Scientific Foundation SPIROSKI, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2021; May 17; 9(A):328-335.
https://doi.org/10.3889/oamjms.2021.6081
eISSN: 1857-9655
Category: *A - Basic Sciences*
Section: *Pathology*

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Evaluation of Early Renal Allograft Dysfunction from Living Donors among Egyptian Patients (Histopathological and Immunohistochemical Study)

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Edited by: Sinisa Stojanoski
Citation: Muhammad MEE, Fadda SAA, Gabal SM, Shaker AM, Mohamad WM. Evaluation of Early Renal Allograft Dysfunction from Living Donors among Egyptian Patients (Histopathological and Immunohistochemical Study). Open Access Maced J Med Sci. 2021 May 07; 9(A):328-335. https://doi.org/10.3889/oamjms.2021.6081
Keywords: Acute rejection; Complement fragment 4d; Histopathology; Renal transplantation
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Received: 23-Mar-2021
Revised: 03-May-2021
Accepted: 07-May-2021
Copyright: © 2021 Maha Emad El-Dein Muhammad, Sawsan A. A. Fadda, Samia M. Gabal, Amr M. Shaker, Wael M. Mohamad
Funding: This research did not receive any financial support
Competing Interests: The authors have declared that no competing interests exist
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Abstract

BACKGROUND: Early renal graft dysfunction is a major problem in the early post-transplantation period and is considered a major cause of graft loss. Clinical diagnosis based on the clinical criteria alone is unreliable; therefore, biopsy remains the gold standard to differentiate between rejection and non-rejection causes.

AIM: This study was designed to identify and differentiate between causes of early graft dysfunction during the first post-transplantation month and to correlate between histological lesions and immunohistochemistry (IHC) for accurate diagnosis and a better outcome.

MATERIALS AND METHODS: A total of 163 renal allograft biopsies, performed in the first post-transplantation month over 6 years, were included in the study. New sections were prepared from the paraffin blocks and stained with conventional stains. Additional sections were prepared and treated by complement fragment 4d (C4d) and cluster differentiation 3 (CD3) antibodies for IHC evaluation.

RESULTS: All the studied cases were from living donors. The mean patient age was 39 years with predominant males. The clinical indication for most biopsies (94.5%) was impaired graft function. Acute rejection (AR) was the main diagnostic category observed in (98/163, 60.1%); out of which, T cell-mediated rejection (TCMR) was observed in (62/98, 63.2%). Drug toxicity was suspected in (53/163, 32.5%), acute tubular injury (ATI) not otherwise specified (nos) in (21/163, 12.9%), and other lesions including thrombotic microangiopathy were observed in the remaining biopsies. The most common cause of graft dysfunction in the 1st and 2nd weeks was AR representing. A significant correlation was seen between mild glomerulitis (g1) and mild peritubular capillaritis (PTC) 1, on the one side, and negative C4d staining, on the other side. No significant correlation was seen between moderate glomerulitis (g2) and moderate ptc2 at one side and positive C4d staining at the other side reflecting the poor association between the microvascular inflammation ("g" and "ptc" scores) and C4d positivity (r = 0.2). Missed mild tubulitis (t1) was found in a single case and missed moderate tubulitis (t2) was found in a single case detected by CD3 IHC.

CONCLUSION: AR and drug toxicity account for the majority of early graft dysfunction, however, other pathological lesions, *per se* or coincide with them may be the cause. The significance of g2 *per se* as a marker for diagnosis of antibody-mediated rejection requires further study. Considering C4d score 1 (by IHC) positive; also requires further study with follow-up.

Introduction

Renal transplantation is the treatment modality of choice for patients with end-stage renal disease (ESRD) throughout the world. Renal allograft dysfunction is a major problem in the early post-transplantation period and is recognized as a major cause of graft loss [1]. Improvements in immunosuppression have reduced acute kidney allograft rejection and clinicians are now seeking ways to prolong allograft survival to 20 years or more. The primary cause of kidney allograft loss is still a chronic rejection, followed by death with a functioning allograft and primary kidney disease recurrence [2]. Thus, overcoming acute kidney allograft rejection remains the most important issue. Kidney allograft rejection can be classified into two types: Antibody-mediated rejection (ABMR) and T cell-mediated rejection (TCMR). Both are diagnosed pathologically based on the Banff 2013/2017 classification. Other important pathological features in addition to rejection include delayed graft reperfusion, with ischemic injury, calcineurin inhibitor toxicity, polyomavirus nephropathy, and recurrence of the primary kidney disease (rare in the first post-transplantation month if the focal segmental glomerulosclerosis [FSGS] is excluded from the study) [3]. For these reasons, it is of considerable interest to identify and differentiate between diverse causes of acute graft failure. Biopsies that show the absence of rejection are useful in that they prevent unnecessary immunosuppressive therapy and thus reduce complications of over immunosuppression [2].

Acute rejection (AR) is simply defined as an acute deterioration in renal allograft function associated with specific pathologic changes in the graft [4]. To acquire information about AR and its complications, it

is important to determine rejection prevalence and its potential causes. The AR may be presented alone or in combination with other causes, which can add to the difficulty in diagnosing the cause. The diagnosis of ABMR is crucial and requires specific treatment, and if improperly managed leads to graft loss. It may affect tubules (Grade I), microvascular compartment (Grade II), or arteries (Grade III). In allograft biopsy, C4d deposition along the peritubular capillaries (PTCs) is considered to be a marker for ABMR. The morphological diagnosis of ABMR consists of various morphological changes together with C4d deposition in the microcirculation of the allograft [5]. It is well known that TCMR is usually diagnosed histologically based on specific patterns of tubulointerstitial lymphocyte infiltration in biopsies according to Banff's criterion. T cells are assumed to be the most deleterious cell type. They are, therefore, still the main diagnostic and therapeutic target in transplantation making their identification crucial in diagnosis [6].

Materials and Methods

Retrieval of cases

This retrospective study included a total of 163 renal allograft biopsies performed in the 1st post-transplantation month. The biopsies were obtained from renal transplant recipients presented clinically by acute deterioration/dysfunction of the renal allograft. The material was collected as formalin-fixed, paraffin-embedded renal tissue sections from January 2014 to June 2019 from the archives of Pathology Department, Kasr AL-Ainy (Cairo University hospital). Exclusion criteria included any biopsy done after the first post-transplantation month or any failed biopsy (non-renal tissue). The available clinical data were obtained from the patient's medical reports including age, sex, cause of ESRD if available, indication for biopsy, post-transplantation time of the biopsy, graft type (living-related vs. living non-related), current therapeutic regimen, and serum creatinine level. The authors obtained the approval of an ethical committee in the faculty of Medicine, Cairo University. A local ethics committee approved the study in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki Declaration/its later amendments. An informed written consent was also obtained from the patients before enrollment.

Histopathological examination

New sections were prepared (about 40 serial sections from each block) and stained with Hematoxylin and Eosin, periodic acid Schiff (PAS), and Masson Trichrome stains. All cases were examined by two pathologists using a light microscope to evaluate the adequacy of the biopsy according to Banff'97 [7]: Biopsy with adequate requirements for scoring (a specimen with at least ten non-sclerotic glomeruli and two arteries), biopsy with minimal requirements for scoring (a specimen having seven glomeruli and one artery), and the rest was considered not suitable for scoring (<7 glomeruli or no arteries or only medulla). Each biopsy was then scored according to Banff' 97 [7] for semi-quantitative grading (03) of tubulitis (t), interstitial inflammation (i), glomerulitis (g), and vasculitis (v) and according to Banff 03 for ptc [8].

Immunohistochemical examination and interpretation

Additional sections (3 μ thick) were prepared from paraffin blocks on charged glass slides using fully automated immunohistochemical system BenchMark XT (Ventana) autostainer, (pH 6) for (1) C4d "polyclonal antibody, ready to use" (Spring Bioscience, Pleasanton, CA, US): An immunological marker for the humoral alloresponse [9] and (2) CD3 "monoclonal antibody, ready to use" (Rabbit monoclonal; Cell Marque, Rocklin, CA, USA): An immunological marker for T cells [10]. Sections from a lymph node with florid reactive follicular hyperplasia were used as a control material for both makers (T-lymphocytes: Positive for CD3. Dendritic cells: positive for C4d. B-lymphocytes: Negative for both CD3 and C4d). Hematoxylin was used as a counterstain in all cases and PAS as an additional counterstain for CD3. C4d staining of the PTCs was graded as C4d score 0 (negative), C4d score 1 (minimal): (1–9%), C4d score 2 (focal): (10–50%), and C4d score 3 (diffuse): (>50%) [6]. Only cases with linear and circumferential deposition of C4d (involving >10% of the cortical PTC or vasa recta) were considered positive (scores 2 and 3), according to Dominy *et al*., 2018, and Kumar *et al*., 2019 [11], [12]. CD3 staining of T lymphocyte cytoplasm facilitates their identification, where a conventional PAS counterstain was then applied to highlight the basement membrane of the renal tubules, thereby facilitating accurate localization of the lymphocytes [13].

Cases were then classified into histological categories (based on the main histological lesions and the immunohistochemistry [IHC] findings), according to Banff'17 [14] into six categories: Normal (category-1), Active ABMR (category-2), Borderline TCMR (category-3), Active TCMR (category-4), interstitial fibrosis and tubular atrophy (category-5), and others: Changes not due to rejection/non-rejection causes (category-6). Drug toxicity was considered in biopsies manifesting small isometric vacuoles associated with epithelial injury (in proximal convoluted tubules), arteriolar myocyte vacuoles, or drug-related thrombotic microangiopathy (TMA) changes in C4d negative cases [15]. Acute tubular injury (ATI) (nos) considered in absence of specific histopathologic and

IHC features [16]. TMA (nos) considered in absence of C4d staining and features of drug toxicity [17].

Statistical analysis

The histopathological and immunohistochemical data were then transferred to the SPSS Software program, version 25 to be statistically analyzed. Simple descriptive statistics (arithmetic mean and standard deviation) were used for the summary of quantitative data and frequencies were used for qualitative data. Estimation of the association between categorical variables was performed using the Chi-square test. $p < 0.05$ is considered as statistically significant. Spearman correlation coefficients were calculated to signify the association between different ordinal variables.

Results

This study included a total of 163 renal allograft biopsies performed in the 1st post-transplantation month. The age of the cases ranged between 9 and 68 years with and male predominance in the studied cases. The etiology of ESRD was unknown in the majority of cases (92.6%). As regard the type of graft, it was documented to be living-related in two cases only (1.2%). The clinical indication for most biopsies (94.5%) was impaired graft function (IGF). The serum creatinine level was reported in 112 cases and ranged between 0.7 and 11 mg/dl. All cases were under standard immunosuppression protocol of prednisolone (10–20 mg/day), Tacrolimus (0.03–0.05 mg/kg/day), and/or mycophenolate sodium (360 mg, 3 or 4 times a day). The demographic and clinical data were detailed in Table 1.

Table 1: Demographic and clinical characteristics of the studied cases

Demographic and clinical characteristics	n (%)
Age	
9–68 years with mean 39.02 ± 12.87	
Gender	
Males	109 (66.9)
Females	54 (33.1)
Etiology of ESRD	
Hypertensive nephrosclerosis	1 (0.6)
Lupus nephritis	2 (1.2)
FSGS	4 (2.4)
Obstructive uropathy	2 (1.2)
Membranous nephropathy	1 (0.6)
Adult polycystic kidney	1 (0.6)
Hereditary nephritis	1 (0.6)
Unknown	151 (92.6)
Post-transplantation interval of the biopsies	
1st week	85 (52)
2nd week	49 (30)
Last 2 weeks	29 (18)
Clinical indication for biopsies	
IGF	154 (94.5)
DGF	9 (5.5)
Adequacy of biopsies according to Banff scoring	
Adequate	81 (50)
Minimal	43 (26)
Inadequate	39 (24)
Serum creatinine	
0.7–11 mg/dl with mean 3.74 ± 1.73 mg/dl	

*ESRD: End stage renal disease, FSGS: Focal segmental glomerulosclerosis, *DGF: Delayed graft function, IGF: Impaired graft function.

AR was observed in 98 cases (60.1%), where the predominant immune injury was TCMR; observed in (62/98, 63.2%). It is worth noting that 47 cases (47/163, 28.8%) showed more than one Banff diagnostic category (mixed pattern). Drug toxicity was suspected in (53/163, 32.5%) and ATI (nos) in (21/163, 12.9%), detailed in Table 2.

Table 2: Categorization of all cases according to Banff

Banff diagnostic category	n	%
Pure ABMR (Banff cat. 2)	19	11.7
Pure borderline for TCMR (Banff cat. 3)	3	1.8
Pure TCMR (Banff cat. 4)	29	17.8
Others (Banff category 6)		
Pure ATI (nos)	21	12.9
Pure Drug toxicity	16	9.8
Pure TMA (nos)	6	3.7
Infarction	15	9.2
Pure oxalosis	2	1.2
Pure AIN	5	3.1
Mixed pattern		
ABMR + borderline (Banff cat. 2 + 3)	3	1.8
ABMR + others (Banff cat. 2 + 6)		
ABMR and drug toxicity	4	2.5
ABMR and AIN	2	1.2
Borderline + others (Banff cat. 3 + 6)		
Borderline and drug toxicity	5	3.1
TCMR + others (Banff cat. 4 + 6)		
TCMR and drug toxicity	25	15.3
TCMR and TMA	6	3.7
TCMR and AIN	1	0.6
TCMR and oxalosis	1	0.6
Total	163	100

*ABMR: Antibody mediated rejection, TCMR: T cell mediated rejection, ATI: Acute tubular injury, TMA: Thrombotic microangiopathy, nos: Not otherwise specified, AIN: Acute interstitial nephritis.

The most common cause of graft dysfunction in the 1st and 2nd weeks was AR representing (59/85, 69%) and (26/49, 53%) of the studied cases, respectively. It is worth noting that (17/28, 60.7%) of ABMR cases and (35/62, 56.5%) of TCMR cases occurred in the 1st week. The majority (71.4%) of ATI (nos) occurred in the 1nd week. All pure TMA cases (100%) and the majority of infarction cases (66.7%) occurred in the 1st week; unlike pure acute interstitial nephritis (AIN) and oxalosis which occurred only in the last 2 weeks; detailed in Table 3.

Table 3: Categorization of all cases corresponding to post-transplantation interval

Diagnostic categories	Post-transplantation interval			
	1st week	2nd week	Last 2 weeks	Total (n)
Acute rejection				
Pure ABMR (Banff cat. 2)	10	7	2	19
ABMR+borderline (Banff cat. 2+3)	3	0	0	3
ABMR+others (Banff cat. 2+6)	4	1	1	6
Pure Borderline (Banff cat. 3)	1	2	0	3
Borderline+others (Banff cat. 3+6)	1	2	2	5
Pure TCMR (Banff cat. 4)	19	6	4	29
TCMR+others (Banff cat. 4+6)	21	8	4	33
Others (Banff cat. 6)				
Pure ATI (nos)	5	15	1	21
Pure drug toxicity	5	6	5	16
Pure TMA (nos)	6	0	0	6
Infarction	10	2	3	15
Pure AIN	0	0	5	5
Pure oxalosis	0	0	2	2
Total (n)	85	49	29	163

ABMR: Antibody mediated rejection, TCMR: T cell mediated rejection, ATI: Acute tubular injury, TMA: Thrombotic microangiopathy, nos: Not otherwise specified, AIN: Acute interstitial nephritis.

C4d immunostaining was performed for all biopsies: Positive C4d staining (scores 2 and 3) was detected in 28 cases (17%), where a significant correlation was seen between g1 and ptc1 at one side and negative C4d staining at the other side (Figure 1). No significant correlation was seen between g2 and ptc2 on one side and positive C4d staining at the other side with a poor association between each of “g” and “ptc” scores and C4d positivity; detailed in (Table 4).

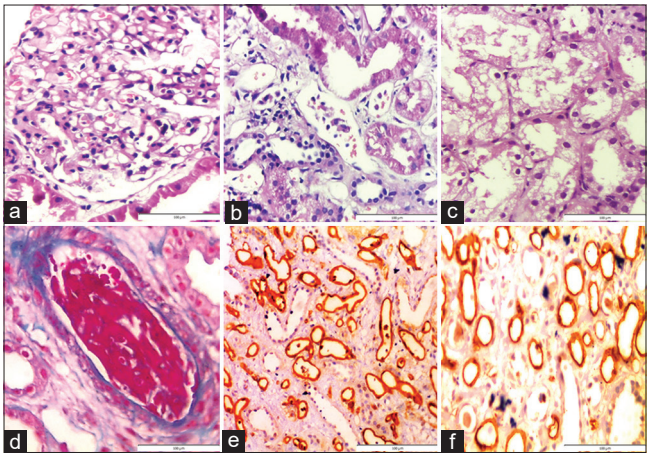

Figure 1: Cases of acute antibody-mediated rejection showing: (a) Mild glomerulitis (g1) (b) Moderate Peritubular capillaritis (ptc2) and interstitial edema. (c) Acute tubular epithelial injury (ATN-like). (a-c: Hematoxylin and Eosin, ×400 [original magnification]). (d) Arteriolar thrombus (Masson Trichrome stain ×400 [original magnification]) (e and f) C4d immunostaining shows diffuse positive circumferential staining of the PTCs (IHC, ×200 [original magnification])

Table 4: Comparison between C4d positivity and microvascular inflammation (MVI) (g and ptc scores)

MVI	C4d (%)		Total (%)	p-value	r
	Negative	Positive			
g1	61 (79)	16 (21)	77 (100)	0.000*	0.2
g2	14 (61)	9 (39)	23 (100)	0.3	
ptc1	50 (82)	11 (18)	61 (100)	0.000*	0.2
ptc2	23 (62)	14 (38)	37 (100)	0.1	

*Statistically significant, g1- <25% of glomeruli with inflammation, g2-25–75% of glomeruli with inflammation, ptc 1- > 10% PTCs with<5 luminal inflammatory cells, ptc 2- > 10% of PTCs with 5-10 luminal inflammatory cells. r=Spearman correlation coefficient (1=perfect association, 0.9–0.8=strong, 0.7–0.6=moderate, 0.5–0.3=fair, 0.2–0.1=poor and 0=no association). N.B: (g0, g3, ptc0 and ptc3 were excluded for statistical purposes; cases with AIN and infarction were also excluded from the study).

CD3 immunostaining confirmed the diagnosis in all TCMR cases (cellular type) as well as all vascular rejection cases (whether TCMR or ABMR) (Table 5). However, "missed" mild tubulitis (t1) was found in a single case (Figure 3c) and of those reported as t1; moderate tubulitis (t2) was found in a single case (Figure 2c).

Table 5: Pediatric age group

Parameters	Value
Biopsy (n)	8
Age	
Range	9–18
Mean ± S.D	14.8 ± 3.4
Post-transplantation interval (w)	
1st week	3
2nd week	2
Other 2 weeks	3
Serum creatinine	
Range	1.7–5
Mean ± S.D	3.4 ± 1.1
Indication for biopsy (n)	
IGF	8
Diagnosis (n)	
ABMR	1
Borderline	2
TCMR	3
Others	2

*n: number, w: week, S.D: Standard deviation, ABMR: Antibody mediated rejection, TCMR: T cell mediated rejection. IGF: Impaired graft function.

Discussion

In the current study, the primary cause of ESRD was unknown in the majority of cases which was following several previous studies [12], [18], [19]. For these patients, the cause of ESKD was unresolved; either their first presentation to our hospital was at a burnt-out phase at which the histology is indeterminate for the primary cause, or the available data about their history were insufficient to conclude a definite primary, that is, lacking immunofluorescence (IF) and electron microscopic study.

However, Barsoum, 2002. and Francis and Fadda, 2003, reported that interstitial nephritis was among the principal causes of ESRD in Egypt. This is often attributed to environmental pollution and medication abuse [20], [21]. In the current study, IGF (94.5%) was the most common indication for allograft biopsies, followed by delayed graft function (DGF) (5.5%). This was closer to the study done by Puntambekar *et al*., 2017, where IGF and DGF were present in 93.8% and 5.5% of the cases, respectively [19].

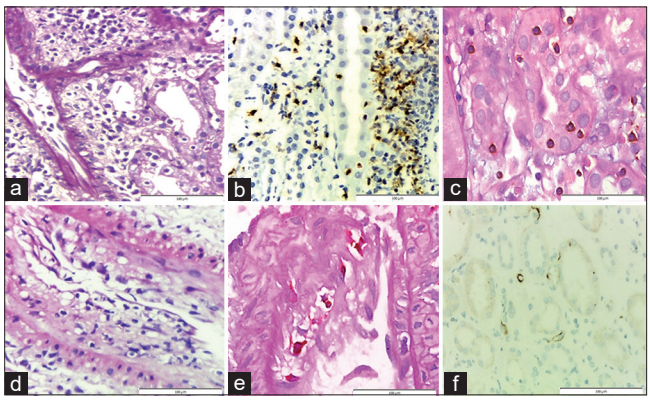

Figure 2: Cases of acute T cell-mediated rejection showing: (a) Moderate interstitial lymphocytic infiltrate with tubular epithelial injury and foci of t2. Note the clear halo surrounding the lymphocytes and mild intimal vasculitis (v1) (Hematoxylin and Eosin [H and E], ×200 [original magnification]). (b) CD3 immunostaining shows predominantly CD3+ interstitial lymphocytic infiltrate and foci of t2 (IHC ×200 [original magnification]). (c) Foci of t2 (CD3 immunostaining and periodic acid schiff (PAS) as a counterstain ×400 [original magnification]). (d) Moderate intimal arteritis (v2) showing subendothelial lymphocytic infiltrate (H and E, ×200 [original magnification]). (e) Mild intimal arteritis (v1). Note the inflammatory cells beneath the endothelial lining (CD3 immunostaining and PAS as a counterstain ×400 [original magnification]). (f) Negative C4d immunostaining of the peritubular capillaries (IHC, ×200 [original magnification])

Devadass *et al*. in 2016 evaluated 65 biopsies, where post-transplantation interval ranged from

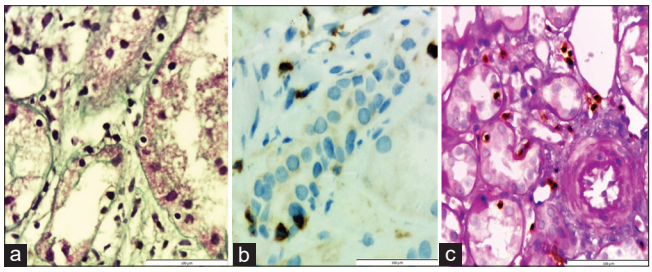

Figure 3: Borderline changes showing: (a) Foci of t1 as well as tubular epithelial injury. (Masson trichrome, ×400 [original magnification]). (b) CD3 immunostaining shows foci of t1 (IHC, ×400 [original magnification]). (c) Foci of t1 (CD3 immunostaining and periodic acid schiff as a counterstain ×200 [original magnification])

5 days to 8 years. Fifty-two percent of the biopsies were performed within the first 6 post-transplantation months. Forty cases (61.5%) were in the rejection category, predominated by ABMR in (35/40, 87.5%), followed by non-rejection causes in 18 cases (27.7%). Cases involving more than one Banff diagnostic category were detected in 25 cases (38.5%). They also reported that the most common cause of graft dysfunction in the 1st week was acute tubular necrosis (ATN) (66.7%), followed by ABMR (33.3%). After the 1st week–6th month, AR (64.5%) was the most common cause followed by acute drug toxicity (12.9%), oxalosis, and infection [22].

In the present study, the cause of early graft dysfunction in 98 cases (60.1%) was AR, which was in accordance with the study conducted by Devadass *et al*., 2016, and Parajuli *et al*., 2019, who confirmed that AR was the most common cause of early graft dysfunction, followed by non-rejection causes [22], [23]. Furthermore, 47 cases (28.8%) showed more than one Banff diagnostic category, which was closer to the study conduct by Devadass *et al*., 2016, but with difference in frequency which may be attributed to difference in the post-transplantation interval and sample size [22]. In the current study, the most common cause of graft dysfunction in the 1st and 2nd weeks was AR representing 69% and 53% of the studied cases, respectively. In the past 2 weeks, non-rejection causes were more common than AR (16/29, 55%), which was in synchrony with the study conducted by Devadass *et al*. 2016 [22].

In contrast to the studies conducted by Koshy *et al*., 2017, and Kumar *et al*., 2019, wherein their studies (56% and 60.4%, respectively) were in the non-rejection category and (44% and 39.6%, respectively) were in the rejection category [12], [24]. Such discrepancy could be attributed to: Differences in the graft type (cadaver and living-related/un-related), immunosuppressive protocols, human leukocyte antigen match, pre-sensitization, and finally post-transplantation interval (since their study included biopsies done during the 1st year and up to 5 years. In the present study, the incidence of rejection decreases, while that of non-rejection causes increases by increasing post-transplantation interval).

The diagnosis of ABMR becomes sophisticated and crucial at the same time. It has become the focus of attention, especially during the past two decades, as it plays an essential role in both short-term and long-term outcomes of the renal allograft and a major cause of late graft loss [25]. According to Banff 2013, three criteria must be fulfilled for the diagnosis of ABMR: (1) Histologic evidence of acute tissue injury (MVI (g+ptc) >0, v>0, TMA, ATN), (2) recent antibody interaction with vascular endothelium (C4d positivity, MVI ≥2, molecular markers), and (3) Donar specific antigens (DSAs) [26]. Part of the difficulty in the diagnosis of ABMR is related to its heterogeneous manifestations and its remarkable dynamic range from fulminant to inactive; also MVI has limited specificity, occurring in other diseases such as TCMR, infection, and renal blood outflow obstruction [27].

Detection of C4d in graft biopsies is emerging as an essential adjunctive tool for the diagnosis of ABMR [28]. Banff 2007 incorporated PTC C4d staining as one of the diagnostic triads for ABMR along with histopathological features of tissue injury and the presence of DSAs [6]. As C4d linked DSAs with histopathology and predicted allograft failure, it became the cornerstone of ABMR diagnosis in clinical practice [29]. Before Banff 2013, C4d positivity by IHC was considered in scores 2 and 3 only, while score 1 was considered negative; in Banff 2013, score 1 was considered positive and Banff 2015 and 2017 supported this concept. However, recent data have questioned the sensitivity and specificity of C4d staining as it is not entirely specific for ABMR and its significance without evidence of rejection is uncertain [11].

In the current study, ABMR was diagnosed in any biopsy with C4d scores 2 or 3 only. Biopsies with score 1 were considered negative; following the studies done by Dominy *et al*., 2018, and Kumar *et al*., 2019 [11], [12]. The reasons for considering score 1 negative in the current study are: First, in some cases, C4d-positive plasma in the capillaries makes the interpretation of PTC staining more difficult (plasma staining is a fixation artifact). Second, in paraffin sections, granular C4d deposits may overlay the specific linear expression in the PTCs, thus interfering with the interpretation of the staining pattern [30]. Third, technical issues related to the type of fixative used and different methods of C4d detection may also interfere with the interpretation of the staining pattern. Forth, C4d deposition in biopsies without evidence of rejection is a common finding and is associated with an increased risk of graft scarring, and may also represent a marker for stable graft accommodation not always associated with MVI, at least in ABO-incompatible renal allografts [31].

In this study, 28/98, 28.6% of all biopsy-proven AR were ABMR as judged by C4d positivity in PTCs and morphological changes. Unfortunately, DSA documentation was not always possible in the present study which might have underdiagnosed the real number of ABMR cases. The overall prevalence of C4d staining in all renal allograft biopsies was 49.1% (80 of 163). C4d positivity was encountered in 17.2% (28 out of 163) which falls in the spectrum of the previous studies' results [32], [33], [34]. In the present work, 21.4% (6 out of 28) and 78.6% (22 of 28) were score 2 and 3, respectively. These findings were closer to those reported by Kumar *et al*., 2019, where 30.8% and 69.2% were scores 2 and 3, respectively [12]. On the contrary, C4d positivity in the study conducted by Tariq and Nasir, 2018, was encountered in (16/60, 26.7%) with score 2 in 20% and score 3 in 80%. The reasons for this discrepancy include techniques applied for immunostaining (IF was used in their study) and sample size [35]. In the current study, all cases of

ABMR showed evidence of MVI; however, Tariq and Nasir, 2018, found that 6.25% of ABMR cases showed only ATI without evidence of MVI [35].

In the present study, a significant correlation was detected between g1 and ptc1 on one side and negative C4d staining on the other side. On the other hand, no significant correlation was detected between g2 and ptc2 at one side and positive C4d staining at the other side reflecting the poor association between MVI (each of "g" and "ptc" scores) and C4d positivity (r = 0.2 for each, respectively). These findings were comparable to the study conducted by Ranjan *et al*., 2008 and Kulkarni *et al*., 2011, where no association was present between "g" score and C4d positivity [32], [36].

In the current study, the major AR category was TCMR accounting for 63.3% of the total rejection cases. This was similar to the findings reported by Koshy *et al*. 2017 where TCMR was the predominant rejection category accounting for 71%. Further, in the studies conducted by Philip *et al*., 2011, and Aryal and Shah, 2012, TCMR was more common than ABMR (46.9% vs. 42% and 39.1% vs. 34.8%, respectively) of total rejection cases [37], [38]. This was closer to the current study, where TCMR far exceeded ABMR (63.3% vs. 28.6%) but with a difference in frequency due to differences in the post-transplantation interval and sample size.

Among the rejection category, borderline changes were the least; encountered only in (11/98, 11.2%). This was closer to the findings reported by Kazi and Mubarak, 2012, and Koshy *et al*., 2017, where borderline changes constituted 11.6% and 12% of all rejection cases, respectively [1], [24]. In the current study, borderline changes were seen in four biopsies, which were not-suitable for Banff scoring either due to absence or paucity of arteries. Besides, three biopsies showed minimal requirements for scoring. Therefore, in seven cases, vascular rejection could not be excluded.

While dealing with renal allograft dysfunction, equal weight should be given to find out the possible etiologies other than rejection. Among the most important reasons for early graft dysfunction other than rejection are the operative complications including vascular thrombosis (which eventually leads to allograft infarction) and peritransplant fluid collection. In the current study, infarction was detected in 15 cases (9.2%). Infarction due to operative causes was present in four cases; clarified by the presence of major vessel thrombosis in the nephrectomy specimen and absence of positive C4d staining. In the rest of cases, there was neither radiological nor pathological evidence of major vessel thrombosis.

In the current study, diagnosis of post-transplantation *de novo* or recurrent glomerulonephritis (GN) faces challenges in identifying and confirming its presence. This may be due to: First, the cause of native ESRD is often uncertain. Second, GN (except FSGS) usually does not occur in the 1st month.

Recognizing early tubulitis and intimal arteritis in conventionally stained sections can be difficult because it necessitates the accurate localization and detection of single lymphocytes. Furthermore, it has been reported that it can be very difficult to distinguish between infiltrating lymphocytes and apoptotic tubular epithelial cells [39]. On the other hand, some centers routinely use and even recommend immune-histochemistry for T cells with a PAS counterstain [13].

The use of IHC stain for T-cells with PAS counterstain in this study allowed the rapid and reliable identification and thus grading of tubulitis as well as the identification of mild intimal arteritis, even if represented by only one lymphocyte, nonetheless, missed' t1 was found only in a single case and t2 was found in a single case, while immunostaining did not detect any case where the grade of tubulitis had originally been overestimated. Elshafie and Furness, 2011 examined 100 cases and reported missed Tubulitis in 68% (t1 in 61% and t2 in 7%), where missed intimal arteritis was found only in one case [40]. The cause of this discrepancy may be because they selected only biopsies previously diagnosed by other pathologists as negative for rejection as well as 26% of the examined biopsies were protocol biopsies, obtained from stable grafts.

Conclusion

Acute rejection and drug toxicity account for the majority of early graft dysfunction, however other pathological lesions, *per se* or coincide with them may be the cause. The routine use of IHC for T cells with PAS as a counterstain is recommended, as it allowed the rapid and reliable identification and grading of early tubulitis as well as the identification of early intimal arteritis, even if represented by a single lymphocyte. The significance of g2 *per se* as a marker for diagnosis of ABMR as well as considering C4d score 1 (by IHC) positive; require further study with follow-up. Finally, adequate allograft biopsy, full clinical data, and presence of histological baseline information of the graft (through zero-hour biopsy) are mandatory for differentiation between the causes of early graft dysfunction and in the establishment of a proper treatment protocol.

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