

The outcomes of renin-angiotensin-aldosterone system inhibition and immunosuppressive therapy in children with X-linked Alport syndrome

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ABSTRACT

Background. Alport syndrome (AS) is characterized by progressive kidney disease. There is increasing evidence that renin-angiotensin-aldosterone system (RAAS) inhibition delays chronic kidney disease (CKD) while the effectiveness of immunosuppressive (IS) therapy in AS is still uncertain. In this study, we aimed to analyze the outcomes of pediatric patients with X-linked AS (XLAS) who received RAAS inhibitors and IS therapy.

Methods. Seventy-four children with XLAS were included in this multicenter study. Demographic features, clinical and laboratory data, treatments, histopathological examinations, and genetic analyses were analyzed retrospectively.

Results. Among 74 children, 52 (70.2%) received RAAS inhibitors, 11 (14.9%) received RAAS inhibitors and IS, and 11 (14.9%) were followed up without treatment. During follow-up, glomerular filtration rate (GFR) decreased <60 ml/min/1.73 m² in 7 (9.5%) of 74 patients (M/F=6/1). In male patients with XLAS, kidney survival was not different between RAAS and RAAS+IS groups (p=0.42). The rate of progression to CKD was significantly higher in patients with nephrotic range proteinuria and nephrotic syndrome (NS), respectively (p=0.006, p=0.05). The median age at the onset of RAAS inhibitors was significantly higher in male patients who progressed to CKD (13.9 vs 8.1 years, p=0.003).

Conclusions. RAAS inhibitors have beneficial effects on proteinuria and early initiation of therapy may delay the progression to CKD in children with XLAS. There was no significant difference between the RAAS and RAAS+IS groups in kidney survival. AS patients presenting with NS or nephrotic range proteinuria should be followed up more carefully considering the risk of early progression to CKD.

Key words: Alport syndrome, cyclosporin A, immunosuppressive therapy, nephrotic syndrome, RAAS inhibitors.

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Alport syndrome (AS) is characterized by progressive kidney disease.^{1,2} There are three types of inheritance patterns; X-linked AS (XLAS) caused by *COL4A5* gene mutations, autosomal recessive AS (ARAS) caused by homozygous or compound heterozygous mutations in the *COL4A3/COL4A4* genes, and autosomal dominant AS (ADAS) caused by heterozygous mutations in the *COL4A3/COL4A4* genes.³ XLAS has a severe clinical course in males and approximately 50% of patients develop kidney failure by the age of 20 years.⁴ There is no specific curative therapy for AS. The treatment aims to delay the progression to chronic kidney disease (CKD). There is increasing evidence that this goal can be achieved with renin-angiotensin-aldosterone system (RAAS) inhibition and optimum results are gathered when treatment is initiated before glomerular filtration rate (GFR) begins to decline.^{5,6} On the other hand, studies evaluating the effectiveness of immunosuppressives (IS) in AS are limited. Studies on the use of cyclosporin A (CSA) in AS have shown that it can slow down disease progression by reducing proteinuria via its effects on the podocyte cytoskeleton and collagen IV cycle. However, an important number of studies do not recommend the routine use of CSA due to its nephrotoxicity.^{7,8}

In this retrospective study, we aimed to analyze the baseline characteristics and outcomes of pediatric patients with XLAS, evaluate the effects of treatment modalities (reduction of proteinuria and changes in GFR), and obtain more information about the potential positive effects of RAAS inhibitors and IS treatment on the clinical course of the disease.

Material and Methods

Patients and data collection

Seventy-four children with XLAS from 13 pediatric nephrology centers in Türkiye, admitted between 2002-2019, were included. Inclusion criteria were XLAS diagnosis based on family history and/or pathological findings

and/or genetic analysis and being under 18 years of age at disease presentation. Patients who were followed up for less than 6 months were excluded. All centers were requested to fill out a standard questionnaire, which included patients' demographic features, clinical and laboratory data (i.e., serum creatinine, serum albumin, spot urine protein to creatinine ratio or 24-hour urine protein quantification, and estimated GFR [eGFR] at first presentation, the onset of treatment, 6th -12th -24th months after treatment, and the last visit), histopathological examinations and genetic test results. Data from medical records were analyzed retrospectively.

The study protocol was approved by the Non-Interventional Clinical Research Ethics Board of Hacettepe University (KA 19073) and written informed consents were obtained from the parents and patients older than 10 years of age. The study was conducted in accordance with the principles of the Helsinki Declaration.

Definitions

XLAS diagnosis was suspected in the presence of persistent hematuria and/or kidney failure and/or hearing loss. The diagnosis was made based on family history and/or pathological findings of AS and/or heterozygous (in females) or hemizygous (in males) pathogenic variants found in *COL4A5*. Nephrotic-range proteinuria was defined as spot urine protein/creatinine ratio of >2 mg/mg or 24-hour urine protein >40 mg/m²/h. Nephrotic syndrome (NS) was defined by nephrotic range proteinuria, hypoalbuminemia (serum albumin <2.5 g/dl), edema, and hyperlipidemia. CKD classification was defined by the Kidney Disease Improving Global Outcomes (KDIGO) Clinical Practice Guideline and the latest KDIGO Consensus Conference.^{9,10} For evaluating kidney prognosis, progression to CKD was defined as a GFR value equal to or less than 60 ml/min/1.73m². eGFR was calculated using the original Schwartz formula.¹¹ eGFR change per year (Δ eGFR/year) was calculated as [eGFR at the last visit - eGFR at the first visit] / Follow-up duration. Genetic variations detected were categorized

into two groups as missense and non-missense mutations (i.e., deletion, duplication, splice site, and non-sense). Patients were divided into three groups in terms of treatment regimens; RAAS inhibitors (RAAS), IS therapy with RAAS inhibitors (RAAS+IS), and the no treatment (NT) group.

Statistical analysis

All data were analyzed using IBM SPSS Statistics for Windows v.21 (IBM Corp., Armonk, NY, USA). The variables were investigated using visual (histograms, probability plots) and analytical methods (Kolmogorov-Smirnov / Shapiro-Wilk's test) to determine whether they were normally distributed. Descriptive statistical analysis methods were used to evaluate demographic and clinical data. Where appropriate, mean, standard deviation (SD), median, and interquartile range (IQR) were calculated for numeric variables. Frequency tables were used to describe categorical data. Categorical variables were compared by Chi-square test and Fisher's exact test where appropriate. Friedman test was conducted to test whether there is a significant change in 24-hour urine protein and eGFR variables due to violation of parametric test assumptions (i.e., non-normal distribution). The nonparametric paired Wilcoxon test was used to assess the differences from the start of treatment to each follow-up time point. Mann-Whitney U test or independent samples t-test was used to compare two independent samples. One-way ANOVA or Kruskal-Wallis tests were used for 3-group comparisons. Survival analysis was performed using the Kaplan-Meier analysis with overall log-rank testing. p values <0.05 in two-tailed tests were considered statistically significant in all analyses. The study had 80% power, with a 5% type-1 error level to detect differences corresponding to a large effect size (a maximum of $f=0.80$ with a minimum coefficient of variation of 40%) among the treatment modalities as statistically significant given the distribution of patients into the three groups.

Results

Patient characteristics

The study included 74 children with XLAS (41 males, 33 females). The median age at first presentation was 6.0 years (IQR 3.4 – 9.9). The median follow-up duration was 4.0 years (IQR 1.7–7.3). Sixty-three (85.1%) patients had proteinuria at first presentation, 11 (14.8%) had nephrotic range proteinuria, and 4 patients (5.4%) presented with NS. Kidney biopsy was performed in 46 (62%) patients, 25 (25/46; 54.3%) of which also had electron microscopic (EM) examination. Histopathological findings were consistent with AS in 22 (47.8%) patients while focal segmental glomerulosclerosis (FSGS) was detected in 9 (%19.6) of the patients. XLAS diagnosis was confirmed with genetic analysis in 56 (75.7%) patients. Of the remaining 18 patients, XLAS was diagnosed with family history and kidney biopsy in 12 (16.2%), and clinical presentation and family history in 6 patients (8.1%). During the follow-up, 7 (9.5%) of the 74 patients progressed to CKD [Stage 3 (n=6), Stage 5 (n=1)]. Demographic and clinical features of male and female patients with XLAS are given in Table I.

Renal outcomes after RAAS inhibitors and IS treatment

Among 74 patients with XLAS, 52 (70.2%) received only RAAS inhibitors (RAAS group), 11 (14.9%) patients received RAAS inhibitors and IS treatment (RAAS+IS group), and 11 (14.9%) patients were followed up without any treatment (NT group). The median age for onset of RAAS inhibition was 9.1 years (IQR 5.0 – 11.7) which did not differ significantly between males and females (8.2 years, IQR 5.0 – 12.3; vs. 9.3 years, IQR 4.5 – 11.4; $p = 0.62$). In the RAAS group (n=52), 39 (75%) received either angiotensin-converting enzyme inhibitor (ACEI) (n=34) or angiotensin receptor blocker (ARB) (n=5) treatment (monotherapy); while 13 (25%) received both ACEI and ARB treatments (dual therapy). In patients treated with dual therapy, ARB was added to ACEIs if proteinuria

Table I. Demographic and clinical characteristics of X-linked Alport syndrome (XLAS) patients.

	Overall (n=74)	Males (n=41) (55.4%)	Females (n=33) (44.6%)
Age at first presentation, median (IQR), yr	6.0 (3.4 – 9.9)	5.3 (3.2 – 10.5)	7.0 (3.4 – 9.6)
Family history, n (%)			
Yes	56 (75.6)	28 (68.3)	28 (84.8)
No	15 (20.3)	12 (29.3)	3 (9.1)
Unknown	3 (4.1)	1 (2.4)	2 (6.1)
Follow-up duration, median (IQR), yr	4.0 (1.7 – 7.3)	4.1 (1.7 – 6.7)	3.9 (1.6 – 8.3)
Urinalysis at first presentation, n (%)			
Hematuria and proteinuria	61 (82.4)	34 (82.9)	27 (81.8)
Hematuria	11 (14.9)	5 (12.2)	6 (18.2)
Proteinuria	2 (2.7)	2 (4.9)	0 (0.0)
Nephrotic syndrome at first presentation, n (%)	4 (5.4)	3 (7.3)	1 (3.0)
Histopathology, n (%)			
Biopsy performed	46 (62)	29 (70.7)	17 (51.5)
Alport	22/46 (47.8)	14/29 (48.3)	8/17 (47.1)
FSGS	9/46(19.6)	7/29 (24.1)	2/17 (11.8)
Mesangial proliferation	8/46 (17.4)	5/29 (17.2)	3/17 (17.6)
Normal	6/46 (13.0)	3/29 (10.4)	3/17 (17.6)
Postinfectious glomerulonephritis	1/46 (2.2)	0/29 (0.0)	1/17 (5.9)
Mutation type, n (%)			
Genetic test performed	56 (75.6)	31 (75.6)	25 (75.7)
Missense	26/56 (46.4)	13/31 (41.9)	13/25 (52.0)
Deletion	16/56 (28.6)	11/31 (35.5)	5/25 (20.0)
Splice-site	10/56 (17.8)	4/31 (12.9)	6/25 (24.0)
Duplication	2/56 (3.6)	2 (6.5)	0/25 (0.0)
Nonsense	2/56 (3.6)	1 (3.2)	1/25 (4.0)
Treatment, n (%)			
No treatment	11 (14.9)	4 (9.8)	7 (21.2)
RAAS inhibitor	52 (70.2)	28 (68.2)	24 (72.7)
RAAS inhibitor + IS	11 (14.9)	9 (22.0)	2 (6.1)
Progression to CKD, n (%)	7 (9.5)	6 (14.6)	1 (3.0)
Age at the onset of CKD, median (IQR), yr	16.2 (15.2 – 16.6)	15.9 (14.5 – 16.7)	16.7 [†]

[†] The age at the onset of CKD of the female patient progressed to CKD (n=1) is given.

CKD: chronic kidney disease, FSGS: focal segmental glomerulosclerosis, IS: immunosuppressive, IQR: interquartile range, RAAS: renin-angiotensin-aldosterone system, XLAS: X-linked Alport syndrome.

persisted despite ACEI treatment. The median age at the onset of ACEI and ARB treatment was 8.6 years (IQR 4.6 – 11.8) and 11.3 years (IQR 10.2 – 13.0), respectively. The median time interval between the onset of ACEI and ARB treatment was 1.5 years (IQR 0.65 – 3.41).

In male patients treated with RAAS inhibitors (n=28), the median proteinuria levels decreased

by about 50% at the end of the 24th month of RAAS inhibitor treatment in both monotherapy and dual therapy groups, respectively (p=0.08 and p=0.08). Also, the median eGFR levels did not show any significant difference at the 24th month of RAAS inhibitor treatment in both monotherapy and dual therapy groups, respectively (p=0.23 and p=0.60). Female patients had lower proteinuria levels than male patients

at the onset of RAAS inhibitor treatment (12.0 vs 27.7 mg/m²/h, $p=0.06$). In female patients treated with RAAS inhibitors ($n=24$), the median proteinuria levels decreased at the end of the 24th month of RAAS inhibitor treatment in both monotherapy and dual therapy groups, respectively ($p=0.04$ and $p=0.59$). Similar to male patients, the median eGFR levels did not differ significantly at the 24th month of RAAS inhibitor treatment in both monotherapy and dual therapy groups, respectively ($p=0.73$ and $p=0.71$). Male and female proteinuria and eGFR values at the onset, 6th, 12th, and 24th of RAAS inhibitor treatment are given in Table II.

IS treatment was given to 11 patients (M/F=9/2) with the diagnosis of NS in 4 patients, rapid progression of post-infectious glomerulonephritis (PIGN) in 1 patient, and AS in 6 patients. Of 4 patients who presented with NS, 2 received steroids only, 1 received steroids and mycophenolate mofetil (MMF) and 1 received steroids and tacrolimus (TAC) before the genetic diagnosis of XLAS. Kidney biopsy findings were consistent with FSGS in all NS patients. Six patients received CSA with the diagnosis of XLAS. CSA treatment was initiated at a median of 1.0 year (IQR 0.3 – 7.1) after the first presentation and was stopped after 0.5, 1.6, 2.1, and 3.3 years due to elevated serum creatinine in four patients and the other 2 patients were still receiving CSA at last visit. In patients with IS treatment, by the end of treatment, while proteinuria was decreased except for one patient (Patient 5), the decline in GFR continued in all except one patient (Patient 7). Features of patients treated with IS are given in Table III.

Median 24-hour urine protein level at first presentation was 40.1 mg/m²/h in the RAAS+IS group, 12 mg/m²/h in the RAAS group and 7.8 mg/m²/h in the NT group ($p=0.016$). Mean eGFR at first presentation was similar between treatment groups ($p=0.42$). Characteristics of patients according to the treatment regimens are given in Table IV.

Progression to CKD and renal survival

After the median follow-up duration of 4.0 years (IQR 1.7 – 7.3), 7 (9.5%) of the 74 patients progressed to CKD [CKD stage 3 ($n = 6$) and CKD stage 5 ($n = 1$)]. Six out of 41 (14.6%) male patients and 1 out of 33 (3%) female patients progressed to CKD ($p=0.09$). The median age at the onset of CKD was 15.9 years (IQR 14.5 – 16.7) in male patients. The age of the female patient was 16.7 years at the onset of CKD. Since there was only one female patient who progressed to CKD, male patients were evaluated in terms of progression to CKD and renal survival. Among male patients with XLAS, the median age at first presentation was higher in patients with CKD than those without (12.4 vs. 5.1 years, $p=0.07$) and the median follow-up duration did not differ significantly between patients with and without CKD (5.0 vs 3.9 years, $p=0.60$). The rate of progression to CKD was significantly higher in patients who had nephrotic range proteinuria and/or NS at first presentation ($p=0.006$ and $p=0.05$, respectively). Median 24-hour urine protein levels at first presentation and the onset of RAAS inhibitor treatment were significantly higher in patients who progressed to CKD than those who did not, respectively ($p=0.001$ and $p<0.001$). The median eGFR level at first presentation was significantly lower in patients who progressed to CKD than those who did not ($p=0.002$). Among the 6 patients who progressed to CKD, 4 were treated with only RAAS inhibitors, and 2 were treated with RAAS + IS ($p=0.57$). The patients who were followed up without treatment did not progress to CKD, however, their follow-up period was significantly shorter compared to other groups ($p=0.02$). Furthermore, among male patients who received RAAS or RAAS+IS therapy, the median age at the onset of RAAS inhibitors was significantly higher in patients who progressed to CKD than those who did not (13.9 vs 8.1 years, $p=0.003$).

Kidney survival analysis was performed in male patients. The overall median kidney survival rate without CKD was 11.6 years (95% CI 9.7–13.6). There was no significant difference

Table II. Proteinuria and eGFR values of patients with RAAS inhibitor treatment, expressed as medians (IQR).

Parameters	Onset	RAAS inhibitor treatment				p-value*	p-value*
		6th month	12th month	24th month	p-value*		
Proteinuria (mg/m²/h)							
Males							
ACEI or ARB (n=19)	24.8 (13.0 – 46.0)	12.0 (6.7 – 36.3)	16.8 (5.1 – 34.3)	11.7 (5.1 – 27.2)	0.09	0.31	0.08
ACEI+ARB (n=9)	26.0 (17.0 – 117.0)	24.8 (10.0 – 98.0)	15.1 (7.8 – 93.1)	13.2 (6.1 – 64.5)	0.60	0.04	0.08
Females							
ACEI or ARB (n=20)	12.4 (7.7 – 15.2)	12.3 (4.9 – 33.2)	9.8 (5.1 – 13.0)	6.3 (5.2 – 12.4)	0.93	0.34	0.04
ACEI+ARB (n=4)	12.0 (9.3 – 63.8)	10.8 (5.3 – 22.7)	13.7 (8.0 – 15.1)	10.9 (1.8 – 16.6)	0.28	0.59	0.59
eGFR (ml/min/1.73m²)							
Males							
ACEI or ARB (n=19)	156.0 (117.0 – 180.0)	130.0 (98.5 – 187.5)	141.0 (94.0 – 175.0)	140.0 (122.2 – 182.5)	0.22	0.21	0.23
ACEI+ARB (n=9)	140.0 (117.5 – 163.0)	139.5 (130.0 – 154.7)	142.0 (104.0 – 159.0)	149.0 (74.5 – 155.7)	0.72	0.49	0.60
Females							
ACEI or ARB (n=20)	154.0 (132.0 – 174.0)	149.5 (118.7 – 170.0)	144.5 (110.0 – 166.7)	146.0 (116.5 – 170.0)	0.65	0.90	0.73
ACEI+ARB (n=4)	155.0 (122.7 – 186.0)	150.0 (110.5 – 180.7)	144.0 (106.0 – 176.0)	158.0 (96.7 – 173.7)	0.46	0.60	0.71

ACEI: angiotensin-converting enzyme inhibitor, ARB: angiotensin receptor blocker; eGFR: estimated glomerular filtration rate, IQR: interquartile range (Friedman test, followed by pairwise comparisons with Wilcoxon test were applied. *The p-values are obtained from the Wilcoxon test and show the difference between the onset of treatment and the specified time.)

Table III. Characteristics of patients treated with immunosuppressives.

Patient No	Sex	Age at first presentation (years)	Mutation type	Proteinuria	NS	Histopathology	IS	Onset of treatment				End of treatment				The last visit	
								Age (years)	Proteinuria (mg/m ² /h)	eGFR (ml/min/1.73m ²)	Age (years)	Proteinuria (mg/m ² /h)	eGFR (ml/min/1.73m ²)	Age (years)	Proteinuria (mg/m ² /h)	eGFR (ml/min/1.73m ²)	
1	M	4.3	Deletion	Nephrotic	No	-	CSA	5.8	1.6 ⁺	202	continued	continued	10.1	0.54 ⁺	180		
2	M	8.3	Deletion	Non-nephrotic	No	FSGS	CSA	8.3	0.34 ⁺	145	continued	continued	14.1	1.4 ⁺	72		
3	M	15.7	Duplication	Nephrotic	Yes	FSGS	Steroid + MMF	15.7	224	106	16.2	135	60	16.3	31.0	44	
4	M	15.8	Splice site	Nephrotic	Yes	FSGS	Steroid + TAC	16.1	148	74	16.9	9.6	44	17.3	80.2	57	
5	M	14.7	Missense	Nephrotic	No	Alport	CSA	15.3	9.2	134	15.8	10.6	73	17.1	135	107	
6	M	0.9	Missense	Non-nephrotic	No	Alport	CSA	10.6	97	163	12.8	36	129	13.0	130	190	
7	F	10.8	Deletion	Non-nephrotic	No	PIGN	Steroid	10.8	22	83	10.9	12	188	11.4	NA	188	
8	M	2.4	Deletion	Non-nephrotic	No	Alport	CSA	2.9	18	162	6.2	12	116	6.8	23.6	147	
9	F	10.2	Deletion	Nephrotic	Yes	FSGS	Steroid	10.2	4.5 ⁺	150	10.8	0.24 ⁺	133	22.9	0.28 ⁺	131	
10	M	5.1	Splice site	Non-nephrotic	No	Alport	CSA	11.4	76	186	13.1	61	75	15.7	109	154	
11	M	12.2	Missense	Nephrotic	Yes	FSGS	Steroid	12.7	134	189	13.2	74	165	13.3	78	141	

CSA: cyclosporin A, eGFR: estimated glomerular filtration rate, IS: immunosuppressive, FSGS: focal segmental glomerulosclerosis, MMF: mycophenolate mofetil, NA: not available, NS: nephrotic syndrome, PIGN: post-infectious glomerulonephritis, TAC: tacrolimus

⁺ Spot urine protein/creatinine (mg/mg) values are given due to the lack of data on 24-h protein levels

Table IV. Characteristics of patients according to treatment modalities.

Characteristics	No treatment (n=11)	RAAS (n=52)	RAAS + IS (n=11)	p-value
Gender, n (%)				
Male (n=41)	4 (36.3)	28 (53.8)	9 (81.8)	0.92
Female (n=33)	7 (63.7)	24 (46.2)	2 (18.2)	
Follow-up duration, yr, median (IQR)	1.7 (0.7 – 2.5)	5.2 (2.6 – 8.2)	4.3 (1.1 – 10.6)	0.02
Age at first presentation, yr, mean ± SD	5.5 ± 3.5	6.5 ± 3.8	9.1 ± 5.3	0.08
Number of patients with missense mutations, n (%)	3 (33.3) [†]	20 (55.5) [†]	3 (27.2) [†]	0.17
24-hour urine protein at first presentation, mg/m ² /h, median (IQR)	7.8 (4.0 – 17.8)	12.0 (5.5 – 24.0)	40.1 (22.0 – 98.0)	0.016
eGFR at first presentation, ml/min/1.73m ² , mean ± SD	156.9 ± 39.7	147.7 ± 45.5	132.7 ± 40.0	0.42
24-hour urine protein at last visit, mg/m ² /h, median (IQR)	6.2 (3.1 – 12.8)	16.8 (10.3 – 34.5)	79.1 (25.4 – 124.7)	0.001
eGFR at last visit, ml/min/1.73m ² , mean ± SD	185.8 ± 25.9	135.7 ± 42.7	128.2 ± 51.9	0.002
Number of patients who developed CKD (GFR <60 ml/min/1.73 m ²), n (%)	0 (0)	5 (9.6)	2 (18.1)	0.34
Age at onset of CKD, yr, mean ± SD	-	15.3 ± 1.6	16.5 ± 0.4	0.33
ΔeGFR / year, ml/min/1.73 m ² , median (IQR) [‡]	12.1 (0.6 – 30.7)	- 2.1 (- 9.0 – 4.4)	- 3.4 (- 12.5 – 3.1)	0.01

CKD: chronic kidney disease, eGFR: estimated glomerular filtration rate, IS: immunosuppressive, IQR: interquartile range, RAAS: renin-angiotensin-aldosterone system, SD: standard deviation,

[†] % values are given as the ratio of the number of patients with missense mutations to the patients with the genetic testing in each group. Genetic testing was performed in 9, 36, and 11 patients in NT, RAAS, and RAAS+IS groups, respectively.

[‡] ΔeGFR / year = (eGFR at the last visit – eGFR at the first visit) / Follow-up duration

One way ANOVA or Kruskal-Wallis tests were used where appropriate.

in kidney survival rates between the RAAS and RAAS+IS groups (Fig. 1). After the first presentation, the 10-year cumulative risk of CKD was 12.3% in patients who received RAAS and 22.2% in patients who received RAAS+IS (p=0.42). There was no significant difference between patients who received ACEI/ARB monotherapy and ACEI+ARB dual therapy in terms of progression to CKD and kidney survival, respectively (p=0.31 and p=0.25). After the first presentation, the 10-year cumulative risk of CKD was 23.6% in patients who received ACEI/ARB monotherapy and 8.3% in patients who received ACEI+ARB dual therapy (p=0.25). Progression to CKD did not significantly differ between patients with missense mutations and non-missense mutations (p=0.52) and no significant difference was found in terms of mutation types (i.e., missense, or non-missense) between NT, RAAS, and RAAS+IS groups (p=0.17). The characteristics of male patients

with and without CKD are summarized in Table V. Kidney survival analysis in male patients showed that only patients with NS had worse kidney survival than those without (Fig. 1).

Discussion

This is the first multicenter retrospective study analyzing the outcomes of RAAS inhibitors and immunosuppressives in Turkish children with XLAS. We observed that RAAS inhibitors had beneficial effects on proteinuria. Also, the median age at the onset of RAAS inhibitor treatment was significantly higher in children who progressed to CKD. Nephrotic range proteinuria and/or NS at presentation were associated with poor prognosis and in the kidney survival analysis of male patients, no significant difference was found in patients who received immunosuppressive therapy or not.

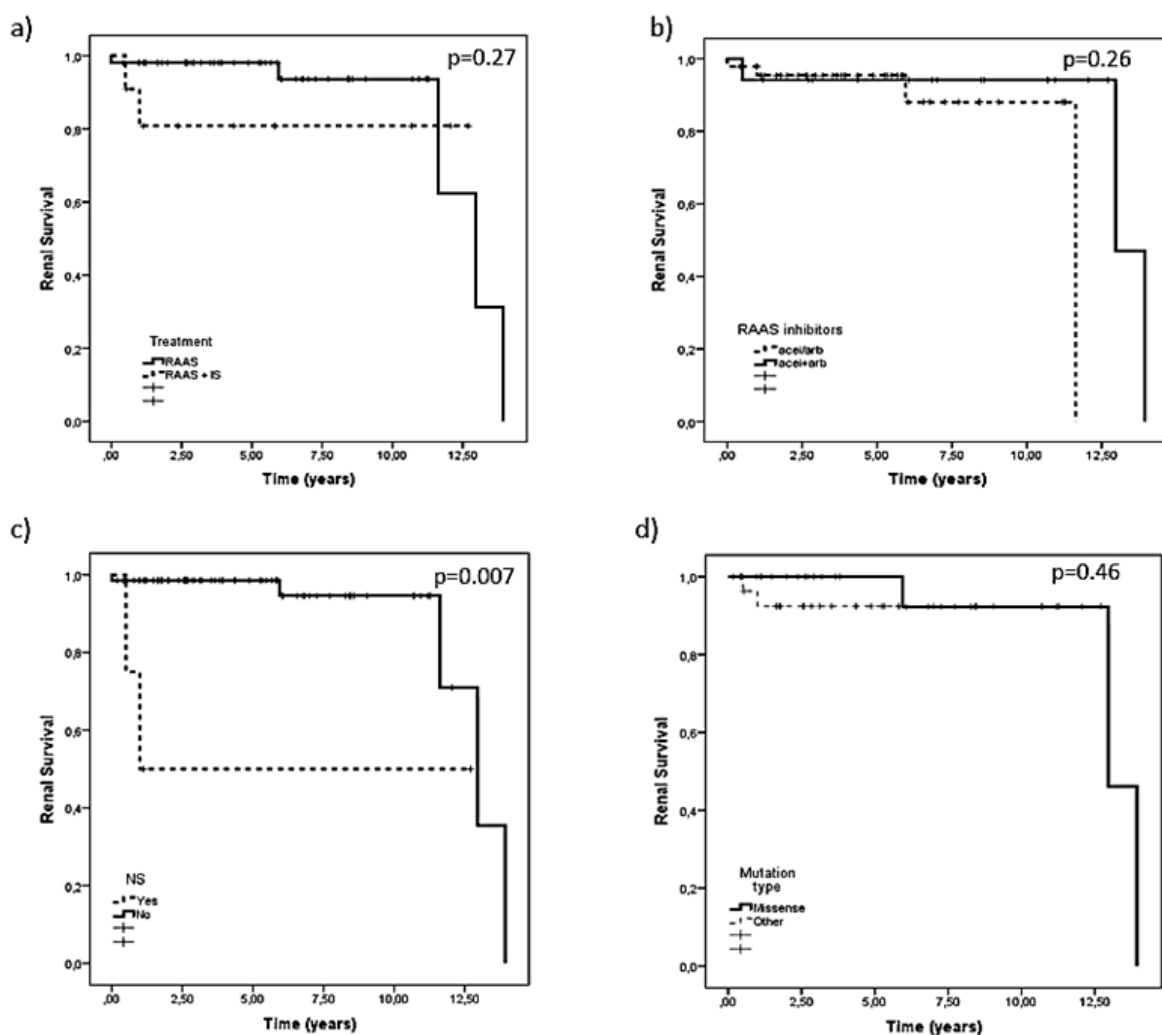


Fig. 1. Time to chronic kidney disease in male XLAS patients. Kidney survival in (a) patients who received RAAS vs. RAAS+IS; (b) patients on ACEI or ARB (monotherapy) vs. ACEI+ARB (dual therapy); (c) patients with or without nephrotic syndrome; and (d) patients with missense vs. other mutations. Note that patients with nephrotic syndrome (NS) at presentation progressed to chronic kidney disease earlier than those without. ACEI: angiotensin-converting enzyme inhibitor, ARB: angiotensin receptor blocker, IS: immunosuppression, RAAS: renin-angiotensin-aldosterone system inhibition, XLAS: X-linked Alport syndrome.

In the literature, it was shown that RAAS inhibition has nephroprotective effects and may delay progression to CKD in patients with AS.^{5,12-14} Firstly, in 2004, Proesmans et al.¹⁵ reported the 5 years results of enalapril treatment in a group of ten children with AS and stated that enalapril reduces urinary protein excretion and preserves glomerular filtration. Subsequently, Webb et al.¹⁶ demonstrated that losartan reduced proteinuria significantly and was well tolerated after 12 weeks of treatment in a group

of 15 children with AS. Zhang et al.¹³ analyzed the long-term efficacy and safety of ACEI and ACEI + ARB treatments in a cohort of 79 children with AS and showed that proteinuria decreased significantly during the first 2 years of treatment. There was no significant difference in anti-proteinuric effects of ACEI and ACEI + ARB treatments in patients with severe or less severe mutations after 1 year of therapy. Webb et al.¹⁷ also analyzed the effects of ACEI versus ARB treatments and reported that enalapril

Table V. The characteristics of male XLAS patients with and without CKD.

Characteristics	Patients with CKD (n=6)	Patients without CKD (n=35)	p value
Age at first presentation, yr, median (IQR)	12.4 (2.3 – 15.7)	5.1 (3.5 – 9.2)	0.07
Follow up duration, yr, median (IQR)	5.0 (1.3 – 12.5)	3.9 (1.7 – 6.7)	0.60
Age at the last visit, yr, median (IQR)	16.6 (15.2 – 18.0)	11.4 (6.9 – 14.0)	0.009
Proteinuria, n (%)			
None (n=5)	0 (0)	5 (14.3)	
Non-nephrotic (n=28)	2 (33.3)	26 (74.3)	0.006
Nephrotic (n=8)	4 (66.7)	4 (11.4)	
NS at first presentation, n (%)			
Yes (n=3)	2 (33.3)	1 (2.9)	
No (n=38)	4 (66.7)	34 (97.1)	0.05
24h urine protein at first presentation, mg/m ² /h, median (IQR)	114.5 (54.0 – 163.7)	15.2 (4.6 – 29.2)	0.001
eGFR at first presentation, ml/min/1.73m ² , median (IQR)	84.5 (58.0 – 135.0)	149.0 (115.0 – 171.0)	0.002
Age at onset of RAAS inhibitor treatment †, yr, mean ± SD	13.9 ± 2.6 †	8.1 ± 4.1 †	0.003
24h urine protein at onset of RAAS inhibitor treatment †, mg/m ² /h, median (IQR)	149.0 (97.2 – 182.7) †	24.4 (14.4 – 34.2) †	<0.001
Mutation type, n (%)			
Missense (n=13)	2 (33.3)	11 (31.4)	
Other (n=28)	4 (66.7)	24 (68.6)	0.46
Treatment, n (%)			
No treatment (n=4)	0 (0)	4 (11.4)	
RAAS (n=28)	4 (66.7)	24 (68.6)	0.57
RAAS+IS (n=9)	2 (33.3)	7 (20.0)	
RAAS inhibitors, n (%)			
No treatment (n=4)	0 (0)	4 (11.4)	
ACEI/ARB (n=25)	4 (66.7)	21 (60.0)	0.65
ACEI+ARB (n=12)	2 (33.3)	10 (28.6)	

ACEI: angiotensin-converting enzyme inhibitor, ARB: angiotensin receptor blocker, CKD: chronic kidney disease, eGFR: estimated glomerular filtration rate, IS: immunosuppressive, IQR interquartile range, NS: nephrotic syndrome, RAAS: renin-angiotensin-aldosterone system, SD standard deviation, XLAS: X-linked Alport syndrome.

† Male patients without treatment (n=4) were not included at this analysis.

Mann-Whitney U test or independent samples t-test was used where appropriate.

and losartan were comparable in reducing proteinuria in children with AS. In agreement with previous studies, we showed that proteinuria levels decreased after both ACEI/ARB and ACEI+ARB treatments while eGFR remained relatively stable. We also observed that there was no significant difference between patients who received monotherapy and dual therapy in terms of kidney survival.

When the effect of RAAS inhibitors on the progression to CKD is concerning; Gross et al.⁵ showed that ACEIs delayed the progression of kidney failure for 3 years in a patient group with impaired renal functions and 18 years in the patient group with proteinuria, while none of the patients in the hematuria and microalbuminuria group progressed to kidney failure. Also, among patients with heterozygous mutations in *COL4A3*, *COL4A4*,

or *COL4A5*, those treated with ACEIs showed a lower and delayed incidence of kidney failure and improved survival than untreated patients.¹⁸ In a study investigating treatment response by genotype, ACEI/ARBs delayed CKD for 17 years in patients with *COL4A5* variants without truncating and 12 years in patients with truncating *COL4A5* variants.¹⁴ The latest guidelines recommend initiating ACEIs at the time of diagnosis in males and when microalbuminuria develops in females with XLAS.⁶ Similarly, among male patients who received RAAS or RAAS+IS therapy in our cohort, we demonstrated that the median age at the onset of RAAS inhibitor therapy was significantly higher in patients who progressed to CKD than those who did not. On the other hand, the median age at disease presentation was higher in patients who progressed to CKD (statistically nonsignificant) and median eGFR at presentation was found to be significantly lower. These findings and our observations suggest that evaluating patients before their eGFR starts to decline and initiating RAAS inhibitor therapy early may help to delay the progression of CKD.

Previously, it has been reported that phenocopy-causing mutations, including *COL4A* mutations, were detected in 3.7% of patients with steroid-resistant NS (SRNS).¹⁹ Also, it has been shown that *COL4A* mutations can be detected in patients with familial or sporadic FSGS.²⁰⁻²² The association between AS and FSGS is not clear yet but it is thought that FSGS phenocopy may be more likely than the development of secondary FSGS in AS patients.²² In our cohort, 4 patients (5.4%) presented with NS and all of them had FSGS histopathologically. It is well known that proteinuria is a risk factor for the development and progression of CKD. However, data on the outcome of AS patients with significant proteinuria at first presentation are limited. In agreement with the study by Ozdemir et al.²³, in this study patients who presented with NS and/or nephrotic range proteinuria progressed to CKD earlier. Taken together, AS should be included in the differential diagnosis of patients presenting with NS and/or nephrotic

range proteinuria, and those AS patients who presented with NS and/or nephrotic range proteinuria should be followed up more carefully.

The effects of immunosuppressives, especially that of CSA, have been investigated in patients with AS. Some animal and human studies reported that CSA reduced proteinuria and slowed progression to CKD, however, due to its nephrotoxic effects, CSA is no longer recommended for routine use.^{7,8,24,25} Petrova et al.²⁶ compared the effects of MMF and placebo in *COL4A3*-deficient (*COL4A3*^{-/-}) mice and showed that MMF improved kidney function, probably by inhibition of tubulointerstitial fibrosis. There is no human study examining the effects of MMF, tacrolimus, or other immunosuppressives in AS patients. In our cohort, 11 patients were treated with IS. We observed that patients who received IS treatment remained relatively stable except for 2 patients who progressed to CKD Stage 3, and it should be noted that one of these patients had a baseline GFR <90 ml/min/1.73m². In kidney survival analysis, there was no significant difference between the RAAS and RAAS+IS groups. Possibly due to the small size of the cohort, our study did not demonstrate an ameliorating or worsening effect of immunosuppressives. With these results, we think that the effects of immunosuppressives in AS are still unclear, therefore studies with larger patient cohorts are needed to reach an evidence-based conclusion.

It has been reported that patients with missense mutations have a better prognosis and progress to CKD later.^{4,27} We observed that there was no difference in kidney survival rates between patients with missense and non-missense variations. This issue remains to be confirmed with larger studies focusing more on the genetic background of the disease.

The strength of our study is that it is the first study that reveals the outcomes of the patients who received RAAS inhibitors and IS therapy in the Turkish pediatric XLAS cohort. The limitations of our study are its retrospective

design, relatively short follow-up duration (i.e., 4.0 years), and relatively small-sized study group. Since we included pediatric patients and the follow-up period was relatively short, the rate of progression to CKD might be lower than in the previous studies. Progression to CKD and kidney survival analysis couldn't be performed in female patients due to the small number of patients who progressed to CKD. Due to the limited size, we are aware that some clinically significant differences might not come out as statistically significant but given the rarity of the disease, we believe that the descriptive statistics we present will be informative for specialists in this field.

Our data showed that RAAS inhibitors have beneficial effects on proteinuria in children with XLAS and early initiation of therapy may delay the progression to CKD. In the kidney survival analysis of males, there was no significant difference between the RAAS and RAAS+IS groups, however, larger scaled prospective studies are still needed to make a definite conclusion regarding kidney survival in AS patients receiving IS therapy. AS should be in the differential diagnosis of patients with NS and/or nephrotic range proteinuria and AS patients presenting with NS or nephrotic range proteinuria should be followed up more carefully due to the risk of early progression to CKD. Early diagnosis and prompt intervention are of high importance to provide appropriate care in children with an AS diagnosis.

Ethical approval

The study protocol was approved by the Non-Interventional Clinical Research Ethics Board of Hacettepe University (KA 19073).

Author contribution

The authors confirm contribution to the paper as follows: research formulation and study design: RT, BG, EDKS, FO and AD; data acquisition: RT, GO, RA, ID, ZBO, SS, AS, OS, AY, AKB, FKE, BKD, SY, YT, AA; genetic analysis: FO, EA;

statistical analysis: MH and GO; data analysis/interpretation: RT, GO, BG, EDKS, FO, AD and MH. All authors contributed important intellectual content during manuscript drafting and/or revision and approved the final version. Furthermore, they all accept responsibility for the overall work, including the accuracy and integrity of all portions of the work.

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Conflict of interest

The authors declare that there is no conflict of interest.

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