. After incubation for 60 - 90 min at 25°C, staining with a vital dye assessed cell injury. A serum/cell reaction was considered positive when reactivity above that seen in the negative control well was observed.

Flowcytometry crossmatch (FCXM)

The FCXM was performed using a Becton Dickinson FACS Scan Flow cytometer. 50 μ l donor cells were incubated with 50 μ l of serum for 30 min at 37 °C. After washing with 2ml PBS, 20 μ l of a fluorescein-conjugated F(ab)'2 goat-anti-human IgG were added, followed by a 30-min incubation at 4°C in the dark. After washing with 2 ml cold PBS, 20 μ l of phycoerythrin-conjugated anti-CD3 mouse monoclonal antibody were added and the tubes were incubated for another 30min at 4°C in the dark. The cells were then washed once with PBS and then resuspended in 500 μ l of sheath fluid until analysed.

A pool of sera negative for anti-HLA antibodies from male blood donors of AB blood type was used as the negative control. The positive control consisted of a pool of sera from highly sensitized patients with 100% PRA. From each sample, 10,000 cells were analyzed in the lymphocyte gate. From the CD3⁺ cells, a histogram of fluorescein isothiocyanate (logarithmic scale) was made.

NB: Nine tubes were used for each test; 3 for positive control tubes, 3 for negative control tubes, and 3 for donor recipient cross match tubes.

Calculation

All FC crossmatch results in this analysis were calculated as follows:

Average Mean test tubes = X.

Average Mean of negative tubes = Y.

If $X > Y + 2SD$ = positive cross match.

SD = difference between negative tubes.

Results

Cross matching of 150 pretransplant renal allograft recipients was done utilizing the standard CDC and AHG-CDC crossmatches. Fifty recipients out of them were additionally monitored by FCXM. This study included

93/150 male recipients (62%) and 57/150 female recipients (38%) with mean age \pm SD of 38.4 ± 1.6 varying from 19-51 years. Collective data for those recipients was shown in table (1).

Table 1. Clinical, virological and crossmatching results from the 150 pre-transplant renal allograft recipients.

Number of recipients	150
Mean age \pm SD [years]	38.4 \pm 1.6
Sex [M/F]	93/57
Recipients blood group [O/A/B/AB]	(30/48/41/31)
Recipients with history of previous renal transplantation	14/136
Recipient Virology [+ve / - ve]:	
HCV Ab	16/134
HBs Ag	-/150
HIV Ab	-/150
CMV [IgM]	-/150
C CDCCXM [+ve / - ve]	37/113
AHG-CDC [+ ve / - ve]	38/112
FCXM [+ ve / - ve]	23/27
Concordant CDCXM / AHG-CDC [+ve/-ve] (total No.: 150)	37/112
Concordant CDCXM/AHG-CDC/FCXM [+ve/-ve] (t (total No.: 50)	12/27

Standard CDCXM showed positive results in 37/150 (24.7%) recipients, while the negative results were reported in 113/150 (75.3%) recipients. AHG-CDC showed 38/150

(25.3%) recipients with positive results, while 112/150 (74.7%) recipients showed negative results (Table 2).

Table 2. Crossmatch results of the standard complement- dependent cytotoxicity crossmatch and the anti-human globulin augmented cytotoxicity in 150 pre-transplant recipients.

Crossmatch results	Positive crossmatch		Negative crossmatch		Total number	P Value
	Number	%	Number	%		
CDCXM	37	24.7	113	75.3	150	N.S
AHG-CDC	38	25.3	112	74.7	150	

P>0.05 is not significant.

On the other hand, comparing the results of CDCXM, AHG-CDC and FCXM in 50 pretransplant renal allograft recipients (Tables 3, 4, 5), CDCXM showed positive results in 12/50 (24%) of recipients, AHG-CDC in 13/50 (26%), and FCXM in 23/50 (46%) of

recipients. These results showed a significant correlation between FCXM and each of CDCXM and AHG-CDCXM while there was no significant correlation between the results of CDCXM and AHG-CDC

Table 3. Crossmatch results of the standard complement- dependent cytotoxicity crossmatch and the anti-human globulin augmented cytotoxicity in 50 pre-transplant recipients.

Crossmatch results	Positive crossmatch		Negative crossmatch		Total number	P Value
	Number	%	Number	%		
CDCXM	12	24	38	76	50	N.S
AHG-CDC	13	26	37	74	50	

P> 0.05 is not significant.

Sensitivity = $t+/t+ + f- = 12/12 + 0 = 100\%$., Specificity = $t-/t- + f+ = 37/37 + 1 = 97.4\%$

PPV = $t+/t+ + f+ = 12/12 + 1 = 92.3\%$., NPV = $t-/t- + f- = 37/37 + 0 = 100\%$.

Table 4. Crossmatch results of the standard complement- dependent cytotoxicity crossmatch and Flowcytometry in 50 pre-transplant recipients.

Crossmatch results	Positive crossmatch		Negative crossmatch		Total number	P Value
	Number	%	Number	%		
CDCXM	12	24	38	76	50	<0.05
FCXM	23	46	27	54	50	

P<0.05 is significant

Sensitivity = $t+/t+ + f- = 12/12 + 0 = 100\%$., Specificity = $t-/t- + f+ = 27/27 + 11 = 71.1\%$

PPV = $t+/t+ + f+ = 12/12 + 11 = 52.2\%$., NPV = $t-/t- + f- = 27/27 + 0 = 100\%$.

Table 5. Crossmatch results of the anti-human globulin augmented cytotoxicity and Flowcytometry in 50 pre-transplant recipients.

Crossmatch results	Positive crossmatch		Negative crossmatch		Total number	P Value
	Number	%	Number	%		
AHG-CDC	13	26	37	74	50	<0.05
FCXM	23	46	27	54	50	

P<0.05 is significant.

From tables 1,2,3,4 and 5 the AHG-CDC showed concordant results with the standard CDCXM in 149/150 cases (99.3%), whereas 37 cases (24.8%) were positive and 112 cases (75.2%) were negative with both techniques. FCXM showed concordant results with CDCXM and AHG-CDC in 39/50 cases (78%), whereas, 12 cases (24%) were positive and 27cases (54%) were negative.

On the other hand, 11/50 of cases (22%) showed discrepant results, as 10 of them were positive FCXM and negative with both CDCXM and AHG-CDC. This percentage appeared to be slightly high to be neglected and showing a significant difference (P< 0.05). Only one case was FCXM and AHG-

CDC positive but CDCXM negative (2%), it gave a non-significant difference in comparison to the total of 50 cases (P> 0.05).

Crossmatch results in relation to donor's blood group were shown in tables 6, 7, 8. Twelve donors out of fifty were with blood group A, 4 of them were positive with both CDCXM and AHG-CDC, while 7 were positive with FCXM. These results showed a statistically non-significant difference (P>0.05). Among 16 donors with blood group B, 6 were positive with both CDCXM and AHG-CDC, and 7 of them were positive with FCXM. These results showed non-significant statistically difference (P>0.05). On the other hand, among 22 donors were blood group O;

two were positive with CDCXM, 3 were positive with AHG-CDC, and 9 were positive with FCXM resulting in a statistically significant difference ($P < 0.05$).

Table 6. Crossmatch results of the standard complement- dependent cytotoxicity crossmatch and the anti-human globulin augmented cytotoxicity in relation to donors blood group.

Crossmatch Donor results Blood group	Total		CDCXM				AHG-CDC				P Value
	No.	%	Positive		Negative		Positive		Negative		
	50	100	No.	%	No.	%	No.	%	No.	%	
A	12	24	4	33.3	8	66.6	4	33.3	8	66.6	N.S
B	16	32	6	37.5	10	62.5	6	37.5	10	62.5	N.S
O	22	44	2	10.1	20	89.9	3	13.6	19	86.4	N.S

$P > 0.05$ is not significant.

Table 7. Crossmatch results of the standard complement- dependent cytotoxicity crossmatch and Flowcytometry in relation to donors' blood group.

Crossmatch Donor results Blood group	Total		CDCXM				FCXM				P Value
	No.	%	Positive		Negative		Positive		Negative		
	50	100	No.	%	No.	%	No.	%	No.	%	
A	12	24	4	33.3	8	66.6	7	58.3	5	41.7	N.S
B	16	32	6	37.5	10	62.5	7	43.8	9	56.2	N.S
O	22	44	2	10.1	20	89.9	9	41.1	13	58.9	<0.05

$P < 0.05$ is significant

Table 8. Crossmatch results of the anti-human globulin augmented cytotoxicity and Flowcytometry in relation to donors' blood group.

Cross match Donor results Blood group	Total		AHG-CDC				FCXM				P Value
	No.	%	Positive		Negative		Positive		Negative		
	50	100	No.	%	No.	%	No.	%	No.	%	
A	12	24	4	33.3	8	66.6	7	58.3	5	41.7	N.S
B	16	32	6	37.5	10	62.5	7	43.8	9	56.2	N.S
O	22	44	3	13.6	19	86.4	9	41.1	13	58.9	<0.05

$P < 0.05$ is significant

Cross match results in relation to patients / donors blood group was shown in [Tables 9, 10, 11]. It was found that 27 recipients out of 50 having the same ABO blood group as their donors. Six of them were positive by both CDCXM and AHG-CDC techniques, and 11 were positive by FCXM technique. Among

the other 23 recipients with different compatible ABO blood group, 6 were positive by CDCXM, 7 were positive by AHG-CDC, and 12 were positive by FCXM with a statistically non-significant difference ($P > 0.05$).

Table 9. Crossmatch results of the standard complement- dependent cytotoxicity crossmatch and the anti-human globulin augmented cytotoxicity in relation to patients / donors blood group.

Crossmatch \ Parameters	Total		CDCXM		AHG-CDC		P Value
	No.	%	Positive No.	Negative No.	Positive No.	Negative No.	
The same blood group	27	54	6 22.2	21 77.8	6 22.2	21 77.8	N.S
Different compatible blood group	23	46	6 26.1	17 73.9	7 30.4	16 69.6	N.S

P> 0.05 is not significant.

Table 10. Crossmatch results of the standard complement- dependent cytotoxicity crossmatch and Flowcytometry in relation to patients / donors blood group.

Crossmatch \ Parameters	Total		CDCXM		FCXM		P Value
	No.	%	Positive No.	Negative No.	Positive No.	Negative No.	
The same blood group	27	54	6 22.2	21 77.8	11 40.7	16 59.3	N.S
Different compatible blood group	23	46	6 26.1	17 73.9	12 52.2	11 47.8	N.S

P> 0.05 is not significant.

Table 11. Crossmatch results of the anti-human globulin augmented cytotoxicity and Flowcytometry in relation to patients / donors blood group.

Crossmatch \ Parameters	Total		AHG-CDC		FCXM		P Value
	No.	%	Positive No.	Negative No.	Positive No.	Negative No.	
The same blood group	27	54	6 22.2	21 77.8	11 40.7	16 59.3	N.S
Different compatible blood group	23	46	7 30.4	16 69.6	12 52.2	11 47.8	N.S

P>0.05 is not significant.

Regarding crossmatch results in 15 renal allograft transplant recipients, no difference was detected in anti-HLA sensitization both pre- and post-transplantation. All patients were CDCXM and AHG-CDC negative; 12 (80%) of them were FCXM negative while 3 (20%) were FCXM positive (Table 12).

Following those patients post-transplantation (0 -8 months), one patient (33.3%) with positive FCXM exhibited early graft loss while three recipients (two of them were FCXM positive) experienced adverse post-transplant immunologic events (Table 13).

Table 12. Flowcytometry versus the anti-human globulin augmented cytotoxicity in 15 patients post-transplantation.

FCXM \ AHG-CDC	Negative (n = 12)		Positive (n = 3)		Total		P Value
	No.	%	No.	%	No.	%	
Positive	—	—	—	—	—	—	N.S.
Negative	12	80%	3	20%	15	100%	

P>0.05 is not significant.

Table 13. Flowcytometry and post-transplantation complications in 15 renal transplant recipients.

	FCXM		P Value
	Positive No %	Negative No %	
Early graft loss.	1 33.3	— —	N.S.
Adverse immunologic events*	2 66.7	1 8.3	
No complications	— —	11 91.7	
Total No. (15)	3 100%	12 100%	

P> 0.05 is not significant

* Adverse immunologic events including drug resistance and / or recurrent rejection episodes.

Discussion

In this study, we compared the results of CDC-XM and AHG-CDCXM utilizing DTT treated sera in detection of anti-HLA antibodies in 150 pre-transplant allograft renal recipients. Also we compared CDCXM and AHG-CDC results with FCXM in 50 recipients out of them. It was possible to ascertain that positive results were caused by IgG Abs. Utilization of AHG ensured that non-complement-fixing as well as complement-fixing IgG Abs were detectable in the CDC XM. Moreover, the development of post-transplantation sensitization and the clinical correlation during the first post-operative two weeks was assessed for 15 pre-transplant renal allograft recipients with CDCXM and AHG-CDC IgG negative of whom 3 were FCXM positive.

Comparison between the results of CDCXM and AHG-CDC techniques in 150 recipients revealed that, CDCXM technique showed positive results in 37 patients (24.7%), and negative results in 113 patients (75.3%). On the other hand, AHG-CDC technique showed positive results in 38 patients (25.3%), and negative results in 112 patients (74.3%). These results reached a non-significance difference ($P > 0.05$). The concordance of the CDCXM and AHG-CDC was 149 / 150 (99.3%). This was granted with

results of Susskind et al., (1998), that approved concordance rates by both methods in 93% of cases. Fuller et al, (1982), revealed that, the AHG-CDC procedure, relative to standard CDC, detects sub-threshold levels of alloantibody. On the other hand, Baron et al, (1998) suggested that a historical positive AHG-CDC XM does not impede long-term allograft survival, and the use of this technique could generate undue prolonged waiting time for renal transplantation candidates. As they found that, patients with a positive historical AHG-CDC XM did not experience a significant greater number of acute rejection episodes than the recipients with a negative historical AHG-XM.

Comparison between the results of CDCXM, AHG-CDC, and FCXM techniques in 50 recipients revealed that, CDCXM technique showed positive results in 12 patients (24%), and negative results in (38) patients (76 %). On the other hand, AHG-CDC technique showed positive results in 13 patients (26%), and negative results in 37 patients (74%). While FCXM technique showed positive results in 23 patients (46%), and negative results in 27 patients (54%). These results reached a statistical significance ($P < 0.05$). The concordance of CDCXM, AHG-CDC and FCXM was 39/50 (78%). Flow cytometry testing of the false positive serum which was CDCXM negative but

AHG-CDC positive sustained the AHG-CDC result, indicating that the discrepancy with CDCXM was caused by greater sensitivity of AHG-CDC.

Based on our results in 50 renal transplant recipients, the sensitivity of AHG-CDC relative to CDCXM (true positive vs. false negative) was 100%; specificity (true negative vs. false positive) was 97.4%; positive predictive value (true positive vs. false positive) was 92.3%; and negative predictive value (true negative vs. false negative) was 100%. (Table 3). Relative to CDCXM, the sensitivity of FCXM was 100%; specificity was 76.3%; positive predictive value was 57.1%; and negative predictive value was 100%. (Table 4). This was in accordance with the results of El-Gezawy (2003), who showed that, CDCXM with positive results in 8/42 recipients (19%) and negative results in 34/42 recipients (81%). On the other hand, FCXM showed 17 recipients (40.5%) with positive results, and 25 recipients (59.5%) with negative results. This difference reached a statistical significant.

In 1997, Buabut et al., reported that, 8/75 (10.7%) tests were negative by CDCXM (5 tests were positive by AHG only and 1 test was positive by FCXM only, and 2 tests were positive by both AHG and FCXM). They were found that, FCXM was the most sensitive technique, followed by AHG-CDC and CDCXM. In addition, AHG technique was more sensitive than CDCXM and does not require sophisticated equipment. Also they reported that, AHG-CDC technique should be appropriate for routine XM, especially in living related kidney transplant. A prospective study of Horsburgh et al, (1997) for anti-donor antibody detection using a variety of cross match techniques in the sera of 247 recipients transplanted in their unit, suggested that the FCXM is more sensitive method than CDCXM.

Regarding the results of crossmatch in relation to donors' blood group, we found that

the positivity of FCXM but not CDCXM nor AHG-CDC, was detected to be significantly increased ($P < 0.05$) with donors' blood group O. Also we found that, there was no statistical significance between the results of cross match in ABO-compatible and those of ABO-incompatible renal graft (Tables 6 through 11). On the other hand, Nasr El-deen (2002), showed a significantly increased number of negative cross match cases whose donor were blood group O. Thus, group O grafts can be transplanted into recipients with blood types A, B, and AB. This is not commonly done, for ethical reasons, so to prevent depletion of available grafts for type O recipients. Uchida et al, (1998), reported that the outcome of ABO-incompatible living renal transplantation is satisfactory and comparable to that of compatible living renal transplantation. Ishikawa et al, (1998), believed that this good outcome is attributable to through elimination of blood group antibodies before transplantation by splenectomy, immunoadsorption and plasma pheresis.

In our study, it was found that, there was no statistical significant analysis between the cross match results and HCV infection. This result may be due to limited number of recipients with HCV infection and needs large number of population to ascertain this role. This agreed with the results of Nasr El-deen, (2002), who reported that, the role of HCV viraemia (antibodies) on the cross match results are not clear.

In this study following results of CDCXM, AHG-CDC, and FCXM in 15 pre-transplant renal allograft recipients, no difference was detected between pre- and posttransplant anti-HLA sensitization within 2 weeks after the operation. The 15 patients were CDCXM and AHG-CDC negative, three of them (20%) exhibited a positive FCXM. Of these positive FCXM patients, one (33.3%) experienced early graft loss not responding to any line of treatment demonstrating antibody-mediated

reaction and finally the patient was put on haemodialysis. This patient was with a history of previous transplantation. The two patients with positive FCXM (66.7%) who maintained their graft experienced more adverse events posttransplant including early steroid – resistant, and recurrent rejection episodes (Tables 12&13). This association between a positive FCXM and poor graft outcomes in our patients could explain that the discrepancy of FCXM with CDCXM and AHG-CDC was caused by greater sensitivity of FCXM technique which is capable of detecting anti-HLA antibodies undetectable by the other two techniques. Consequently, those patients in whom CDCXM or /and AHG-CDC were negative, a positive FCXM may be associated with an increased risk of early graft loss and may represent a relative contraindication to transplantation. On the other hand, one patient in whom FCXM was negative exhibited rejection episodes which could be experienced by other contributing non immunological graft adverse events. However, the number of specimens evaluated in this study as regards following the clinical relevance of FCXM in terms of graft survival were relatively small as well as the period of post-transplant follow up was a little short (0 – 8 months).

Garovoy et al (1983) reported that flow cytometry could detect low level of HLA antibodies not detected by conventional cytotoxicity. In preliminary study of 23-cadaver renal transplant, they found 10 which were positive in FCXM but negative by CDCXM, and 5 were rejected within 3 months of transplantation. This number of failure was higher than the 2 seen in the group of 13 patients who were both FCXM and CDCXM negative. Results offered an explanation for graft failure in only 25% of the cases studied. Chapman et al, (1985) performed a retrospective FCXM on 16 patients who had early graft failure despite a negative CDCXM and found that 4 had a positive FCXM, 3 with peak sera only and

one with a serum taken on the day of transplantation. Thus, at best, the FCXM result offered an explanation for graft failure in only 25% of the cases studied.

Kimball et al, (1998) showed that, on performing FCXM technique before and after transplantation, pre- and post-transplant antibody levels were relatively unchanged among negative and B- positive FCXM patient groups. In contrast, patients with T-positive FCXM produce cytotoxic IgG against class I after transplantation, which may have contributed to the severe graft dysfunction experienced by this group. They conclude that, FCXM is a useful tool to stratify primary renal transplant candidates in terms of potential risk for severe rejection. Furthermore, demonstration of pre-operative flow reactivity against class I may identify a subgroup of patients at risk for early elaboration of cytotoxic alloantibody. Also, Bryan et al, (1998) reported that, the use of FCXM as the exclusion criterion for cadaveric renal retransplantation yield an improved long-term graft outcome over that obtained when only the AHG cross match was used and has improved survival of re-graft recipients to the level of primary renal transplant population.

O'Rourke et al, (2000) agreed that, FCXM was developed as a more sensitive assay than the standard CDCXM for detection of anti-donor antibodies that mediate hyper acute rejection and graft loss in the early post-transplant period in renal transplant recipients. Piazza et al, (2001) reported that on screening renal transplant patients 1-year after surgery by means of flow cytometry cross-match, and all transplants were followed-up for 2-years or until graft removal. The results were that, FCXM monitoring identified donor-specific antibodies (DS-Abs) in 29/120 (24.2%) of transplanted patients. Correlation with clinical data a high incidence of acute rejection in (DS-Abs) –positive patients compared to negative patients. Furthermore, graft failure

occurred more frequently among FCXM-positive patients than among negative patients. They conclude that, FCXM technique is precious tools for investigating the activation of humoral response against HLA antigens of the graft in renal transplantation. On the other hand, Chirstiaan et al, (1996) and Maarten et al, (1996), showed that, there was no advantage of FCXM over CDCXM in renal allograft recipients on 1-year graft survival rejection and 1-year patient survival. Slavcev et al., (1998) found no correlation between a positive FCXM result before transplantation and the occurrence of immunological complications after transplantation. Also Naser El-deen, (2002) revealed that, there is no statistical difference between the results of FCXM and standard CDCXM. It should be stressed, that even though the sensitivity of the FCXM exceeds CDCXM and AHG-CDC, not all positive FCXM assays are associated with poor graft outcome (Cook et al, 1998). When assessing whether an allogenic organ should be transplanted, several other factors besides an FCXM should be considered. The main advantages of FCXM mentioned were increased sensitivity. It has shown to be up to 100 times sensitive than usual serologic methods. It allows the simultaneous analysis of complement- activating and non- activating allo antibodies, the class of donor-specific antibodies [IgG and / or IgM], the kind of donor target cells [T and / or B lymphocytes], adjective analysis and the test requires half the time of cytotoxicity. It generate cross match, where CDCXM cannot because of low cell viability. Moreover, the computer printouts represent a permanent record of the cross match for retrospective review, which is a valuable to rule out an antibody in cases of primary non-function (Scomic et al, 2001). The main disadvantages are the large number of cells needed to perform a test, the large volume of serum required and the initial cost of the apparatus (Radcliffe and Gray, 1996).

When assessing whether an allogenic organ should be transplanted, several other factors besides an FCXM should be considered, such as the status of the primary or regrant patients, titre and fine specificity of circulating antibodies (e.g., HLA versus non- HLA); and parameters involving the donated organ such as age, sex, and cause of death of the organ donor. Thus, a positive FCXM is no an unequivocal contraindication to transplantation and must be weighed in the context of other variables such as those listed above (Scomic et al, 2001).

In conclusion, we have shown that a non-significant correlation exists between crossmatch results of CDCXM and AHG-CDC techniques. However, AHG-CDC is more sensitive than CDCXM and does not require sophisticated equipment. Our results have also demonstrated that a significant proportion of negative CDC crossmatch renal pretransplant recipients possess donor-specific antibodies detectable by FCXM. These individuals are at increased risk for early graft loss due to antibody –mediated rejection and experience an increased rate of adverse immunologic events posttransplant. These findings suggest that in renal transplant recipients, a positive FCXM in the face of a negative CDCXM or AHG-CDC cross match may represent a relative contraindication to transplantation. However, the number of specimens evaluated in this study is relatively small, and further studies with larger number of cases are recommended.

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