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Dysregulation of Interleukin 23 Receptor Expression in Kidney Allografts Associated with Composite Outcome

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Abstract

Background: Interleukin 23 (IL-23) and interleukin 23 receptor (IL-23R) play a role in the pathogenesis of multiple autoimmune processes and renal inflammation, but research has yet to clarify the histological association of IL-23/IL-23R and transplant kidney allografts.

Methods: Between July 2009 and August 2011, 31 renal transplant recipients who received sonography-guided kidney allograft biopsy were enrolled in this retrospective study. The patients were divided into two groups including group A (patients reaching composite outcome) and group B (patients not reaching composite outcome). The composite outcome was defined as serum creatinine (Scr) doubling and lower estimated glomerular filtration rate (eGFR). Specimens of 31 patients were examined by the immunohistochemical stain of IL-23 and IL-23R in allograft kidneys, and clinico-pathological associations were evaluated.

Results: Of the 31 patients, group A had 15 patients (48.3%) and group B had 16 patients (52.7%). Group A had significantly higher SCr, lower eGFR, and low serum albumin (p=0.024). Univariate analysis showed that group A was negatively associated with atrophic glomerular mesangial cell cytoplasmic IL-23R expression (p=0.044). The decreased expression of IL-23R could be due to higher acute antibody-mediated rejection with heavy proteinuria in our study. In other words, the more the glomerular damage due to antibody-mediated rejection, the less the expression of IL-23R in atrophic glomerular mesangial cell cytoplasma.

Conclusions: The patients with composite outcome may have decreased expression of IL-23R in atrophic glomerular mesangial cell cytoplasm.

Keywords: IL-23; IL-23 receptor; Immunohistochemistry stain; Renal transplantation

Introduction

A kidney transplant is the best choice for the treatment of end stage kidney disease. Rejection of transplanted tissues involves the interplay between mechanisms that maintain tolerance to the graft and factors that promote rejection. Acute rejection continues to be one of the most important causes of graft loss and involves the cellular and/or humoral immune response [1]. Cellular rejection is characterized morphologically by the presence of mononuclear cells in the interstitial, tubular, and glomerular compartments [1-3]. Moreover, humoral rejection is associated with vascular involvement (vasculitis), deposition of immunoglobulins (C4d deposition in peritubular capillaries), and activation of the complement cascade [4-6].

Patients with acute allograft rejection present with an acute rise in the Scr and lower eGFR. A rising Scr level, however, is a relatively late development in the course of a rejection episode and usually indicates the presence of significant histological damage [1]. New or worsening proteinuria may also be present with/without decreased serum albumin level, especially in the case of acute humoral rejection [6].

T lymphocytes not only play an essential role in the initiation of the cascade of mechanisms underlying rejection but also participate in mechanisms that maintain graft tolerance [7,8]. Naive CD4+ helper T cells have been shown to develop into at least 4 types of committed helper T cells, namely, T helper (Th) 1, Th2, Th17, and regulatory T cells [3,5,9]. Interleukin 23 (IL-23) affects interferon-γ production (IFN-γ) by T and natural killer cells, activates memory T cells, stimulates Th1 cell responses, and enhances inflammation by stimulating the production of proinflammatory cytokines [10,11].

IL-23 mediates these effects through binding a receptor composed of IL-12Rb1 and interleukin 23 receptor (IL-23R), with the latter being located on chromosome 1p31.3. IL-23R, which is the initial sensor of the IL-23 signal, also determines Th17cell expansion and in turn serves

as an important gate for Th17 cell mediated autoimmune responses [12]. An accumulating body of literature reports that the presence of IL-23R gene polymorphism is associated with various autoimmune diseases such as rheumatoic arthritis, Crohn's disease, Grave's ophthalmopathy and graft-versus-host [13-16]. Recently, Tsai et al. [17] revealed an association between interleukin 23 receptor polymorphism and kidney transplant outcomes.

Based on the aforementioned background research, we hypothesize that IL-23 and IL-23R are related to the immuno-modulatory functions of transplanted kidneys. The aim of this study is thus to examine the clinico-pathological correlation of expressions of IL-23/IL-23R in kidney transplant allografts.

Materials and Methods

Study design and patients

From July 2009 to August 2011, pathological specimens from 31 renal transplantation recipients who received sonography-guided kidney allograft biopsy were retrospectively recruited. Institutional review board approval of Chung Shan Medical University Hospital was obtained for the review of patients' medical records, data analysis and pathological specimens staining, and the need for informed consent was waived.

Patient age, gender, body mass index, status of cigarette smoking, hypertension, diabetic mellitus, hepatitis B, hepatitis C, blood pressure, dialysis mode before transplantation, immusupressant drug (tacrolimus and cyclosporin) and drug trough level were recorded. Labotory data including SCr, eGFR, hemoglobin, hemoglobin A1c (HBA1C), lipid titer, albumin, uric acid and dipstick urine protein. eGFR was calculated by the abbreviated Modification of Diet in Renal Disease formula (aMDRD): eGFR=186 x (serum Creatinine)-1:154 x (age)-0:203 x (0.742 if female). Chronic kidney disease was defined by K/DOQI guidelines [18].

Primary composite outcome

The primary outcome measured in this study was a composite end-point of time to first event with a doubling of SCr or declining of eGFR of more than 30%. The composite group (group A) was defined as reaching the doubling value of baseline SCr or declining of baseline eGFR of more than 30%. The group B was defined as not reach composite outcome. A baseline SCr/eGFR was defined as the best stable level of SCr/eGFR within six months between allograft biopsy, and terminal SCr/eGFR was defined as the following of SCr/eGFR to September 15, 2013.

Doubling of SCr was defined as the first date to have the doubling value of baseline SCr, and the declining of eGFR was defined as the first date when eGFR declined 30% from baseline eGFR. Doubling of SCr was chosen as the primary composite outcome because it is a well-defined, clinically relevant outcome [19,20]. As recently recommended by the US Food and Drug Administration (FDA), we used the 30% decline in eGFR as an endpoint for kidney failure [21].

Clinical pathological diagnosis

The clinical pathological diagnosis was acute tubular injury (n=5), mild acute cellular rejection (n=8), severe acute cellular rejection (n=13) and antibody mediated rejection (ABMR) (n=5). The severe acute cellular rejection was defined as Banff type acute cellular rejection IA, IB and IIA [22]. If there were more than two pathological diagnoses of ex calcineurin inhibitor nephrotoxicity, chronic fibrosis change, de novo glomerulonephritis, dibetic nephropathy or polyomavirus nephropathy in one renal specimen, we chose the major pathological result as the pathological diagnosis in this study.

Tissue processing

Pathologic material was processed by conventional histological procedures. The specimens were collected by sonography-guided kidney allograft biopsy. Each section was at least 2×0.5 cm2. The formalin-fixed, paraffin-embedded tissues were cut into 4-mm hematoxylin- and eosin-stained (H&E staining) sections and examined to evaluate the glomerular, renal tubular, and interstitial conditions. The scoring of fibrosis was based on Banff scoring for chronic lesions [22] with interstitial fibrosis score and glomular fibrosis score.

Each patient who recieved kidney allograft biopsy was received medical therapy according to the pathological diagnosis. When the renal insuffiency recovered, the patient was discharged with an outpatient department follow up. The remaining biopsy tissue was stained by innumohistochemical (IHC) of IL-23 and IL-23R. The study protocol was approved by the local Ethics Committee.

Immunohistochemical staining

Paraffin embedded kidney tissue sections (4-mm) on poly-1-lysine-coated slides were deparaffinized. After treatment with 3% H2O2 in methanol, the sections were hydrated with gradient alcohol and PBS, incubated in 10 mM citrate buffer, and finally heated at 100 uC for 20 min in PBS. Slides were incubated with the anti-IL-23 and IL-23R antibody (Santa Cruz, CA, USA) for 20 min at room temperature, and then with a horseradish peroxidase (HRP)/Fab polymer conjugate for another 30 min. Then, slides were thoroughly washed three times with PBS, and the sites of peroxidase activity were visualized using 3, 3-diamino-benzidine tetrahydrochloride as a substrate and hematoxylin as the counter stain.

Semi-quantitative grading

All IHC stain data were independently scored by two blinded pathologists using the following scale: 0=no staining, 1=mild staining, 2=moderate staining, 3=high staining (Figure 1). Every slide was examined entirely for nuclear and cytoplasmic IL-23/IL-23R stains in the normal and atrophic renal tubules, in the normal and atrophic glomeruli, and in the renal interstitium. Each 2×0.5 cm2 section contained at least 10 glomerular areas, and the actual number of examined glomeruli was based on the sectioned tissue size. The results of nuclear and cytoplasmic staining were recorded separately. The intensity of IL-23 and IL-23R staining was classified as with staining (1, 2 and 3) or without staining (0).

Statistical analysis

Continuous and categorical data were expressed as median (25%-75%) and as proportions, respectively. Categorical variables were analyzed by the chi-square test or Fisher's exact test. Spearman's rank correlation coefficient was used for association between clinical variables when reaching composite outcome. A p-value less than 0.05 was considered statistically significant. All data were analyzed using SPSS version 14.0 statistical software.

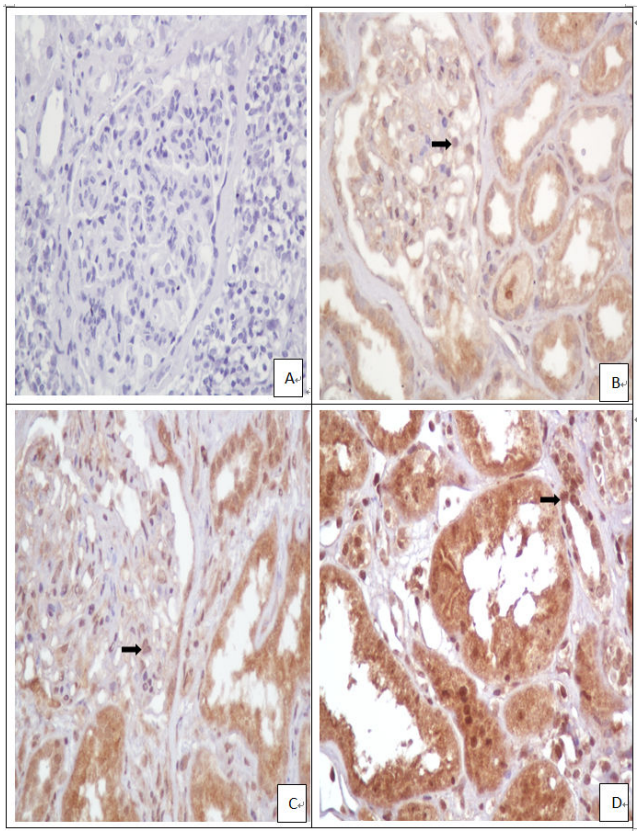

Figure 1: Representative panels showing different expression intensity of IHC IL-23R stain quantitative grading in glomeruli and renal tubules (IHC stain, × 400). (A) no IHC IL-23R stain in glomeruli and tubules, (B) mild IHC IL-23R stain in normal glomeurli and tubules , (C) moderate IHC IL-23R stain in atrophic glomeruli and tubules, and (D) high IHC IL-23R stain in normal renal tubules. The arrow indicates the IHC stain in a glomerular mesangial cell and renal tubular cell.

Results

We classified the 31 patients devided into group A (n=15) and group B (n=16). There were significant differences in the eGFR (ternimal), SCr (ternimal) and albumin level between those two groups. Our results indicate that group A had low ternimal eGFR (15.6 mL/min for group A, 58.5 mL/min for group B, p<0.001), high ternimal SCr (3.3 mg/dl for group A, 1.2 mg/dl for group B, p<0.001) and lower albumin levels (3.5 g/dL for group A, 3.8 g/dL for group B, p=0.025) (Table 1).

	Patients reaching composite outcome (group A)		**Patients not reaching composite outcome (group B)**		**P value**
	median	25%-75%	median	25%-75%	
Patient number (n)	15		16		
Age (year)	57.3	52.6 - 60	55.85	48.8 – 62.23	0.861
Gender F/M (n)	5/10		5/11		1
HTN (n, %)	12 (80)		11 (68.8)		0.685
DM (n, %)	5 (33.3)		7 (43.8)		0.822
HBV (n, %)	1 (6.7)		2 (12.5)		1
HCV (n, %)	6 (40)		2 (12.5)		0.113
Smoke (n, %)	6 (40)		7 (43.8)		0.833
BMI (kg/m^2)	27	20.8 – 30.6	24.9	22.75 - 26.78	0.338
HD/PD (n)	12/3		13/3		1
Dialysis duration (y)	1	0.7 - 2.0	2	0.93 – 4.38	0.216
Pathologic diagnosis					0.302
(1) Acute tubular injury (n, %)	1 (6.7)		4 (25)		
(2) Mild cellular rejection (n, %)	4 (26.7)		4 (25)		
(3) Severe cellular rejection (n, %)	6 (40)		7 (43.8)		
(4) Antibody rejection (n, %)	4 (26.7)		1 (6.3)		
Drug (Tacrolimus/ Cyclosporin)	14/1		13/3		0.6
SBP (mmHg)	129	120 - 144	132	120.5 - 150	0.379
DBP (mmHg)	74	60 - 82	81	70.75 - 93	0.11
eGFR (biopsy) (ml/min)	31	24 - 39	34.5	31.25 - 44.75	0.202
eGFR (baseline) (ml/min)	53	41 - 58	60	51 - 67.75	0.093
eGFR (terminal) (ml/min)	15.6	8.6 – 36.1	58.5	44.75 – 70.58	< 0.001*
SCr (biopsy) (mg/dl)	2.2	1.8 - 2.8	1.8	1.7 - 2.28	0.216
SCr (baseline) (mg/dl)	1.4	1.3 - 1.8	1.2	1.1 - 1.4	0.066
SCr (terminal) (mg/dl)	3.3	2.0 - 6.8	1.2	1.03 - 1.6	< 0.001*
Hemoglobin (g/dl)	10.8	9.6 - 13	11.65	8.88 - 13.43	0.892
HbA1C (%)	6.4	6.08 - 7.08	6.3	5.9 - 7.9	0.914
Low-density lipoprotein (mg/dl)	86.5	74.3 – 121.5	84	51 - 132	0.683
Total cholesterol (mg/dl)	161	132 - 187	179.5	112.5 - 243.25	0.682

Triglyceride (mg/dl)	112	93 - 181	138.5	113.5 – 269.25	0.318
Albumin (mg/dl)	3.5	3.15 - 3.75	3.8	3.6 - 4.1	0.025*
Uric acid (mg/dl)	7.1	5.8 - 8.3	6.5	5.7 - 7.68	0.65
Urine dipstick protein (mg/dL)					0.296
0 mg/dL		5 (33.3)	10 (62.5)		
30-100 mg/dL		7 (46.7)	5 (31.25)		
>100 mg/dL		3 (20)	1 (6.25)		
Composite outcome: first event with doubling of SCr or declining eGFR more than 30%. HTN, hypertension; DM, diabetes mellitus; HBV, hepatitis B; HCV, hepatitis C; BMI, body mass index; HD, hemodialysis; PD, peritoneal dialysis; SBP, systolic blood pressure; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration; SCr: serum creatinine; HbA1C: hemoglobin A1c.					
*: p<0.05 indicates significance					

Table 1: Demographic and clinical characteristics of patients divided by patients reaching composite outcome (left) and patients not reaching composite outcome (right).

We compared the association between group A and group B with the intensity of IL-23/IL-23R expression in each component of the specimen in Table 2. However, there was no relationship found for thedifferent regions of renal tissues including the glomerular, tubular and intertisium.

	Score	**Patients reaching composite outcome (group A)**	**Patients not reaching composite outcome (group B)**	**P value**
IFS	Without	5 (33.3)	9 (60)	0.143
	With	10 (66.7)	6 (40)	
GFS	Without	12 (80)	15 (100)	0.224
	With	3 (20)	0	
IL23 (Gn)n	Without	6 (54.5)	9 (81.8)	0.361
	With	5 (45.5)	2 (18.2)	
IL23 (Gn)c	Without	9 (81.8)	5 (45.5)	0.183
	With	2 (18.2)	6 (54.5)	
IL23 (Ga)n	Without	12 (92.3)	6 (100)	1
	With	1 (7.7)	0	
IL23 (Ga)c	Without	10 (76.9)	6 (100)	0.517
	With	3 (23.1)	0	
IL23 (ATn)n	Without	15 (100)	15 (93.8)	1
	With	0	1 (6.3)	
IL23 (ATn)c	Without	0	1 (6.3)	1
	With	15 (100)	15 (93.8)	
IL23 (ATa)c	Without	0	1 (33.3)	0.3
	With	7 (100)	2 (66.7)	
IL23 (IT)c	Without	14 (93.3)	11 (68.8)	0.172
	With	1 (6.7)	5 (31.3)	
IL23R (Gn)n	Without	4 (44.4)	3 (30)	0.65
	With	5 (55.6)	7 (70)	
IL23R (Gn)c	Without	8 (88.9)	8 (80)	1
	With	1 (11.1)	2 (20)	
IL23R (Ga)n	Without	9 (69.2)	5 (71.4)	1
	With	4 (30.8)	2 (28.6)	
IL23R (Ga)c	Without	13 (100)	5 (71.4)	0.111
	With	0	2 (28.6)	
IL23R (ATn)n	Without	9 (60)	8 (50)	0.576
	With	6 (40)	8 (50)	
IL23R (ATn)c	Without	2 (13.3)	3 (18.8)	1
	With	13 (86.7)	13 (81.3)	
IL23R (ATa)n	Without	3 (50)	3 (75)	0.571
	With	3 (50)	1 (25)	
IL23R (ATa)c	Without	1 (16.7)	1 (25)	1
	With	5 (83.3)	3 (75)	
IL23R (IT)n	Without	10 (66.7)	13 (81.3)	0.433
	With	5 (33.3)	3 (18.8)	
IL23R (IT)c	Without	10 (66.7)	9 (56.3)	0.552
	With	5 (33.3)	7 (43.8)	
Composite outcome: first event with doubling of SCr or declining eGFR more than 30% IFS, interstitial fibrosis score; GFS, glomerular fibrosis score; (Gn)n, nuclear staining intensity of normal glomerulus mesangial cell; (Gn)c, cytoplasmic staining intensity of normal glomerulus mesangial cell; (Ga)n, nuclear staining intensity of atrophy glomerulus mesangial cell; (Ga)c, cytoplasmic staining intensity of atrophy glomerulus mesangial cell; (ATn)n, nuclear staining intensity of normal renal tubule; (ATn)c, cytoplasmic staining intensity of normal renal tubule; (ATa)n, nuclear staining intensity of atrophy renal tubule; (ATa)c, cytoplasmic staining intensity of atrophy renal tubule; (IT)n, nuclear staining intensity of interstitium; (IT)c, cytoplasmic staining intensity of interstitium.				
p<0.05 indicates significance.				

Table 2: Intensity of IL 23/IL-23 receptor expression in different regions of renal tissues divided by patient reach composite outcome (left) and patient did not reach composite outcome (right).

The Spearman's correlation between the clinical variables and primary composite outcome revealed that group A was negatively

associated with eGFR (ternimal) (r=0.78, p<0.001), positively associated with SCr (terminal) (r=0.784, p<0.001), and negatively associated with albumin level (r=0.427; p=0.024) (Table 3).

	R	P value
Patient number (n)	0.032	0.862
Age (year)	-0.222	0.905
Gender F/M (n)	0.128	0.491
HTN (n, %)	0.081	0.666
DM (n, %)	-0.099	0.598
HBV (n, %)	0.314	0.085
HCV (n, %)	-0.38	0.839
Smoke (n, %)	0.177	0.341
BMI (kg/m2)	0.016	0.933
HD/PD (n)	-0.23	0.213
Pathology diagnosis	0.281	0.125
Drug	0.18	0.332
SBP (mmHg)	-0.166	0.371
DBP (mmHg)	-0.296	0.106
eGFR (biopsy) (ml/min)	-0.238	0.196
eGFR (baseline) (ml/min)	-0.307	0.093
eGRF (terminal) (ml/min)	-0.78	<0.001*
SCr (biopsy) (mg/dl)	0.232	0.21
SCr (baseline) (mg/dl)	0.342	0.06
SCr (terminal) (mg/dl)	0.784	<0.001*
Hemoglobin (g/dl)	0.029	0.877
HbA1C (%)	0.025	0.898
Low-density lipoprotein (mg/dl)	0.078	0.686
Total cholesterol (mg/dl)	-0.079	0.671
Triglyceride (mg/dl)	-0.184	0.322
Albumin (mg/dl)	-0.427	0.024
Uric acid (mg/dl)	0.091	0.638
Urine dipstick protein (mg/dL)	0.309	0.09
Composite outcome: first event with doubling of SCr or declining eGFR more than 30%. HTN, hypertension; DM, diabetes mellitus; HBV, hepatitis B; HCV, hepatitis C; BMI, body mass index; HD, hemodialysis; PD, peritoneal dialysis; SBP, systolic blood pressure; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration; SCr, serum creatinine; HbA1C, hemoglobin A1c.		
*: p<0.05 indicates significance		

Table 3: Spearman's correlation association between clinical variables when reaching composite outcome

Table 4 shows the Spearman's correlation between pathological variables and primary composite outcome. Univariate analysis indicated that IHC IL-23R expression intensity stain was negatively associated with atrophic glomerular mesangial cell cytoplasm (r=0.454, p=0.044).

	R	P value
IFS	0.267	0.153
GFS	0.333	0.072
IL23 (Gn)n	0.293	0.186
IL23 (Gn)c	-0.378	0.083
IL23 (Ga)n	0.16	0.513
IL23 (Ga)c	0.294	0.222
IL23 (ATn)n	-0.177	0.341
IL23 (ATn)c	0.177	0.341
IL23 (ATa)c	0.509	0.133
IL23 (IT)c	-0.311	0.089
IL23R (Gn)n	-0.15	0.541
IL23R (Gn)c	-0.122	0.62
IL23R (Ga)n	0.023	0.924
IL23R (Ga)c	-0.454	0.044*
IL23R (ATn)c	0.074	0.694
IL23R (ATa)n	0.25	0.486
IL23R (ATa)c	0.102	0.779
IL23R (IT)n	0.167	0.371
IL23R (IT)c	-0.107	0.567
Composite outcome: first event with doubling of SCr or declining eGFR more than 30%. IFS, interstitial fibrosis score; GFS, glomerular fibrosis score; (Gn)n, nuclear staining intensity of normal glomerulus mesangial cell; (Gn)c, cytoplasmic staining intensity of normal glomerulus mesangial cell; (Ga)n, nuclear staining intensity of atrophy glomerulus mesangial cell; (Ga)c, cytoplasmic staining intensity of atrophy glomerulus mesangial cell; (ATn)n, nuclear staining intensity of normal renal tubule; (ATn)c: cytoplasmic staining intensity of normal renal tubule; (ATa)n, nuclear staining intensity of atrophy renal tubule; (ATa)c, cytoplasmic staining intensity of atrophy renal tubule; (IT)n, nuclear staining intensity of interstitium; (IT)c,cytoplasmic staining intensity of interstitium.		
*: p<0.05 indicates significance		

Table 4: Spearman's correlation association about pathological variables when reaching composite outcome.

Figure 2 illustrates the different expression intensities of H&E stain (panel A, C, E) and IL-23 IHC stain (panel B, D, F) in pateints reaching composite outcome; H&E and IL-23R IHC stain in patients not reaching composite outcome is shown in Figure 3. Finally, a decreased intensity of IL-23R expression in atrophic glomerular mesangial cell cytoplasma in group A (panel A) can be seen in Figure 4.

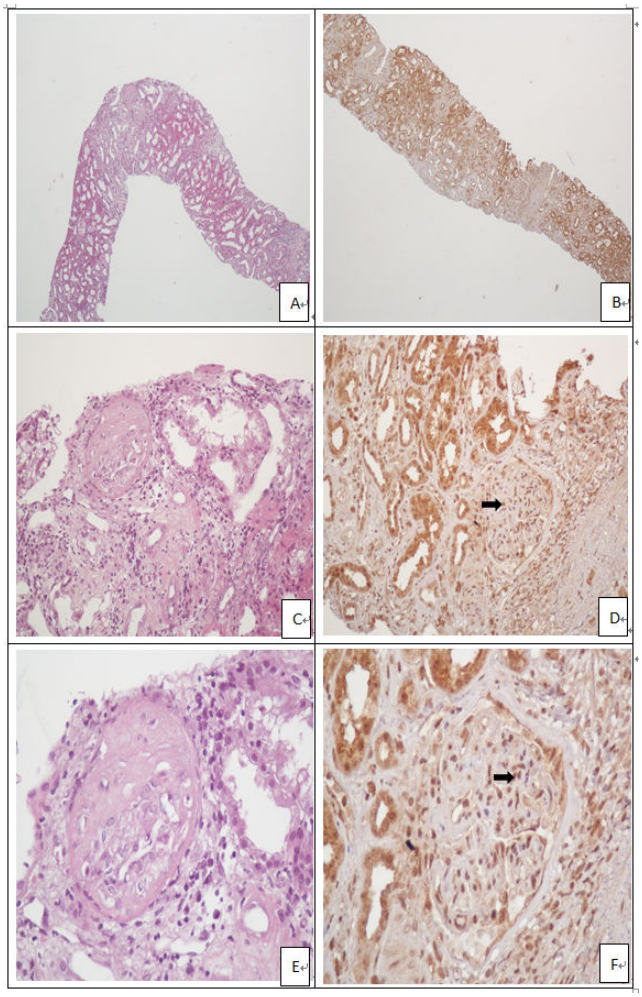

Figure 2: Representative panels showing a different intensity of IL-23 expression by IHC stain compared to H&E stain in a patient reaching composite outcome (group A). (A) H&E stain, x 20, (B) IHC IL-23 stain, x 20, (C) H&E stain, x 200, (D) IHC IL-23 stain, x 200, (E) H&E stain, x 400, (F) IHC IL-23 stain, x 400. The arrow indicates the IL-23 IHC stain in a glomerular mesangial cell.

Discussion

Our results demonstrate that patients reaching the composite outcome were negatively associated with atrophic glomerular mesangial cell cytoplasmic IL-23R expression (p=0.044). However, IL-23 expression was not associated with the outcome in any of the renal tissue, including the glomerular, tubule and interstitium.

In the clinical variable analysis, the group A was negatively associated with terminal eGFR (r=0.78, p<0.001), positively associated with terminal SCr (r=0.784, p<0.001), and negatively associated with albumin level (r=0.427; p=0.024). Because the definition of composite outcome was doubling volume of SCr or a declining baseline eGFR of more than 30% in our study, the results of terminal Scr and eGFR could be due to the definition. Furthermore, compared with group B, group A had more ABMR (26.7% vs. 6.3%) in the pathologial diagnosis and higher urine dipstick protein (>100mg/dL, 20% vs. 6.25%). Given that AMBR usually presents with transplant glomerulopathy and proteinuria [22,23], the lower albumin level in group A (3.5 mg/dl vs. 3.8 md/dl, p=0.025) could be caused by a higher ABMR with transplant glomerulopathy and high urine dipstick protein in our study.

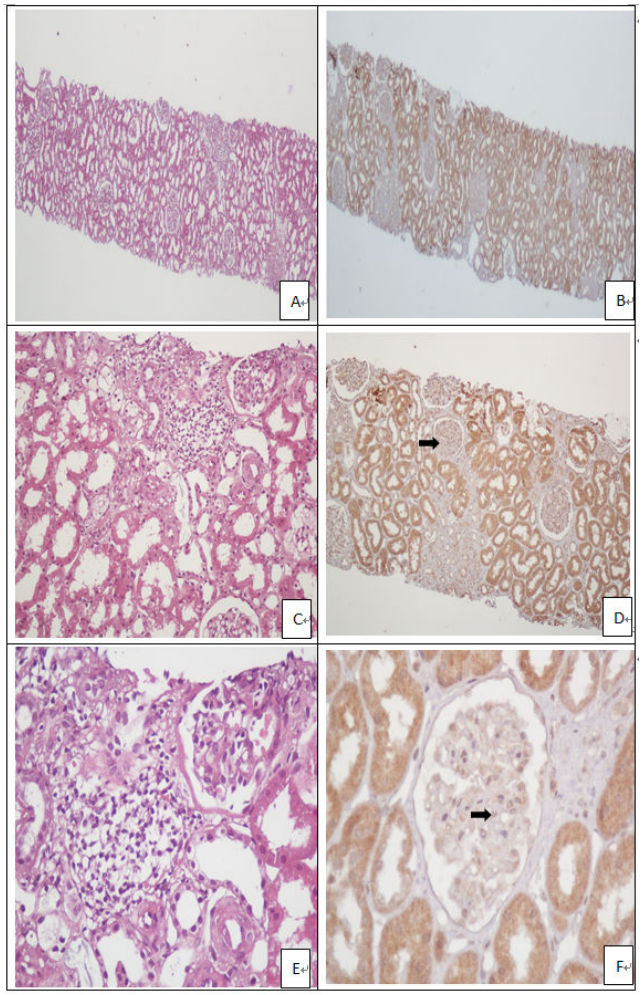

Figure 3: Representative panels showing a different intensity of IL-23R expression by IHC stain compared to the H&E stain in a patien not reaching composite outcome (group B). (A) H&E stain, x 20, (B) IHC IL-23R stain, x 20, (C) H&E stain, x 200, (D) IHC IL-23R stain, x 200, (E) H&E stain, x 400, (F) IHC IL-23R stain, x 400. The arrow indicates the IL-23R IHC stain in a glomerular mesangial cell.

There have been several reports about interlukin marker expression in acute allograft rejection. Byung at al. revealed that higher infiltration by Th17 cells is associated with severe acute T-cell-mediated graft rejection. Higher infiltration of Th17 is significantly associated with the severity of allograft dysfunction and tissue injury [24]. IL-17 expression by tubular epithelial cells in renal transplant recipients with acute antibody-mediated rejection has also been observed, with IL-17 tubular expression being directly and significantly correlated with the extension of C4d deposits [25].

Although interlukin expression was found to be approximally expressed in the renal tubule and interstitium in previous research, several studies have revealed the correction of IL-23 and glomeruli disease. Paust et al. reported that the IL-23/Th17 axis contributes to renal injury in experimental glomerulonephritis, with IL-17 enhancing the production of the proinflammatory chemokines CCL2/MCP-1, CCL3/MIP-1, and CCL20/LARC in mouse mesangial cells. They further found that IL-23 p19-/- mice developed less severe nephritis as measured by renal function, albuminuria, and frequency of glomerular crescent formation [26]. IL-23 receptor expression has also been shown to be up-regulated in lupus nephritis; the greater the increase in serum level of IL-23R, the greater the glomerular damage of lupus nephritis in mice [27].

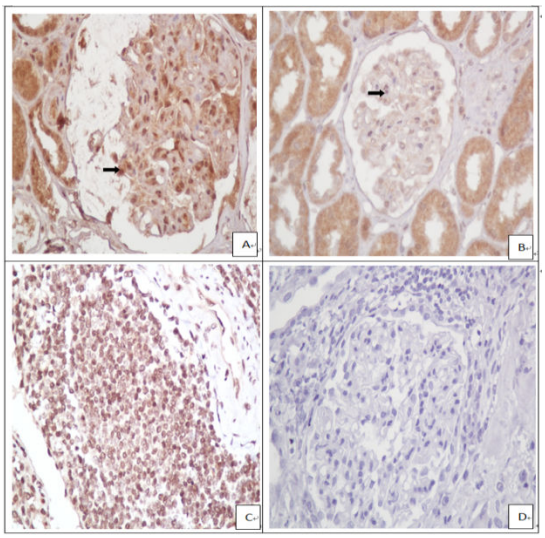

Figure 4: Representative panels showing a decreased intensity of IHC IL-23R expression in atrophic glomerular mesangial cell cytoplasma in a patient reaching composite outcome (group A) (IHC stain, x400). (A) IHC IL-23R stain in a patient reaching composite outcome (group A), (B) IHC IL-23R stain in a patient not reaching composite outcome (group B), (C) positive control of IL-23R IHC stain, (D) negative control of IL-23R IHC stain. The arrow indicates the IL-23R IHC stain in an atrophic glomerular mesangial cell.

In our study, the patients reaching the composite outcome had a decline of IL-23R expression in atrophic glomerular mesengial cell cytoplasm. ABMR was more evident with transplant glomerupathy [22]. Since our group A had a high ABMR ratio in the pathological diagnosis and greater deterioration of renal function, there could be more severe glomeruli demange with the presentation of atrophic glomeuli, and for this reason, less IL-23R was expressed in the atrophic glomerular mesangial cell cytoplasma. In other words, the more the allograft glomerular damage, the less the IL-23R expression in atrophic glomerular mesangial cell cytoplasm.

The limitation of this study was the small patient number, which caused inconsistency of the p values for the IL-23R expression in the glomerular cytoplasma of atrophic mesangial cells as seen between Table 2 (p=0.111, by Fisher's exact test) and Table 4 (p=0.044, by Spearman's correlation). To increase the patient number could further clarify the role of interleukin 23 receptor expression in kidney allografts.

In summary, our analysis of the expression of IL-23R in allograft specimens indicates a possible role of IL-23R over mesangial cytoplasm of atrophic glomerular cells. However, because most of the pathologic changes of acute allograft rejection were involved in the renal interstitium, the finding of IL-23R expression over glomeruli needs further study for clarification.

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