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Original Article

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Prediction of Recurrence and Graft Outcomes in Kidney Transplant Recipients with IgA Nephropathy: Role of Genetic and Oxidative Stress Markers

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ABSTRACT

Background: IgA nephropathy (IgAN) recurrence after kidney transplantation (KTx) could cause allograft loss. New biomarkers are needed to assess the risk of IgAN recurrence and allograft dysfunction following KTx.
Methods: Thirty-seven KTx recipients who underwent KTx between 2002-2011 with biopsy-confirmed pre-transplant IgAN were evaluated in this retrospective study. Demographics, pathological and clinical/laboratory findings, and biomarkers, including levels of advanced oxidative protein products (AOPP) and galactose-deficient IgA1 and 4 tag single-nucleotide variants (tSNVs) associated with IgAN, were recorded. The primary outcome was IgAN recurrence. Biopsy-proven allograft rejection (BPAR) and death-censored graft failure (DCGF) were secondary outcomes.
Results: 72.9% of patients were male. The mean age of KTx recipients was 37.4 ± 9.9 years. 45.9% of them experienced a recurrence of IgAN, during a median of 125 months of follow-up. There were more male donors in the recurrent IgAN group (P = .022). The median levels of UPCR were higher (P = .002) and estimated glomerular filtration rate (eGFR) were lower (P = .007) at the time of sampling in the recurrent group. Donor sex (male) (P = .018, HR: 3.853, 95% CI: 1.256-11.826) and eGFR at sampling (P = .039, HR: 0.967, 95% CI: 0.937-0.998) were predictors of IgAN recurrence in Cox regression analysis. Advanced oxidative protein products (median 78.4 vs 67.35 (µmol/L)) and Gd-IgA1 (median 5390.3 vs 4732.5 ng/µL) levels were insignificantly elevated in the patients with IgAN recurrence. Statistical differences were not present in terms of secondary outcomes between the patients with and without IgAN recurrence. Single-nucleotide variants were not statistically associated with the recurrence of IgAN.
Conclusions: Prospective biomarker/genetic marker studies, including large patient cohorts, are required to evaluate the risk of IgAN recurrence and allograft function after KTx.
Keywords: IgA nephropathy, biomarker, kidney transplantation, single nucleotide variants, oxidative stress

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INTRODUCTION

IgA nephropathy (IgAN) is one of the leading etiologies of kidney failure.[1,2] Various clinicopathological variables affecting the outcome of IgAN, such as hypertension, degree of proteinuria, decreased estimated glomerular filtration rate (eGFR) at the time of IgA recurrence, crescents, and chronic lesions in histopathology, in addition to several biomarkers of disease activity for the progression of IgAN, have been studied so far.[3-7] Among these, a few new biomarkers deserve to be examined thoroughly in patients undergoing kidney transplantation (KTx). Abnormal O-glycosylation of IgA1 plays an important role in IgAN pathogenesis; hence, serum galactose-deficient IgA1 (Gd-IgA1) levels could be valuable biomarkers that can reflect disease activity.[8,9] Another candidate biomarker for disease progression is elevated serum levels of advanced oxidation protein products (AOPP), demonstrating enhanced oxidative stress, which could

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induce inflammation and the progression of IgAN.[10,11] Innate immunity and both alternative and lectin complement pathways are significant components of the inflammatory response in IgAN, and a few genetic susceptibility loci have been discovered in innate immunity and complement-associated genes such as tumor necrosis factor ligand superfamily member 13 (T*NFSF13*), HORMA domain containing 2 (*HORMAD2*), human alpha defensin (*DEFA*), and complement factor H (*CFH)*, which may have a role in the pathogenesis of IgAN through an exaggerated inflammatory response.[12,13]

Kidney transplantation is the optimal treatment for kidney failure due to IgAN, although recurrence of the disease after transplantation is quite common, occurring in 20-60% of patients.[14-17] The prognosis of KTx in patients with IgAN is similar to that of KTx recipients without IgAN at 10 years after transplantation. However, disease recurrence is a poor prognostic risk factor for graft survival.[18,19] Different studies have focused on the prediction of IgAN recurrence after KTx and discovered various clinical, histopathological, and non-invasive serological prognosticators, such as the presence of crescents on native biopsies and younger age at disease onset,[20] KTx from living related donors,[21] serum IgA levels after 6 months of KTx,[22] and high levels of serum Gd-IgA1, IgG-Gd-IgA1, and CD89-IgA1 complexes.[23] In addition, higher degrees of normalized Gd-IgA1 specific IgG at the time of diagnosis and KTx were shown to predict recurrence.[24] However, a considerable number of these studies have reported contradictory results.[25] IgA nephropathy is genetically complex with a polygenic trait.[26] However, the genetic link between oxidative stress, Gd-IgA1, and recurrent IgAN after KTx has not yet been studied. Therefore, by conducting a retrospective study among KTx patients whose primary diseases were biopsy-proven IgAN, it was aimed to examine the 4 tag single-nucleotide variants (SNVs) associated with IgAN, AOPP, and Gd-IgA1 and evaluate their role in predicting recurrence of IgAN after KTx and allograft outcomes.

MATERIAL AND METHODS

Patients and Follow-up Protocol

In this retrospective study, 37 adult patients with kidney failure caused by primary IgAN were enrolled. These patients received KTx between 2002 and 2011 in the tertiary care center. All KTx recipients were categorized into 2 groups: recurrent IgAN (n = 17, 45.9%) and non-recurrent IgAN (n = 20, 54.1%). In 21 KTx recipients who required a kidney biopsy, serum samples for all biomarkers (tSNVs, AOPP and Gd-IgA1) were obtained from patients at the time of or after graft biopsy. In the remaining 16 patients, samples were collected once during clinical follow-up. The prepared aliquots for biomarkers were deposited in the biobank for analysis. Medical records were noted from patients' files, and clinical information including patient age/sex, donor type/age/sex, human leukocyte antigen (HLA) mismatches, biopsy-proven allograft rejection (BPAR), and induction and maintenance immunosuppressive treatment regimens were recorded. After sampling, all participants were followed at the clinic. Laboratory data of serum creatinine, albumin, and quantitative measurements of proteinuria were recorded. The urinary protein-to-creatinine ratio (UPCR) was used to evaluate quantitative proteinuria. Allograft biopsy was done in patients with graft dysfunction and/or ≥ 1 g/day of proteinuria. Time to IgAN recurrence was measured as the duration in months from kidney transplantation (KTx) until the allograft biopsy that confirmed a histopathological diagnosis of IgAN. The follow-up time (months) included the interval between transplantation and the last control (until March 2020) or initiation of renal replacement therapy or decease of the KTx recipient. All procedures involving human participants were conducted in accordance with the ethical standards of institutional and national research committees and the Declaration of Helsinki.[27] Informed consent was obtained from all patients. All subsequent data collection, including biomarkers, and analyses were conducted after ethics committee approval. This study was approved by Istanbul University, Istanbul Faculty of Medicine Clinical Research Ethics Committee (decision date and number: 2013/1832).

Immunosuppressive Therapy

Standard-dose anti-thymocyte globulin (ATG Fresenius, 2 mg/kg/day for 3 to 7 days) was administered as induction treatment in deceased-donor KTx (Dd-KTx). In living-donor KTx (Ld-KTx), one of the following 3 induction strategies was selected according to the immunologic risk assessment: standard-dose ATG, basiliximab (20 mg IV on days 0 and 4), or no induction. All patients received intraoperative intravenous methylprednisolone 500 mg, and they were subsequently treated with maintenance immunosuppressants, including a calcineurin inhibitor (CNI) (tacrolimus or cyclosporine), an antiproliferative agent [mycophenolate mofetil/sodium (MMF) or azathioprine (AZA)], and steroid. Calcineurin inhibitors and antiproliferative drugs were added 2 days and 1 day before the operation in Ld-KTx, respectively. The drug level targets (C0) for tacrolimus and cyclosporine were 8-12 and 200-300 ng/mL in the first 3 months, and 4-8 and 50-150 ng/mL after 3 months, respectively. Azathioprine and MMF dosages were 1.5 mg/kg/day and 2000 mg/day (1440 mg/day for mycophenolate sodium), respectively. Intravenous methylprednisolone 120 mg was applied on day 1 after KTx, followed by 20 mg/day maintenance prednisolone treatment in the first month, 10 mg/day in the second and third months, and

MAIN POINTS

- IgA nephropathy (IgAN) recurrence is a common entity after kidney transplantation.
- New markers are required to predict IgAN recurrence and the prognosis of allograft survival in patients with IgAN.
- The AA genotype of rs6677604 of *CFH* and the AA genotype of rs2412971 of *HORMAD2* were not detected in any patients with biopsy-proven acute rejection and graft loss in the study, despite not being statistically significant. Additional research involving larger sample sizes is needed to investigate the possible effectiveness of these markers.

5 mg/day prednisolone after the third month of KTx. The immunosuppressive treatment regimen after the first 3 months of KTx was recorded as maintenance treatment. In case of necessity, treatment strategies were altered according to immunologic risk status and complications after KTx. Calcineurin inhibitors and antiproliferatives were decreased or stopped in some KTx recipients due to drug-related side effects and infections during follow-up.

Histopathologic Evaluation

Adequate allograft biopsy specimens with ≥8 glomeruli and ≥2 arterioles were examined. 3-4 µm sections were evaluated with immunohistochemical staining and histopathologic examination. 0.4 to 0.6 cm non-fixed biopsy material was frozen for immunofluorescence (IF) (IgG, IgA, IgM, fibrinogen, C3, and C1q) staining in liquid nitrogen. Additionally, tissues were embedded in paraffin after fixation with Hollande's fixative and prepared for light microscopic examination with Congo red, hematoxylin-eosin, periodic acid-Schiff, Masson trichrome, and methenamine silver staining. Banff diagnostic criteria at the time of biopsies were used for pathologic evaluation.[282930-31] Immunofluorescence staining was staged as 0 to 3, based on the intensity of staining. C4d immunohistochemical staining was performed on paraffin-embedded tissues, with linear or circumferential staining in peritubular capillaries (C4d > 0) considered positive.[282930-31] Recurrent IgAN was defined as dominant/co-dominant IgA staining in the glomeruli with IF examination (the degree of IgA staining should be greater than 1+). IgA staining distribution should include the mesangial area with or without capillary loops (the staining pattern should not be diffuse, pure membranous, global granular, or linear basement membrane staining).

Single-Nucleotide Variants in IgAN-Related Genes

Genomic DNA data were extracted from the peripheral blood samples. Genotyping was performed by direct Sanger sequencing for tag SNVs (tSNVs) rs3803800 of *TNFSF13*, rs2738048 of *DEFA*, rs2412971 of *HORMAD2*, and rs6677604 of *CFH* with the designed Polymerase chain reaction (PCR) primers.

Advanced Oxidative Protein Products

Measurement of serum AOPP level (µmol/L) (human ELISA kit, CUSABIO, Wuhan Huamei Bio-tech, Inc., China) was performed by enzyme-linked immunosorbent assay (ELISA) using a spectrophotometer ELx800 BIO TEK Instruments, Winooski, Vermont,Inc, US. Inter- and intra-assay coefficient variables (CVs) were <12% and <8%, respectively.

Galactose-Deficient IgA1

Serum Gd-IgA1 levels were evaluated with the KM55 ELISA assay from Immuno-Biological Laboratories (IBL) Co, Japan. This assay uses a monoclonal antibody specifically designed to recognize the galactose-deficient region of human Gd-IgA1. Galactose-deficient IgA1 serum levels (ng/µL) were quantitatively measured using a lectin-independent method, referencing a standard curve based on optical density at 492 nm, utilizing 4-parameter logistic curve fitting. Inter- and intra-assay CVs were <12% (7.5-11.9%) and <10% (8.7-9.9%), respectively.

Study Outcomes

IgA nephropathy recurrence was the primary outcome. Biopsy-proven allograft rejection and death-censored graft failure (DCGF) were defined as secondary outcomes.

Statistical Analysis Methods

The results are reported as mean ± standard deviation (SD) or as median (interquartile range [IQR25-75]) according to the distribution pattern of the data. Groups of study were compared using the *t*-test or Mann–Whitney *U* test. Differences in the proportions of diverse patient groups were compared by Chi-squared or Fisher's exact test. Spearman analysis was conducted to show the correlation between AOPP and Gd-IgA1 levels and the correlation of these 2 markers with baseline and last eGFR levels. Univariate and multivariate Cox regression analyses were used to analyze graft loss and IgAN recurrence in terms of hazard ratio (HR) and 95% confidence intervals (CI), and variables with p levels <0.2 in univariate analyses were selected for multivariate analysis. Statistical analyses were performed using Statistical Package for Social Sciences (SPSS) Windows, version 26.0 (IBM SPSS Corp.; Armonk, NY, USA). A *P*-value <.05 was deemed statistically significant.

RESULTS

Baseline and Follow-up Characteristics of Patient Groups

Twenty-seven patients (72.9%) were male, and the mean age was 37.4 ± 9.9 years. The median follow-up time was 125 months (IQR 25-75: 97.5-151 months). Distributions of sex, age, donor age, donor type (LD-KTx/DD-KTx), duration of follow-up, family history of kidney disease, presence of diabetes mellitus, history of transfusion and pregnancy, pre-transplant PRA, previous KTx, number of HLA mismatches, and the types of induction and maintenance treatment were all similar between the study groups (Table 1). None of the patients experienced mortality during the follow-up period. Twenty-one patients underwent allograft biopsies during follow-up. Pathological diagnoses were IgAN recurrence in 12 patients, IgAN recurrence and acute T-cell mediated rejection in 2, IgAN recurrence and acute antibody-mediated rejection in 1, IgAN recurrence and chronic antibody-mediated rejection in 1, IgAN recurrence and CNI toxicity in 1, chronic antibody-mediated rejection in 2, acute antibody-mediated rejection in 1, and CNI toxicity in 1 patient. There were more male donors in the recurrent IgAN group (*P* = .022). The baseline UPCR levels were higher (*P* = .002) and serum eGFR levels at the time of sampling were lower (*P* = .007) in the recurrent group. Additionally, the last control serum creatinine and UPCR levels were higher, and the last serum eGFR levels were lower in KTx recipients with IgAN recurrence. No significant differences were observed between the recurrent (17 patients) and non-recurrent IgAN (20 patients) groups in terms of the

Table 1. Baseline and Follow-up Characteristics of Recurrent and Non-Recurrent IgAN Patients (n = 37)

Characteristics	Non-Recurrent IgAN (n = 20)	Recurrent IgAN (n = 17)	*P*
Recipient age at KTx (years), median (IQR: 25-75)	34.5 (29.25-40)	39.5 (31.5-47.25)	.322
Recipient sex (male), n (%)	16 (80)	11 (64.7)	.460
Donor age at Ktx (years), median (IQR: 25-75)	49.5 (39.25-54.75)	51 (45.25-64)	.314
Male donor, n (%)	5 (25)	11 (64.7)	**.022**
Living donor, n (%)	17 (85)	16 (94.1)	.609
Duration of post-tx follow-up (months), median (IQR: 25-75)	104 (99.5-137.75)	132.5 (91.75-162.25)	.692
Family history of kidney disease, n (%)	3 (15)	3 (17.6)	1.000
Transfusion, n (%)	7 (35)	4 (23.5)	.495
Diabetes mellitus, n (%)	3 (15)	4 (23.5)	.680
Pregnancy history, n (%)	4 (20)	4 (23.5)	1.000
Presence of pretransplant PRA, n (%)	0 (0)	2 (11.7)	.204
Previous KTx, n (%)	2 (10)	1 (5.9)	1.000
HLA mismatches, median (min-max)	3 (0-5)	3 (0-6)	.986
Sampling time for AOPP and Gd-IgA1 (post-Ktx months), median (IQR: 25-75)	35.5 (2.25-47.5)	53 (36.75-67.5)	**.022**
Sampling time AOPP (µmol/L), median (IQR: 25-75)	67.35 (38.13-139.9)	78.4 (41.2-195.5)	.903
Sampling time Gd-IgA1 (ng/µL), median (IQR: 25-75)	4732.5 (1067.1-8865.7)	5390.3 (2370-11893.5)	.428
Sampling time Hemoglobin (g/dL), median (IQR: 25-75)	13.45 (12.5-13.93)	12.45 (11.2-14)	.127
Sampling time UPCR (mg/mg), median (IQR: 25-75)	0 (0-1.8)	0.4 (0.005-2.8)	**.002**
Sampling time creatinine (mg/dL), median (IQR25-75)	1.25 (1.2-1.7)	1.5 (1.33-1.8)	.071
Sampling time eGFR (mL/min/1.73 m^2), median (IQR: 25-75)	68.5 (44.75-77.5)	48.5 (38.75-57.25)	**.007**
Biopsy-proven allograft rejection rate, n (%)	3 (15)	4 (23.5)	.680
Graft loss, n (%)	4 (20)	5 (29.4)	.703
Last serum creatinine (mg/dL), median (IQR: 25-75)	1.48 (1.01-2.32)	2.4 (1.42-4.32)	**.043**
Last UPCR (mg/mg), median (IQR: 25-75)	0.2 (0.1-0.48)	1.1 (0.2-2.7)	**.010**
Last eGFR, (mL/min/1.73 m^2), median (IQR: 25-75)	55 (28.75-77.75)	32 (16-47.5)	**.019**
Induction regimen, n (%)			
No	13 (65)	12 (70.6)	.521
ATG	3 (15)	1 (5.9)	
Basiliximab	5 (25)	3 (17.6)	
Last Maintenance Immunosuppressive Regimen, n (%)			
Triple	16 (80)	11 (64.7)	.460
Double	42 (20)	6 (35.3)	
FK + MMF + steroid	12 (60)	10 (58.8)	
CsA + MMF + steroid	2 (10)	0 (0)	
MMF + steroid	2 (10)	5 (29.4)	
EVO + MMF + steroid	1 (5)	0 (0)	
AZA + steroid	0 (0)	1 (5.9)	.319
EVO + MMF	1 (5)	0 (0)	
CsA + AZA + steroid	1 (5)	0 (0)	
FK + AZA + steroid	0 (0)	1 (5.9)	
CSA + steroid	1 (5)	0 (0)	

Abbreviations: AOPP; advanced oxidative protein products, ATG; anti-thymocyte globulin, AZA; azathioprine, CsA; cyclosporine, eGFR; estimated glomerular filtration rate, EVO; everolimus, FK; Tacrolimus, Gd-IgA1; galactose-deficient IgA1, HLA; human leukocyte antigen, IgAN; IgA nephropathy, IQR: interquartile range, KTx; transplantation, MMF; mycophenolate derivates, UPCR; urine protein to creatinine ratio. (Bold values indicate $p<0.05$).

secondary outcomes. Seven of 37 patients (18.9%) experienced BPAR. Graft loss was observed in 9 patients (24.3%) during the follow-up period. The recurrent IgAN group had higher levels of both AOPP (median 78.4 vs. 67.35 µmol/L) and Gd-IgA1 (median 5390.3 vs. 4732.5 ng/µL) compared to the non-recurrent group, although these differences did not reach statistical significance. Notably, the proportion of male donors was significantly greater in the recurrent group (64.7% vs. 25%, P = .022). Additionally, the sampling times for AOPP and Gd-IgA1 were statistically longer in the recurrent group (median 53 vs. 35.5 months, P = .022). Furthermore, baseline UPCR and serum creatinine levels were elevated, while baseline eGFR levels were lower in the recurrent patient group than non-recurrent group, with statistical significance. These findings, along with other features, are detailed in Table 1.

Single Nucleotide Variants

There were no significant differences identified in the SNVs associated with IgAN recurrence, BPAR, and graft loss (Tables 2-4). However, the AA genotype of rs6677604 in *CFH* and the AA genotype of rs2412971 in *HORMAD2* were absent in all patients who experienced BPAR or graft loss (Tables 3 and 4).

Correlation Analyses for AOPP and Gd-IgA1 Levels

A significant correlation was present between the AOPP and Gd-IgA1 levels with the Spearman correlation test (P < .001, r = 0.58). However, no significant correlations were shown between AOPP or Gd-IgA1 levels and baseline eGFR (P = .586, r = −0.093 and P = .627, r = −0.033, respectively). Also, the last eGFR levels did not have significant correlations with AOPP or Gd-IgA1 levels (P = .873, r = 0.027 and P = .736, r = −0.027, respectively).

Clinical and Laboratory Features of Patient Groups with Rejection and Graft Loss

The comparison of the patients with and without rejection showed that sampling time creatinine and rates of graft loss were statistically higher (P = .005, P = .001, and P = .045, respectively) in the rejection group. In addition, levels of Gd-IgA1 at the time of sampling were significantly higher in patients with rejection than in those without rejection (median 9486.4 *vs.* 3709.1 (ng/µL), P = .044). Patients with graft loss had a statistically higher percentage of BPAR (44.4 % vs. 10.7%, P = .045) and a shorter duration of follow-up (median 103.5 vs. 136.6 months, P = .026). The other laboratory/clinical features of groups with rejection and graft loss are provided in Tables 3 and 4.

Regression Analyzes for IgAN Recurrence and Graft Loss

Male donor sex (P = .018, HR: 3.853, 95% CI: 1.256-11.826) and eGFR at sampling (P = .039, HR: 0.967, 95% CI: 0.937-0.998) predicted IgAN recurrence in multivariate analysis (Table 5). No variables had a statistically significant impact on graft loss (Table 6).

DISCUSSION

Post-transplant recurrence of IgAN is generally considered a poor prognostic factor for graft survival.[18,19] Forty-six percent of the patients in the study group experienced recurrence of IgAN during follow-up. Similar to previous studies, the patients with recurrent IgAN had lower eGFR levels than those in the non-recurrent group at the last control. Multivariate regression analysis indicated that the eGFR at the time of sampling and male donor sex were independent risk factors for IgAN recurrence. Previously, it was shown that combined recurrent IgAN with rejection had unfavorable outcomes compared to IgAN recurrence alone in grafts, as expected.[32] In the study, BPAR and graft loss rates were not different in the recurrent and non-recurrent groups. These two findings could be attributed to the limited sample size of the study. Although a strong correlation between Gd-IgA1 and AOPP levels was observed, these markers were not associated with IgAN recurrence or graft survival. Genetic variants, including 4 tSNVs in 4 reported IgAN-associated genes, were not statistically good predictors of IgAN recurrence and graft survival. However, no KTx recipients with genotype "AA" rs6677604 of *CFH* and genotype "AA" rs2412971 of *HORMAD2* developed graft rejection or failure. Even though this observation did not reach statistical significance, additional well-designed studies are required to investigate the relationships between SNVs and allograft function.

Table 2. Single Nucleotide Variants in the Recurrent and Non-recurrent Groups

SNV Types	Non-recurrent IgAN (n = 20)	Recurrent IgAN (n = 17)	*P*
rs6677604, n (%)			
AA	2 (10)	1 (5.9)	.25
GG	10 (50)	13 (76.5)	
AG	8 (40)	3 (17.6)	
rs2412971, n (%)			
AA	3 (15)	3 (17.6)	.866
GG	10 (50)	7 (41.2)	
AG	7 (35)	7 (41.2)	
rs2738048, n (%)			
CC	2 (10)	2 (10.8)	.866
TT	10 (50)	7 (41.2)	
CT	8 (40)	8 (47)	
rs3803800, n (%)			
AA	6 (30)	3 (17.6)	.683
GG	9 (45)	9 (52.9)	
AG	5 (25)	5 (29.4)	

Abbreviations: IgAN; IgA nephropathy, SNV; single nucleotide variant.

Table 3. Single Nucleotide Variants, Galactose-Deficient IgA1 and AOPP Levels, and Clinical Features in Rejection and Non-rejection Groups

SNV Types	Rejection (n = 7)	Non-Rejection (n = 30)	*P*
rs6677604, n (%)			
AA	0 (0)	3 (10)	.663
GG	5 (71.4)	18 (60)	
AG	2 (28.6)	9 (30)	
rs2412971, n (%)			
AA	0 (0)	6 (20)	.429
GG	4 (57.1)	13 (43.3)	
AG	3 (42.9)	11 (36.7)	
rs2738048, n (%)			
CC	0 (0)	4 (13.3)	.557
TT	4 (57.1)	13 (43.3)	
CT	3 (42.9)	13 (43.3)	
rs3803800, n (%)			
AA	1 (14.3)	8 (26.6)	.781
GG	4 (57.1)	14 (46.8)	
AG	2 (28.6)	8 (26.6)	
Gd-IgA1 (ng/µL), median (IQR: 25-75)	9486.4 (6918.4-23179.3)	3709.1 (1143.3-7208.9)	**.044**
AOPP, median (µmol/L) (IQR: 25-75)	172.6 (111.7-251.1)	64.2 (38.9-106.7)	.103
Sampling time for AOPP and GdIgA1 (months), median (IQR: 25-75)	42 (35.25-63.75)	41 (10.5-60.75)	.342
Graft loss, n (%)	4 (57.1)	5 (16.7)	**.045**
IgAN recurrence, n (%)	4 (57.1)	13 (43.3)	.680
Donor age at KTx, median (IQR: 25-75)	60.5 (41.25-63.5)	50 (39.75-54.25)	.393
Recipient age at KTx, median (IQR: 25-75)	37 (34.75-44.75)	34.5 (30-47.25)	.801
eGFR (ml/min/1.73 m^2) at sampling, median (IQR: 25-75)	38 (21-55.75)	59.5 (46.5-75)	**.015**
Serum creatinine (mg/dL) at sampling, median (IQR: 25-75)	2.1 (1.58-3.38)	1.4 (1.2-1.63)	**.005**
UPCR (g/g) at sampling, median (IQR: 25-75)	0.4 (0.3-2.6)	0 (0-0.4)	.133
HLA mismatches, median (min-max)	3 (3-5)	3 (0-6)	.272
Follow-up time (months), median (IQR: 25-75)	113 (78.25-140.5)	125.5 (100.8-156.5)	.121
Living donor, n (%)	6 (85.7)	27 (90)	1.000
Male donor, n (%)	3 (42.9)	13 (43.3)	1.000
Male recipient, n (%)	7 (100)	20 (66.7)	.155
Family history of kidney disease, n (%)	2 (28.6)	4 (13.3)	.315
Transfusion, n (%)	2 (28.6)	9 (30)	1.000
Diabetes mellitus, n (%)	2 (28.6)	5 (16.7)	.596
Pregnancy history, n (%)	0 (0)	8 (26.7)	.308
Presence of pretransplant PRA, n (%)	0 (0)	2 (6.7)	1.000
Previous KTx, n (%)	0 (0)	3 (10)	1.000
Induction regimen, n (%)			
No	4 (57.1)	21 (70)	
ATG	2 (28.6)	2 (6.7)	.238
Basiliximab	1 (14.3)	7 (23.3)	

Abbreviations: AOPP; advanced oxidation protein products, ATG; anti-thymocyte globulin, eGFR; estimated glomerular filtration rate, Gd-IgA1; galactose deficient IgA1, HLA; human leukocyte antigens, IgAN; IgA nephropathy, IQR; interquartile range, KTx; kidney transplantation, PRA; panel reactive antibodies, SNV; single nucleotide variant, UPCR; urine protein to creatinine ratio. (Bold values indicate $p<0.05$).

Table 4. Single Nucleotide Variants, Galactose-deficient IgA1 and AOPP Levels, and Clinical Features in Graft Loss and Non-graft Loss Groups

SNV Types	Graft Loss (n = 9)	Non-Graft Loss (n = 28)	*P*
rs6677604, n (%)			
AA	0 (0)	3 (10.7)	.590
GG	6 (66.7)	17 (60.7)	
AG	3 (33.3)	8 (28.6)	
rs2412971, n (%)			
AA	0 (0)	6 (21.4)	.316
GG	5 (55.5)	12 (42.9)	
AG	4 (44.5)	10 (35.7)	
rs2738048, n (%)			
CC	1 (11.1)	3 (10.7)	.995
TT	4 (44.4)	13 (46.4)	
CT	4 (44.4)	12 (42.9)	
Gd-IgA1 (ng/µL), median (IQR: 25-75)	6322.3 (1924.4-8619.9)	4732.5 (1111.3-11893.5)	.832
AOPP, median (µmol/L) (IQR: 25-75)	70.13 (37.7-170.8)	75.7 (41.2-141.4)	.436
Sampling time for AOPP and GdIgA1 (months), median (IQR: 25-75)	46.5 (32.25-62.5)	39.5 (8.75-59.5)	.242
Biopsy-proven acute rejection, n (%)	4 (44.4)	3 (10.7)	**.045**
IgAN recurrence, n (%)	5 (55.5)	12 (42.9)	.703
Donor age at KTx, median (IQR: 25-75)	48.5 (32-61.75)	50.5 (40.25-56.5)	.723
Recipient age at KTx, median (IQR: 25-75)	37 (31.25-52.25)	35 (30-43)	.832
eGFR (m/min/1.73 m^2) at sampling, median (IQR: 25-75)	39.5 (24.5-73.75)	58.5 (48-73.5)	.083
Serum creatinine (mg/dL) at sampling, median (IQR: 25-75)	1.65 (1.25-3.08)	1.4 (1.2-1.7)	.088
UPCR (g/g) at sampling, median (IQR: 25-75)	0.95 (0.1-2.1)	0 (0-0.3)	.060
HLA mismatches, median (min-max)	3 (3-3)	3 (0-6)	.479
Follow-up time (months), median (IQR: 25-75)	103.5 (85.5-125.75)	136.5 (100.3-159.5)	**.026**
Living donor, n (%)	9 (100)	24 (85.7)	.554
Male donor, n (%)	4 (44.4)	12 (42.9)	1.000
Male recipient, n (%)	8 (88.9)	19 (67.9)	.393
Family history of kidney disease, n (%)	1 (11.1)	5 (17.9)	1.000
Transfusion, n (%)	4 (44.4)	7 (25)	.404
Diabetes mellitus, n (%)	2 (22.2)	5 (17.9)	1.000
Pregnancy history, n (%)	0 (0)	8 (28.6)	.159
Presence of pretransplant PRA, n (%)	0 (0)	2 (7.1)	1.000
Previous KTx, n (%)	2 (22.2)	1 (3.6)	.141
Induction regimen			
No, n (%)	6 (66.7)	19 (67.9)	
ATG, n (%)	2 (22.2)	2 (7.1)	.360
Basiliximab, n (%)	1 (11.1)	7 (25)	

Abbreviations: AOPP; advanced oxidation protein products, ATG; anti-thymocyte globulin, eGFR; estimated glomerular filtration rate, Gd-IgA1; galactose deficient IgA1, HLA; human leukocyte antigens, IgAN; IgA nephropathy, IQR; interquartile range, KTx; kidney transplantation, PRA; panel reactive antibodies, SNV; single nucleotide variant, UPCR; urine protein to creatinine ratio.

Table 5. Multivariate Cox Regression Analysis for Risk Factors in IgA Nephropathy Recurrence

			95% CI	
	P	HR	Lower	Upper
Recipient age at KTx	.269	1.033	0.975	1.096
Donor age at KTx	.116	1.047	0.989	1.109
Donor sex (male)	.018*	3.853	1.256	11.826
eGFR at sampling	.039*	0.967	0.937	0.998
Proteinuria at sampling	.541	1.064	0.872	1.298
No rejection	.332	0.492	0.117	2.061

*$P < .05$
Abbreviations: CI; confidence interval, eGFR; estimated glomerular filtration rate, HR; hazard ratio, KTx; kidney transplantation.

A meta-analysis showed that younger recipient age, younger donor age, male recipient sex, and transplantation from related donors could be risk factors for recurrent IgAN.[33] The study group mainly consisted of male transplant recipients (73%), who were mostly transplanted from their living relatives. No differences in recipient sex were noted in the recurrent and non-recurrent groups. However, the recurrent IgAN group had a significantly higher percentage of male donors. While the proportion of living-related donors was greater in the recurrent group, this difference did not reach statistical significance. Furthermore, the study did not reveal any differences in patient and recipient ages related to IgAN recurrence.

Steroid-free or sirolimus-based regimens and ATG-free induction treatments have been shown to be risk factors for IgAN recurrence.[33,34] In addition, MMF may be associated with less recurrence of IgAN.[33] However, recurrence rates of IgAN were similar between MMF and AZA treatments in another study.[35] Differences in sample size and ethnicity might have caused these contradictory results. In this cohort, there were no differences in maintenance immunosuppressive treatments or induction regimens between the study groups. It may stem from the limited patient sample size.

In the study by Camilla et al[36], higher levels of Gd-IgA1 and AOPP could be related to the progression of IgAN. Levels of these markers correlated with proteinuria, and the authors showed a correlation between AOPP and Gd-IgA1. In the study, both markers were increased in the recurrent IgAN group relative to patients without IgAN recurrence. Nevertheless, the differences between the recurrent and non-recurrent groups were not statistically significant. Notably, a significant correlation was present between the AOPP and Gd-IgA1 levels. In addition, Gd-IgA1 levels were significantly increased in the rejection group compared to those in the kidney transplant group without rejection.

Table 6. Multivariate Cox Regression Analysis for Risk Factors in Graft Loss

			95% CI	
	P	HR	Lower	Upper
eGFR at sampling	.445	0.986	0.950	1.023
Proteinuria at sampling	.144	1.224	0.933	1.606
No rejection	.094	0.281	0.064	1.242

Abbreviations: CI; confidence interval, eGFR; estimated glomerular filtration rate, HR; hazard ratio.

The significant limitations of the study involve its retrospective design and the restricted sample size. Also, the heterogeneous sampling time of the AOPP and Gd-IgA1 levels is another major drawback. In addition, the patients mostly consisted of male living donor transplant recipients, and the absence of zero-hour and follow-up protocol biopsies, which could lead to a missed diagnosis of subclinical IgAN recurrence in the non-recurrent group, are the other significant limitations of the research. However, the long follow-up time and presence of serum biomarkers, including Gd-IgA1, AOPP, and 4 tSNVs, are the strengths of this study.

In conclusion, IgAN is associated with a substantial risk of recurrence after KTx. Prospective biomarker and genetic marker studies, including large sample sizes, are required for the evaluation of the risk of recurrence of IgAN and allograft functions after renal transplantation.

Data Availability Statement: The data that support the findings of this study are available on request from the corresponding author.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of Istanbul University Faculty of Medicine (Date: 2013, Number: 1832).

Informed Consent: Written informed consent was obtained from the patients who agreed to take part in the study.

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