Urinary CXCL 10 is a Monitoring Marker for Acute Rejection in Pediatric Kidney Transplant Recipients

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Declarations

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Consent for participation: Informed consent was obtained from children care givers prior to inclusion in the study

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Abstract

Background: Early detection and proper management of acute graft dysfunction particularly acute rejection (AR) to improve outcomes are still needed. Urinary CXCL10 chemokine could provide a non-invasive monitoring marker for graft function. The aim of this study is to investigate the association of urinary CXCL10 levels with AR and to evaluate its prognostic value and utility as follow up marker after antirejection treatment.

Methods: Data of ninety-seven transplanted children was obtained and analyzed. Urine samples for CXCL 10 testing by ELISA technique were obtained from all patients. Follow up CXCL 10 evaluation for recipients with AR was performed one month later**.**

Results: Urine CXCL 10 level was significantly elevated in rejection group (n=41; 42.3%) than control group (n=56; 57.7%) (Median (IQR) =350 (151-2104) versus 55 (38-82) pg/ml, p<0.001). ROC curve analysis demonstrated that at a cut-off value of 87.5 pg/ml of CXCL10 level; AR could be predicted with 100% sensitivity, 78.6% specificity, 95% CI of (0.899-0.983) and AUC of 0.94. CXCL 10 levels significantly declined in rejection group after receiving antirejection therapy (p<0.001). Elevated

CXCL 10 levels significantly associated with chronic graft dysfunction 6 months later to its initial assessment (p=0.03).

Conclusion: Urine CXCL 10 is a reliable marker of acute allograft rejection after transplantation in pediatrics. Levels of urine CXCL 10 in rejection patients significantly decline after receiving antirejection therapy. Elevated urine CXCL 10 levels significantly associated with poor short term graft outcome.

Key words: Cellular rejection; CXCL10; graft outcome; monitoring; non-invasive.

Introduction:

Kidney transplantation (KT) is the preferred treatment for pediatric end-stage kidney disease (ESKD). However; complications are not uncommon following pediatric KT; hence its early detection and proper management to improve outcomes are mandatory [1].

Despite advances in immunosuppression (IS); acute allograft rejection (AR) remains one of the key factors affecting patient and graft survival in pediatric KT [2]. Current methods used to predict and/or to monitor AR have different limitations; rising of creatinine level may only be detected after graft damage has occurred and sometimes this rising may be related to cyclosporine (CsA) nephrotoxicity or infections [3]. Additionally, histological analysis of biopsy is invasive method and does not always give conclusive results in early rejection [4]. Although surveillance biopsy appears to assist in management of pediatric kidney transplant recipients (KTRs), the benefit as an invasive tool to improve long term graft outcome after subclinical rejection (SCR), to be evaluated [5].

Urinary CXC chemokine ligand 10 (CXCL10) is a promising early noninvasive diagnostic biomarker for (sub) clinical allograft rejection in adult and pediatric KTRs [6]. Levels of CXCL10 in urine have been closely correlated with the extent of subclinical tubulitis suggesting an important role for CXCL10 as urinary biomarkers of early rejection [7]. Furthermore, urinary CXCL10 was reported to normalize with treatment of AR [8] while its persistent elevation was associated with both chronic graft dysfunction (CGD) and decline in allograft function [9].

This study aims to validate urinary CXCL10 as non-invasive biomarker of AR, also to study its prognostic value and its utility as a follow-up marker after the therapeutic intervention of AR in pediatric KT.

Patients and Methods:

Patients:

The study included 97 pediatric KTRs who were recruited during their follow up at Kidney Transplantation Outpatient Clinic, Cairo University Children`s Hospital (CUCH), Cairo, Egypt. Included patients were recipient of living donor renal allograft for their first time aged between 2 and 18 years. Pediatric KTRs with CGD [10] only or acute graft dysfunction (AGD) due to any cause other than AR (as viral, bacterial infections or dehydration) were excluded from the study.

Enrolled patients (n=97) were categorized into cases (AR group) and controls (non-AR group) based on presence or absence of clinical [2] and pathological [11] evidences of AR on initial assessment:

(1) Cases (rejection group n=41): patients with AR (alone or on top of CGD)

(2) Control (non-rejection group n=56): patients with stable graft function [12].

Methods:

This is a cross-sectional study that was conducted between 2018 & 2020. The study protocol was approved by Research Ethical Committee of Pediatric Department, Faculty of Medicine, Cairo University (N: I - 250318). The study design confirmed to

the requirements of Revised Helsinki Declaration of Bioethics. Informed consent was obtained from all individual participants included in the study.

Baseline demographic, clinical and transplantation related data of included patients were gathered: age, sex, original renal disease, weight, height, body mass index (BMI), and blood pressure (BP) assessment. Donor issues was reviewed including age, sex, relation to the recipient, as well as review of IS regimen (including initial maintenance drugs/ protocol and current IS therapy), pathological type of current AR episode, antirejection treatment and response to therapy (decline of serum creatinine to its previous baseline), review of post transplantation hypertension, proteinuria, IFTA, laboratory investigations including: complete blood picture by coulter counter, kidney function test (blood urea nitrogen and serum creatinine), serum electrolytes (potassium, sodium, calcium, and phosphorus) and random blood sugar.

- **Urine CXCL 10 assessment:**

Urine CXCL 10 was assessed for all included patients (n=97) at initial evaluation. In patients diagnosed clinically as having AR upon enrollment into the study; initial CXCL 10 assessment was performed at the same day of performing indication graft biopsy.

- For patients proved pathologically to have AR (Rejection group n=41); follow up assessment of CXCL 10 after treatment with antirejection therapy (one month later to initial assessment) was performed.
- For patients with stable graft function (control group n=56); follow-up was done by monitoring for occurrence of clinical/pathologically proven rejection during the next month following initial CXCL10 assessment.

Early morning urine samples were collected from patients. Urine samples then were centrifuged at 3000 r.p.m (Jouan, GR412, Herblain, France) for 20 min at 4 °C. Supernatants were separated and stored at −80 °C till used for quantification of CXCL10 by the enzyme-linked immunosorbent assay (ELISA), using human CXCL10 Quantikine ELISA kit according to the manufacturer's instructions. The optical density of the samples was determined at 450 nm/540 nm using the microplate reader (Synergy 2, BioTek® Instrument, Inc, USA). A standard curve was generated using the CXCL10 standards and the concentration of samples was calculated using the Gen5® (version 1.08) software. Each sample was assayed in duplicate and the average value of the CXCL10 protein was used for statistical analysis.

Prognostic value of urine CXCL10 levels on short term graft outcome was assessed by evaluation of graft function (in term of serum creatinine and e GFR) six months later to initial CXCL 10 assessment.

Statistical analysis:

Data was coded and entered using the statistical package for the Social Sciences (SPSS) version 26 (IBM Corp., Armonk, NY, USA). Data was summarized using mean and standard deviation (SD) for normally distributed quantitative variables or median and interquartile range (IQR) for non-normally distributed quantitative variables and frequencies and percentages for categorical variables. Comparisons between groups were done using unpaired t test in normally distributed quantitative variables while non-parametric Kruskal-Wallis test and Mann-Whitney test were used for non-normally distributed quantitative variables. For comparison of serial measurements within each patient the non-parametric Wilcoxon signed rank test was used for comparing categorical data, Chi square test was performed. Exact test was used instead when the expected frequency is less than 5. Correlations between quantitative variables were done using Spearman correlation coefficient. ROC curve was constructed with area under curve analysis performed to detect best cutoff value

of significant parameters for detection of rejection. P-values less than 0.05 were considered as statistically significant.

Results:

The mean age of the study group was 12.1 (± 2.8) years, with male / female ratio of 77/20 and median post transplantation duration of 3 years. The primary renal disease was obstructive uropathy in 25.7%, glomerulopathy in 21.6%, nephronopthesis in 10.3%, unknown in 25.7% and other diagnoses (polycystic kidney, antiphospholipid disease, wilms tumor, primary hyperoxaluria, Bardet Biedl syndrome, cystinosis, vasculitis & caroli disease) in 16.5% of included patients. All patients received a living-donor kidney transplant. The vast majority of the included recipients (85.4% of the rejection group and 94.6% of the control group) received the transplant from their parents. The remainders were non-related living transplant recipients. Donors of rejection group were significantly older than those of the control group (p=**0.031**). Patients who received CSA as an initial CNI were more among rejection than the control group (66% versus 45% with p=0.029). Demographic, clinical, laboratory and KT related data of the study groups are illustrated by table 1.

Pathological findings, interpretation, therapeutic intervention of concomitant AR episode in rejection group are demonstrated in table 2. All patients with rejection received pulse steroid therapy as the first line antirejection therapy. Twenty-nine patients (70.7%) of rejection group received other modalities of antirejection treatment in addition to pulse steroids based on interpretation of their pathological data (table 2). At 6 month follow up assessment of rejection group (n=41); 8 patients developed CGD (19.5%), 3 patients (7.3%) reestablished regular dialysis (graft failure) while serum creatinine declined to its previous baseline in 30 patients.

Urine CXCL 10 in rejection group was significantly more than that in non-rejection group (p<0.001). Figure 1 demonstrates the area under the curve (AUC), specificity and sensitivity among rejection and control groups. The highest sensitivity (100%) and specificity (78.6%) was observed for the rejection group vs. control group, at a cut-off value of 87.5 pg/ml of urinary CXCL10 level with 95% CI of (0.899-0.983) and AUC of 0.94.

Urine values of CXCL 10 significantly declined after receiving antirejection therapy in rejection cases (41 patients) (p=<0.001) however they were still significantly more than their values in control group (p=0.016). When using urine CXCL 10 cutoff value that was extracted by this study (87.5 pg/ml) in comparison between the study groups; we found that frequency (&%) of elevated levels was significantly more in rejection group than controls (p<0.001), and significantly more in rejection group at the time of rejection (initial assessment) than in the same group after receiving antirejection treatment (follow up assessment) (p<0.001). Nevertheless; frequency (& %) of urine CXCL 10 levels > 87.5 pg/ml was more in rejection group after receiving antirejection therapy than control group (p=0.002) signifying that even if CXCL 10 levels decline with treatment of AR, they do not reach their control values before the incidence of AR within one month follow up (table 3).

Table 4 demonstrates comparison between CXCL 10 levels in different patient categories. Urine CXCL 10 levels were found to increase significantly in the setting of low GFR (<60 ml/min/1.73m), presence of proteinuria and high CNI trough level (p<0.001, 0.016 and 0.049 respectively). None of the included patients had active CMV infection at assessment while one patient had positive PCR for BK. No significant difference in CXCL 10 levels was detected between recipients with and

without active BK virus infection (CXCL10 median 250 pg/ml in BK positive and 103 pg/ml in BK –ve cases with p=0.495).

CXCL 10 levels significantly elevated in TCMR when compared to ABMR (CXCL10 median (IQR) 1725 (530-2527) in TCMR group vs 152 (113-184) in ABMR with p=0.02) and significantly declined in pathological (glomerulutis) or serological (DSA) evidence of ABMR (p= 0.049 and 0.001 respectively). Higher levels of CXCL 10 (>200 pg/ml) were prevalent among TCMR patients (85.7% of them had high CXCL 10 levels) (Table 5). Patients with CGD had significantly elevated levels of CXCL 10 than those with stable graft function (p=0.03).

CXCL 10 levels correlated positively with serum creatinine and negatively with GFR among the total sample as well as within rejection group (table 6). Within rejection group; CXCL 10 levels correlated positively with chronic sum of pathological scoring system (p=0.023, CC=0.495) and with follow up GFR 6 month later (p=0.011, CC=0.395), while negatively correlated with serum creatinine after antirejection therapy (p=0.006, CC=-0.420).

To study role of CXCL 10 values in prediction of short term graft outcome; follow up of graft function 6 months later was analysed. CXCL 10 levels were significantly more in patients with CGD than patients with stable graft function (p=0.03), with no significant difference between patients with CGD and patients with graft failure (p=1). No significant differences in follow up CXCL 10 levels between patients with stable graft function, CGD and graft failure within rejection group only (p=0.577).

Notably; among the control group (n=56), ten patients developed AR one to three weeks later to time of CXCL10 sampling. We observed that CXCL10 levels of those 10 patients at time of assessment (before developing AR) were higher than that of others in the same group [CXCL10 median was 181 (IQR 146-195) pg/ml in those 10 patients versus 50.5 (IQR 35-66) pg/ml in other patients of the group with p-value <0.001]. So CXCL10 level may predict that patients will develop AR.

Table (1): Demographic, clinical, laboratory and KT related data of the study groups

		Rejection group (n=41)		Control group (n=56)		P value
Sex (Count-%)	Male	28	68.3%	49	87.5%	**0.021**
	Female	13	31.7%	7	12.5%	
Age mean (±SD) years		12.95	(±2.69)	11.57	(±2.85)	**0.018**
Weight median (IQR) kg		37	(30-50)	30	(25-40.5)	**0.034**
Weight for age centile (Count-%)	<3rd percentile	11	26.8%	25	44.6%	0.188
	between 3rd and 97th	29	70.7%	29	51.8%	
	>97th	1	2.4%	2	3.6%	
Height mean (±SD) cm		137.66	(±14.15)	130.41	(±16.17)	**0.024**
Height for age centile (Count-%)	<3rd percentile	24	58.5%	32	57.1%	0.891
	between 3rd and 97th	17	41.5%	24	42.9%	
	>97th	0	0.0%	0	0.0%	
BMI median (IQR) kg/m^2		19.2	(16.5-24)	17.4	(16-23.1)	0.231
BMI for age (Count-%)	Healthy	20	48.8%	32	57.1%	0.846
	Underweight	5	12.2%	5	8.9%	
	Overweight	4	9.8%	5	8.9%	
	Obese	12	29.3%	14	25.0%	
Donor relation	Unrelated	6	14.6%	3	5.4%	0.161
	Related	35	85.4%	53	94.6%	
Donor sex	Male	29	70.7%	37	66.1%	0.627

	Female	12	29.3%	19	33.9%	
Donor age (years)	Median (IQR)	38	(35-42)	36	(35-38)	**0.031**
Donor age category	< 40 years	28	68.3%	49	87.5%	**0.021**
	> 40 years	13	31.7%	7	12.5%	
Initial IS drugs	CSA,MMF,PRED	27	65.9%	25	44.6%	**0.029**
	TAC,MMF,PRED	13	31.7%	31	55.4%	
	#TAC, m-TOR,PRED	1	2.4%	0	0.0%	
Current IS drugs	CSA,MMF	2	4.9%	2	3.6%	0.210
	TAC,MMF	36	87.8%	54	96.4%	
	TAC,AZA	1	2.4%	0	0.0%	
	TAC,M-TOR	2	4.9%	0	0.0%	
HB (g/dl)	Mean ±SD	11.13	±1.17	11.25	±1.13	0.625
TlC (x10³/µL)	Mean ±SD	6.43	±1.37	7.29	±1.67	**0.009**
PlT (x10³/µL)	Mean ±SD	183.56	±28.23	179.11	±15.24	0.320
Na (mEq/l)	Mean ±SD	140.54	±2.67	139.98	±2.06	0.251
K (mEq/l)	Mean ±SD	4.05	±0.33	3.95	±0.29	0.123
S. Albumin(g/dl)	Mean ±SD	3.88	±0.42	4.17	±0.27	**< 0.001**
Calcium(mg/dl)	Mean ±SD	8.47	±0.47	8.68	±0.47	**0.028**
ALT (U/L)	Mean ±SD	42.27	±5.85	32.36	±4.12	**< 0.001**
RBS(mg/dl)	Mean ±SD	107.34	±9.72	98.77	±15.94	**0.003**
S. Creatinine	Median (IQR)	1.40	1.20-1.70	0.70	0.70-0.8	**< 0.001**
Urea (mg/dl)	Median (IQR)	45.00	40-55	33	30-35	**< 0.001**
GFR at assessment	Median (IQR)	50.00	38.5-58.3	87.5	77.4-96.8	**<0.001**
IFTA	No	22	53.7%	48	85.7%	**0.001**
	Yes	19	46.3%	8	14.3%	
*Assessment CNI through level	Low	4	9.8%	2	3.6%	0.29
	Desired	34	82.9%	52	92.9%	
	High	3	7.3%	2	3.6%	
Post TX HTN	No	28	68.3%	47	83.9%	0.069
	Yes	13	31.7%	9	16.1%	
Current proteinuria	No	28	68.3%	56	100%	**<0.001**
	Yes	13	31.7%	0	0.0%	

BMI (body mass index), CSA (cyclosporine), TAC (tacrolimus), AZA (azathioprime), MMF (mycofenolate mofetile), mTOR (mammalian target of rapamycine), PRED (prednisone), IS (immunosuppression).

MMF was replaced by mTOR in this patient one month after TX due to suspected malignancy. Adjuvant therapy included MMF, mTOR or AZA

HB (hemoglobin), TLC (total leukocytic count), PLT (platelets), Na sodium, K potassium Ca calcium, AlT alanine aminotranseferase, RBS (random blood sugar).

IFTA (interstitial fibrosis and tubular atrophy), CNI (calcinurine inhibitors), TX (transplantation), HTN (hypertension).

*Desired CNI levels: {Desired TAC through level: Month 1 (10 to 12)-Month 2 and 3 (8 to 10)-Month 4, 5 and 6 (6 to 8)-After Month 6 (4 to 6). Desired cyclosporine level: Less than 1month (200 to 250)-1 to 2 months (150 to 200) - 2 to 3 months (100 to 150) - Greater than 3 months (80 to 100)}

Table (2): Pathological findings, interpretation, therapeutic intervention of current acute rejection episode in rejection group (n=41)

		Count	%
Pathological type of rejection (interpretation)	ABMR	14	34.1%
	Cell mediated	14	34.1%
	Mixed	13	31.7%
Pathological findings:			
Tubulitis		41	100%
Interstitial inflammation		38	92.7%
Peritubular capillaritis		14	34.1%
Glomerulitis		13	31.7%
Microvascular inflammation		27	65.8%
Arterial hyalinosis		23	56.1%
IFTA		19	46.3%
C4D Positive		13	31.7%
Banff score	Inadequate	20	48.8%
	Adequate	21	51.2%
Therapeutic intervention	Pulse steroids only	12	29.3%
	IVIG	13	31.7%
	Rituximab	1	2.4%
	ATG	4	9.8%
	IVIG + Rituximab	2	4.9%
	IVIG + Rituximab+PE	4	9.8%
	ATG +PE	2	4.9%
	PE	3	7.3%

ABMR (antibody mediated rejection), IVIG (intravenous immunoglobulin), PE (plasma exchange), ATG (antithymocyte globulin), IFTA (interstitial fibrosis/ tubular atrophy)

Table (3): Comparison between rejection group before/ after antirejection therapy and control group as regard urine CXCL 10 levels

	Control group (n=56)	**Rejection group before treatment (n=41)**	**Rejection group after treatment (n=41)**
CXCL 10 (pg/ml) Median (IQR)	55 (38-82)	350 (151-2104)	88 (55-108)
P value	<0.001	<0.001	0.016 vs control
	Control group (n=56)	**Rejection group before treatment (n=41)**	**Rejection group after treatment (n=41)**
CXCL 10≤ 87.5 pg/ml Number (%)	44 (78.6%)	0 (0%)	20 (48.8%)
CXCL 10>87.5 pg/ml Number (%)	12 (21.4%)	41 (100%)	21 (51.2%)
P value	<0.001	<0.001	0.002 vs control

Table (4): Comparison of different patient categories as regard their CXCL 10 levels

	CXCL 10 levels (pg/ml) Median	P value
GFR <60 (n=11)	172 (130-1958)	**<0.001**
GFR>60 (n=86)	65 (39-148)	

Proteinuria (n=13)	117 (140-350)	**0.016**
No proteinuria (n=84)	82 (46-216)	
Low CNI trough level (n=6)	143 (109-169)	**0.049**
*Desired CNI trough level (n=86)	87 (49-195)	
High CNI trough level (n=5)	237 (182-250)	
Interstitial inflammation (n=38)	440 (151-2104)	0.944
No interstitial inflammation (n=3)	172 (130-3045)	
Peritubular capillaritis (n=14)	311 (140-2308)	0.444
No peritubular capillaritis (n=27)	1090 (184-1961)	
Glomerulitis (n=13)	159 (119-1961)	**0.049**
No glomerulitis (n=28)	1220 (250-2357)	
ABMR (n=14)	152 (113-184)	**0.001**
TCMR (n=14)	1725 (530-2527)	
Mixed (n=13)	1694 (159-2447)	
IFTA (n=19)	140 (66-350)	0.075
No IFTA (n=22)	86 (44-237)	
DSA +ve (n=17)	155 (119 – 272)	**0.001**
DSA –ve (n=24)	1959 (252 – 2652)	
Stable graft function (n=30)	85.5 (49-237)	**0.033**
Chronic graft dysfunction (n=8)	260 (145-2369)	
Graft failure (n=3)	140 (113-154)	

GFR (glomerular filtration rate), CNI (calcinurine inhibitor), ABMR (antibody mediated rejection), TCMR (T cell mediated rejection), IFTA (interstial fiberosis/ tubular atrophy), DSA (donor specific antibody)

*Desired CNI levels: {Desired TAC through level: Month 1 (10 to 12)-Month 2 and 3 (8 to 10)-Month 4, 5 and 6 (6 to 8)-After Month 6 (4 to 6). Desired cyclosporine level: Less than 1month (200 to 250)-1 to 2 months (150 to 200) - 2 to 3 months (100 to 150) - Greater than 3 months (80 to 100)}

Table (5): CXCL 10 level category in patients with different pathological types of rejection

		ABMR (n=14)		**TCMR** (n=14)		**MIXED** (n=13)		**P value**
		Count	**%**	**Count**	**%**	**Count**	**%**	
CXCL10 (pg/ml)	**< 87.5**	0	0%	0	0.0%	0	0.0%	**0.002**
	87.5- 200	11	78.6%	2	14.3%	4	30.8%	
	> 200	3	21.4%	12	85.7%	9	69.2%	

Table (6): Correlation between CXCL 10 levels and different variables

	Total sample (n=97) CXCL 10 pg/ml		**Rejection group (n=41) CXCL 10 pg/ml**		**Control group (n=56) CXCL 10 pg/ml**	
	CC	**P value**	**CC**	**P value**	**CC**	**P value**

Age (year)	0.100	0.329	-0.082-	0.612	-0.154-	0.257
Height (cm)	0.124	0.227	0.132	0.412	-0.193-	0.153
Weight (kg)	0.067	0.512	-0.107-	0.505	-0.136-	0.318
BMI (kg/m^2)	0.010	0.925	-0.281-	0.075	0.031	0.821
Post TX duration (year)	0.016	0.878	-0.339-	**0.030**	-0.169-	0.214
S. Creatinine (mg/dl)	0.566	< **0.001**	0.345	**0.027**	-0.114-	0.403
GFR (ml/min/1.73 m^2)	-0.605-	< **0.001**	-0.400-	**0.010**	-0.082-	0.547
Urea (mg/dl)	0.583	< **0.001**	0.097	0.545	0.039	0.778
HB (gm/dl)	-0.125-	0.221	-0.137-	0.394	-0.109-	0.426
TlC ($x10^3$/mcl)	-0.236-	**0.020**	-0.233-	0.143	0.084	0.537
PlT ($x10^3$/mcl)	0.036	0.727	0.028	0.861	0.022	0.872
Serum Na (meq/l)	0.084	0.412	0.310	0.049	-0.038-	0.781
Serum K (meq/l)	0.090	0.380	-0.301-	0.056	0.130	0.339
Serum Albumin (gm/dl)	-0.207-	**0.041**	0.215	0.177	-0.017-	0.903
Serum Ca (mg/dl)	-0.008-	0.935	0.168	0.295	0.273	**0.042**
AlT (U/L)	0.582	< **0.001**	0.050	0.754	0.068	0.617
RBS (mg/dl)	0.378	< **0.001**	-0.270-	0.088	0.410	**0.002**
*Acute pathological sum	-	-	0.126	0.585	-	-
*Chronic pathological sum	-	-	0.495	**0.023**	-	-
S. Creatinine (mg/dl) after therapy	-	-	-0.420-	**0.006**	-	-
S. Creatinine (mg/dl) 6 month later	-	-	-0.293-	0.063	-	-
GFR 6 month later	-	-	0.395	**0.011**	-	-

CC (Correlation coefficient), BMI (body mass index), TX (transplantation),
HB (hemoglobin), TLC (total leukocytic count), PLT (platelets), Na (sodium), K (potassium), Ca (calcium), AlT (alanine aminotranseferase), RBS (random blood sugar).
*Only 21 patients of the rejection group had their graft biopsy sufficient for pathological Banff Scoring.

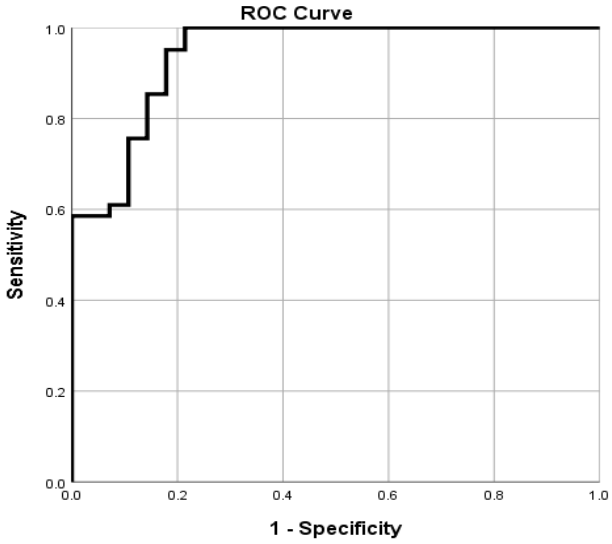

Figure (1): ROC curve of urinary CXCL10 levels. The true positive (sensitivity) and false positive (1–specificity) for urinary CXCL10 levels as a marker for AR are shown.

Discussion:

Advances in IS drugs and clinical care have helped to reduce the rate of AR; nevertheless, it remains a major challenge after KT [13]. CXCL10 is a member of the CXCR3 chemokine family that mediates inflammation by inducing chemotaxis of effector cells. Interferon-γ is overexpressed in AR and induces the production of CXCL10, resulting in leukocyte recruitment [14].

The results of this study confirmed that urine CXCL 10 level is significantly elevated in the setting of AR ($p<0.001$). The follow-up of urine CXCL 10 levels in the rejection patients illustrated a significant decline after receiving antirejection therapy ($p<0.001$). Surveillance of control group revealed that patients who developed AR within one month had significantly elevated CXCL 10 levels than those who did not develop AR ($p<0.001$). Elevated CXCL 10 levels are significantly associated with CGD six months later to its assessment ($p=0.03$) denoting its prognostic value.

Many studies have been conducted to evaluate the diagnostic role of CXCL 10 in AR in pediatric [15-17], adult [18, 19], both pediatric and adult [20] transplant recipients, whether or not normalized to urine creatinine levels [21]. Moreover, the prognostic value of CXCL 10 has been also discussed by previous studies [22, 23].

Levels of urine CXCL 10 in the present study are close to those reported by Ciftci and Raza studies. We reported CXCL 10 median (IQR) of 350 (151-2104) pg/ml in rejection group versus 55 (38-82) in non-rejection control group with $p<0.001$. Similarly, Ciftci reported CXCL10 levels of 242.34 pg/ml at the time of rejection and 64.25 pg/ml in non-rejection transplant patients ($p<0.001$) [18]. Also, Raza and his co-worker reported levels of 228 pg/ml in their rejection, 60 pg/ml in non-rejection recipients ($p<0.001$) and 10.5 pg/ml in healthy controls [19].

In the present study; subgroup analysis of patients with AR revealed that patients with TCMR had significantly elevated CXCL 10 levels than other pathological types of rejection ($p=0.001$). Among our rejection patients; those with glomerulities in their graft pathology and DSA in their serological analysis had significant simultaneous low urine CXCL 10 levels in comparison to those without glomerulitis or circulating DSA ($p=0.049$, 0.001 respectively).

Although CXCL 10 had been previously linked to TCMR [24], our finding of low CXCL 10 levels with glomerulitis does not go with the published data of Blydt-Hansen et al. They found that isolated microvascular inflammation, including the subgroup with ABMR and Banff g score component, was independently associated with elevated urinary CXCL10: Creatinine. They suggested that urinary CXCL10: Creatinine may have a dual use for the detection of both subclinical ABMR and TCMR [15]. Also; Rabant et al, observed that urinary CXCL10 levels correlated with ongoing ABMR in a large cohort of highly sensitized and well phenotyped kidney transplant recipients [21]. Perhaps this entity needs further researches to evaluate role of CXCL 10 in diagnosis of ABMR.

A large multicenter CTOT-04 study found urinary CXCL10 mRNA to be significantly associated with AR as part of a three-gene signature. Although pediatric KTRs (48.3 ±13.2 years, age range, 2 months–77 years) were enrolled, the amount remains unclear [25]. The studies of Suthanthiran and co-workers did not separately characterize their pediatric subgroups**.**

In the present study, we performed a ROC curve analysis to apply the value of CXCL 10 in clinical practice that revealed a cut-off value > 87.5 pg/ml; CXCL 10 that can predict AR with 100% sensitivity, 78.6% specificity with 95% CI of (0.899-0.983) and AUC of 0.94.

Similarly, Ho et al. have also reported greater sensitivity (86.4%) and specificity (91.3%) with rejection at CXCL 10 levels >100 pg/ml [7]. Also an adult CXCL 10 cut-off value of 66.46 ng/ mL with a sensitivity of 78% and specificity of 84% was reported by Ciftci et al., for the diagnosis of AR [18].

On the other hand, our CXCL 10 levels of AR were higher than what was reported by Raza and his colleagues. The results of their ROC analysis showed a sensitivity and specificity of 72 and 71% respectively at 27.5 pg/ml [19]. Also, Jackson et al. have shown 80% sensitivity and 76% specificity at 28 pg/ml [20].

In the present study, levels of urine CXCL 10 among rejection group significantly declined after receiving antirejection therapy. Nevertheless, follow-up CXCL 10 was still significantly more than its values in the non-rejection group (88 (55-108) pg/ml after antirejection therapy and 55 (38-82) in non-rejection patients with p=0.016). Similarly, other studies also indicated a patient's response to antirejection therapy by down regulating the CXCL10 levels [19, 26, 27].

A similar pattern of CXCL10 levels has also been reported in other studies that showed high CXCL10 concentrations (309±506 and >100 pg/ml) in rejection episodes and low levels in chronic, stable and control cases [7, 28].

We also reported that CXCL 10 levels significantly elevated in patients developed CGD than patients with stable graft function (p=0.03) 6 months later. No significant differences in follow up CXCL 10 levels between stable graft function, CGD and graft failure patients (p=0.577). High CXCL 10 levels have been linked to poor graft outcome by many studies [18, 19, 21, 23, 29]. In Rabant at al. study, they performed A Kaplan–Meier analysis of post-ABMR, death–censored graft survival showed that increased urinary CXCL10: Cr ratios at the time of biopsy correlated with graft survival [21]. Mockler et al, found that CXCL10: Cr is associated with time to 50% eGFR decline (HR = 1.04, P = .02), and time to allograft failure (HR = 1.05, P < .01) [23].

Our results have been recently confirmed by Blydt-Hansen and his co-worker; they reported urinary CXCL10: Creatinine as a superior parameter to serum creatinine monitoring for detection of AR and for identifying high risk patients whom biopsy is indicated based on relevant CXCL 10 thresholds [16]. Similarly; Lamarche et al., concluded while evaluating the decision-making impact of CXCL10 testing in a pediatric cohort**,** that urinary CXCL10/Cr improves probability estimates for risk of rejection and therefore decreases the high variability in decision making on biopsy indication [17].

This study has a number of limitations; first, the small sample size that limited the value of subgroup analysis. Second; the lack of universal fixed time intervals for assessment after KT. Third, the relation between CXCL10 levels and SCR was not clear due to lack of protocol biopsy performed to patients who had elevated levels at assessment; and finally, urinary CXCL 10 levels were not corrected to urine creatinine. Nevertheless, these limitations can be easily overcome with further recommended studies.

Conclusions: Urine CXCL 10 is a reliable marker for diagnosis and follow up acute allograft rejection after KT in pediatrics. Levels significantly decline after receiving antirejection therapy. Thus, it could be used as a non-invasive screening and monitoring tool for renal allograft. Elevated urine CXCL 10 levels are associated with poor short-term graft outcome.

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