Angiotensin II receptor 1 antibodies associate with post transplant focal segmental glomerulosclerosis and proteinuria

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Abstract

Background Angiotensin II type 1 receptors (AT1Rs) are expressed on podocytes, and play an essential role in the maintenance of vascular homeostasis. The presence of AT1R antibodies (AT1R-Abs) leads to activation of these receptors resulting in endothelial cell dysfunction and podocyte injury. We assessed the correlation between AT1R-Abs and the risk of post transplant FSGS. Methods This is a retrospective study included all kidney transplant recipients with positive AT1R-Abs (≥9 units/ml), who were transplanted and followed at our institution between 2006-2016. We assessed the development of biopsy proven FSGS and proteinuria by urine protein to creatinine ratio of ≥ 1 g/g. Results We identified 100 patients with positive AT1R-Ab at the time of kidney transplant biopsy or proteinuria. 49 (49%) recipients (FSGS group) had biopsy-proven FSGS and/or proteinuria. Pre-transplant hypertension was present in 89% of the FSGS group compared to 72% in the non-FSGS group, p =0.027. 43% of the FSGS group were on angiotensin converting enzyme inhibitors or angiotensin receptor blockers prior to transplant, compared to 25.5% in the non-FSGS group, p =0.06 . Primary idiopathic FSGS was the cause of ESRD in 20% of the FSGS group, compared to 6% in the comparison group; p =0.03. The allograft loss was significantly higher in the FSGS group 63% compared to 39% in non-FSGS. Odds ratio and 95% confidence interval were 2.66 (1.18 -5.99); p =0.017. Conclusions Our data suggests a potential association between AT1R-Abs and post transplant FSGS leading to worse allograft outcome. Therefore, AT1R-Abs may be considered a biomarker for FSGS post transplant.

Background

Angiotensin II type 1 receptors (AT1Rs) are widely expressed across endothelial cells and renal podocytes. In previous reports, angiotensin II type 1 receptor antibodies (AT1R-Abs) have shown to be associated with vascular rejection of renal allografts in the absence of human leukocyte antigen (HLA) antibodies [1,2]. In animals, AT1R-Abs have been reported in association with malignant hypertension, pre-eclampsia[3] and post transplant focal segmental glomerulosclerosis (FSGS)[4]. In one case, a patient with positive AT1R-Abs presented with new onset collapsing FSGS and antibody mediated rejection one month after renal transplantation[5]. Although the exact mechanism of injury in human is not known, it is thought that AT1R-Ab can cause activation of the AT1R receptors leading to podocyte injury, glomerular endotheliosis and proteinuria. In animal models and cultured podocyte studies the AT1R-Abs prevented the mRNA expression of the slit diaphragm molecules leading to proteinuria[6].

FSGS is a histopathologic diagnosis, classified as idiopathic (primary) or secondary. Post transplant FSGS can be recurrent or de-novo in nature. Recurrent FSGS is very common with 30–40% recurrence rate post transplant. Not all patients respond to treatment and some progress leading to allograft failure and loss[7].

The pathogenesis of recurrent FSGS is not well understood; however established data suggest that podocyte injury is secondary to circulating factor/s [8]. In a case report,recurrence of FSGS in renal allograft was reversed with complete resolution of proteinuria after re-transplantation into a different

recipient [9]. Several factors have been investigated as potential causes of primary and recurrent FSGS, such as soluble urokinase type plasminogen activator (suPAR), cardiotrophin-like cytokine-1 (CLC-1), etc. no one factor was validated in a large cohort.

A recent study showed an association between pre-transplant AT1R-Abs in patients with primary FSGS and the risk of post transplant FSGS [10].

In this study, we aim to assess the association between the presence of AT1R-Abs and the development of post transplant FSGS and proteinuria.

Materials And Methods

Study population

The study was approved by the Institutional Review Board (IRB) at Johns Hopkins Hospital. This is a retrospective study that included allrenal transplant recipients with AT1R-Abs concentrations ≥ 9 Units/ml, transplanted and followed at our institution between 2006 and 2016. Data were collected throughout transplant period until last available follow up (ending December 2019) or until graft loss.

AT1R-Abs testing:

AT1R-Ab testing was performed using quantitative ELISA (CellTrend GmbH, Luckenwalde, Germany) as described before [11] using sera collected at time of graft dysfunction. Briefly, serum was diluted of a 1:100,added to the 96-well polystyrene microtiter plate coated with human AT1R derived from transfected Chinese hamster ovary cell extracts and incubated at 4 °C for 2 hours. Following wash steps, a horseradish peroxidase-conjugated goat antihuman IgG detection antibody was added, followed by an 1 hour incubation., of 3,3',5,5-tetramethylbenzidine (TMB) substrate was then added to the reaction mix Presence of antibody bound to AT1R was detected by a colorimetric change. A standard curve was generated to allow the quantitation of AT1R-Ab, using a control sample at varying concentrations (2.5, 5, 10, 20, and >40 U/ml).If available, pre-transplant sera were also tested retrospectively. AT1R-Ab concentrations of ≥ 9 units/ml were reported as positive.

Outcomes definitions:

The primary outcome was the development of FSGS lesion or proteinuria. FSGS was defined by renal allograft biopsy detection of FSGS lesions by light microscope (LM) or presence of 20% or more effacement of the podocyte foot processes by electron microscope (EM). Proteinuria was defined by urine protein creatinine ratio of ≥ 1 g/g.

Secondary outcomes were to compare renal allograft loss, death censored renal allograft loss, renal allograft survival time and all cause mortality between patients meeting FSGS criteria and patients who did not. Renal allograft loss was defined as eGFR less than 15 ml/min/1.73 m^2 for three or more

consecutive months, re-transplantation, the need for long-term dialysis, or death. Death censored allograft loss excluded patients who died with functional renal allograft.

Statistical analysis:

Baseline demographic characteristics and transplant characteristics for renal allograft transplant recipients in both groups (FSGS Vs no FSGS) data were presented as proportions with percentage (%) or median with inter quartile range. Statistical analyses were performed using MedCalc 19.1.5.

Statistical differences were assessed by t-test for parametric data, Mann-Whitney test for non-parametric data and Pearson Chi square or Fisher's exact test for categorical variables, as appropriate. In Kaplan Meier curve logrank test was used to calculate the*p* value. Forest plot was used to present secondary outcomes, with odds ratio and 95% confidence interval.

Results

We identified 100 kidney transplant patients with positive AT1R-Abs during the study period. Median follow up time was 64 months (30–93). Out of 100 patients with AT1R-Abs, 37 patients (37%) were found to fit criteria for biopsy proven FSGS. Additional 12 patients (12%) had persistent proteinuria more than 1 g/g (by urine protein to creatinine ratio) fitting selection criteria for FSGS. In total, 49 out of 100 patients (49%) fit the criteria for FSGS (FSGS group). 51 patients (51%) did not have biopsy proven FSGS or significant proteinuria, thus were assigned to the comparison group (No FSGS), Fig. 1.

Baseline characteristics are summarized in table 1. The median age of patients with FSGS was 51 years (44.75–61.25) and was 55 years (44.25–61) in patients without FSGS. Female patients were 57% in the FSGS group and 70% in the comparison group. Regarding race, 61% were white, 29% black, 10% other race in FSGS group compared to 70% white, 20% black, 10% other race in the comparison group. Pre-transplant hypertension was present in 89% of the FSGS patients group, and 72% in the comparison group; the difference was statistically significant with p = 0.027. There was no statistical difference in the prevalence of pre-transplant diabetes between the two groups, 26.5% in FSGS group versus 23.5% in the comparison group. 43% of patients in the FSGS group were on angiotensin converting enzyme inhibitors (ACEI) or angiotensin receptor blockers (ARBs) prior to transplant, compared to 25.5% in the comparison group, p = 0.06. Primary renal disease in patients with FSGS was primary FSGS in 20% of the patients, compared to 6% in the comparison group; the difference was statistically significant with p = 0.03.

Allografts' characteristics are summarized in table 2. There was no statistical difference in the source of donated kidney between the two groups; 69.5% were from living donors in the FSGS group, and 82.5% in comparison group (p = 0.12). Induction immunosuppression was rabbit- anti-thymocyte globulin (rATG) in 86% in FSGS group, and was 80% in the comparison group. 89% of patients in the FSGS group and 98% in the comparison group received standard maintenance immunosuppression (mycophenolate mofetil, tacrolimus andprednisone). 28.5% of patients in the FSGS group had previous one renal transplant, while 37% of the comparison group had previous one transplant. Previous two or more transplants were in 20%

in the FSGS group, and 10% in the comparison group. Donor specific antibodies (DSAs), via flow cytometry at the time of biopsy, were negative in 28.5%, low level in 22.5% and positive in 49% in the FSGS group. In the comparison group, donor specific antibodies were negative in 37%, low level in 23% and positive in 40%, p = 0.57. Biopsy proven antibody mediated rejection (ABMR) was present in 30.5% in FSGS group compared to 25.5% in the comparison group, p = 0.57. Biopsy proven cell mediated rejection (CMR) was present in 10% in FSGS group compared to 8% in the comparison group. Mixed cellular and antibody mediated rejection was present in 2% in the FSGS group and in 4% in the comparison group. None of these differences in rejection rates were statistically significant.

Secondary outcomes are summarized in table 3. Renal allograft losses were more observed in the FSGS group, 63% compared to 39% in the comparison group with odds ratio (95% confidence interval) of 2.66 (1.18–5.99) p = 0.017. Death censored renal allograft loss was more observed in the FSGS group 59%, versus 25.5% in the comparison group. Odds ratio (95% confidence interval) of 4.23 (1.81–9.90) p = 0.0009 which also showed statistically significant difference. All cause mortality was less in the FSGS group compared to the other group (16.3% and 25.4% respectively), however the odds ratio and confidence interval 0.57(0.21–1.52) failed to show statistical significance, p = 0.26. Figure 2 shows Kaplan-Meier curve comparing renal allograft survival in patients in FSGS group. Figure 3 shows Forest plot of secondary outcomes (renal allograft loss, death censored renal allograft loss and all cause mortality), presented as odds ration and 95% confidence interval, with calculated p value.

We assessed the correlation between the levels of AT1R-Abs and the risk of developing FSGS. There was no statistically significant difference in the levels of AT1R-Abs and the development of FSGS, table 4.

Discussion

Widely expressed in podocytes, angiotensin II type 1 receptors (AT1Rs) play an essential role in the maintenance of vascular homeostasis and several cellular and tissue functions in physiological state [10]. As demonstrated by animal models, AT1Rs hinder the mRNA expression of the slit diaphragm molecules, and their antagonists ameliorate proteinuria by preventing a reduction in the functional molecules of the slit diaphragm [6], which may lead to FSGS.

Despite successof renal transplant in many FSGS patients, the risk of recurrence remains high and is estimated to be 30–40%. This risk can increase especially amongst patients with idiopathic (primary) FSGS or those with history of recurrence after previous transplant. Recurrence often leads to allograft failure and loss. Several markers were suggested to be associated with FSGS and its recurrence. Serum soluble urokinase receptor (suPAR) and apolipoprotein A1 (ApoA-1) are examples; however additional factors are likely to exist. Given the wide expression of AT1Rs in podocytes, we evaluated the association of their antibodies (AT1R-Abs) and the risk of developing FSGS in renal transplant recipients. In our study, about half of patients with positive AT1R-Abs were found to have biopsy proven FSGS and/or significant proteinuria at the time of the AT1R-Abs detection, which could not be attributed to other causes. In those

patients, renal allograft survival was significantly lower compared to those without FSGS or proteinuria (37% compared to 61%).

Mujtabaet al tested pre-transplant sera of 28 patients with history of primary FSGS for anti-AT1R levels as a biomarker for risk of recurrence of FSGS. Sera from 11 patients with polycystic kidney disease were used as controls. Twelve patients had biopsy proven post-transplant FSGS recurrence at 1.5 months. Anti-AT1R antibodies levels in patients with FSGS were significantly higher in patients who developed FSGS recurrence compared to those who did not. The authors concluded that pre-transplant anti-AT1R-Abs levels might be a helpful biomarker in identifying patients at high risk of post-transplant FSGS recurrence[10]. In our study we assessed a larger sized population with positive AT1R-Abs and included patients with various causes of ESRD. Our study demonstrated that even without a primary diagnosis of FSGS, de novo FSGS can develop in the presence of AT1R-Abs. Our cohort included a total of 13 primary FSGS patients with positive AT1R-Abs, 10 of them developed recurrent FSGS. Also, in our study, the AT1R-Ab levels were not statistically different in patients with FSGS and patients without FSGS; which does not support Mujtaba et al finding of correlation between level of AT1R-Abs and risk of recurrence of primary FSGS.

We found that pre-transplant hypertension prevalence was more amongst patients who developed post transplant FSGS. Hypertension per se can be associated with secondary FSGS due to hyperfiltration. Despite the absence of statistical significance, pre transplant use of ACEI or ARB was more amongst AT1-Abs positive patients who developed FSGS, which is possibly reflecting the higher prevalence of pre-transplant hypertension in that population.

In a retrospective study by Pascual et al, the authors compared the risk of recurrence of FSGS and other forms of glomerulonephritis, the rate of FSGS was lower in patients who received induction therapy with polyclonal rabbit anti-thymocyte globulin, compared with alemtuzumab and an interleukin-2 receptor antagonist[12]. Another study showed no difference in graft loss due to recurrent FSGS between those treated different anti-metabolites[13], however mTOR inhibitors are known to be a cause of secondary FSGS [14]. In our study, there were no statistically significant differences in the induction or in the maintenance immunosuppression between patients who developed FSGS and those who did not.

Secondary FSGS can also be a sequel of transplant rejection especially when chronic, and can be associated with chronic transplant glomerulopathy and proteinuria. In this study, the biopsy proven allograft rejection and presence/absence of donor specific antibodies were comparable in both groups with FSGS and without FSGS. It remains unclear why some patients with positive AT1R-Abs would develop post transplant FSGS and why some would not. This could be explained by other factors or possible “second hit” that yet to be identified.

The real challenge resides in the management of patients with positive AT1R-Abs. Patients with acute non HLA vascular rejection induced by AT1R-Abs are often (and not surprisingly) treated with “all in” strategy, where pulse steroids, plasmapheresis, IVIG with or without rituximab are given. That could be

related to delay in diagnosis as not all patients are tested for non HLA antibodies unless requested. In addition, ARBs are added (if possible) which are likely of some benefit [1]. The story may not differ here. ARBs are- till the moment- the mainstay in management of AT1R-Abs, however it may not be unreasonable to be more aggressive when FSGS presents with sudden onset nephrotic range proteinuria and try serial plasmapheresis with post treatment IVIG in attempt to remove any other circulating factor/s or the AT1R-Abs themselves. In a case report, de novo collapsing FSGS in AT1R-Ab positive patient was successfully treated with plasmapheresis and losartan resulting in complete resolution of proteinuria[5]. However, the effectiveness of such measures is yet to be determined.

The strength of this study is that it is the first study to evaluate the association of AT1R-Abs and development of post transplant FSGS in patients with various causes of ESRD. It provides an insight about a possible “villain” involved the pathogenesis of post transplant FSGS whether recurrent or de novo. It also shows worse allograft outcomes when AT1R-Ab associates with FSGS.

This study has several limitations. The retrospective nature of this study may have potential bias. It involved more living kidney transplants and more female recipients, which do not represent the demographics of transplants in the U.S population. The timing of the renal allograft biopsies and/or laboratory work up was not standardized and was highly variable. Additionally, the histopathology of renal biopsies especially the degree of podocyte foot process effacement on EM can be variably estimated according to the reading pathologist. Nevertheless, our study is the first and largest with findings that may explain some cases of recurrent and de novo FSGS and proteinuria post kidney transplant. Finally, our study lacks a control group. Although, a control group will add a significant strength to our manuscript, however, only patients who we had a strong suspicion of having AT1R-Ab were tested. Therefore, we were unable to find a matching group in whom the test was negative and compared with our study cohort. However, we think although there is no matching control group, comparing to published data, AT1R-Ab seems to correlate highly with FSGS than many investigated permeability factors.

Conclusion

AT1R-Abs may play a role in the pathogenesis and the development of recurrent and de novo FSGS and proteinuria post renal transplantation. The early detection may predict the risk for developing post transplantFSGS; thus prompting closer follow up and initiating proper management. More studies are neededto confirm our findings of the role of AT1R-Abs in post transplant FSGS and impact of interventions targeting these antibodies on renal allograft outcome.

Abbreviations

AT1R, Angiotensin II type 1 receptor

FSGS, focal segmental glomerulosclerosis

HLA, human leukocyte antigen

suPAR, soluble urokinase type plasminogen activator

eGFR, estimated glomerular filtration rate

ESRD, end stage renal disease

LM, light microscope

EM, electron microscope

rATG, rabbit anti-thymocyte globulin

DSA, donor specific antibody

IQR, Inter Quartile Range

ApoA-1, apolipoprotein A1

ABMR, antibody mediated rejection

CMR, cell mediated rejection

Declarations

AUTHORSHIP

Mohammad Abuzeineh participated in design of the work, data collection, data analysis and interpretation, drafting the article, final approval of the version to be published.

Amtul Aala participated in data collection, data analysis and interpretation, final approval of the version to be published.

Sami Alasfar participated in critical revision of the article, final approval of the version to be published.

Nada Alachkar participated in design of the work, data collection, data analysis and interpretation, drafting the article, final approval of the version to be published.

Disclosures:

The authors of this manuscript have no conflict of interest to disclose as described by the Transplantation.

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None

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Tables

Table 1

	FSGS n = 49	No FSGS n = 51	*p* value
Median age, years (IQR[a])	51 (44.75 to 61.25)	55 (44.25 to 61.00)	0.7667
Gender	Females 28/49 (57%) Males 21/49 (43%)	Females 36/51 (70%) Males 15/51 (30%)	0.1614
Race	White 30/49 (61%) Black 14/49 (29%) Other 5/49 (10%)	White 36/51 (70%) Black 10/51(20%) Other 5/51 (10%)	0.5563
Pre-transplant hypertension	44/49 (89%)	37/51 (72%)	*0.0279*
Pre-transplant diabetes	13/49 (26.5%)	12/51 (23.5%)	0.7289
Use of ACEI[b] or ARB[c] prior to transplant	21/49 (43%)	13/51 (25.5%)	0.0668
Primary renal disease is primary FSGS	10/49 (20%)	3/51 (6%)	*0.03*

Baseline characteristics. There were no statistically significant differences between FSGS group and comparison group in the median age, gender, race, pre-transplant diabetes or use of ACEI or ARB prior to transplant. Pre-transplant hypertension was more in the FSGS group compared to the comparison group, the difference was statistically significant. Primary FSGS as a cause of primary renal disease was more in patients with FSGS compared to comparison group, the difference was statistically significant.

[a]Inter Quartile Range

[b] Angiotensin converting enzyme inhibitors

[c]Angiotensin receptor blockers

Data are presented as proportions followed by percentage (%) unless otherwise mentioned.

Table 2

	FSGS n = 49	No FSGS n = 51	*p*value
Type of donation	DKT[a]: 15/49 (30.5%) LKT[b]: 34/49 (69.5%)	DKT: 9/51 (17.5%) LKT: 42/51 (82.5%)	0.1291
Induction immunosuppression	rATG[c]: 42/49 (86%) Other: 7/49 (14%)	rATG: 41/51 (80%) Other: 10/51 (20%)	0.4787
Maintenance immunosuppression	Standard[d]: 44/49 (90%) Non standard: 5/49 (10%)	Standard: 50/51 (98%) Non standard: 1/51 (2%)	0.0827
Previous one renal transplant	14/49 (28.5%)	19/51 (37%)	0.3559
Previous two or more renal transplants	10/49 (20%)	5/51 (10%)	0.1376
Presence of donor specific antibodies	Negative 14/49 (28.5%) Low positive 11/49 (22.5%) Positive 24/49 (49%)	Negative 19/51 (37%) Low positive 12/51 (23%) Positive 20/51 (40%)	0.5697
Biopsy proven ABMR[f]	15/49 (30.5%)	13/51 (25.5%)	0.5684
Biopsy proven CMR[g]	5/49 (10%)	4/51 (8%)	0.6800
Biopsy proven AMR and CMR (mixed)	1/49 (2%)	2/51 (4%)	0.5815

Allografts' characteristics. There were no statistically significant differences between FSGS group and comparison group in type of donation, induction immunosuppression, maintenance immunosuppression, number of previous transplants, presence of donor specific antibodies, presence of biopsy proven AMR or CMR or mixed rejection.

[a]Deceased Kidney Transplant

[b] Living Kidney Transplant

[c]Rabbitantithymocyte globulin

[d] Standard immunosuppression: Mycophenolate mofetil, tacrolimus, prednisone. Non standard: any other

[e]HLA antibody testing was performed with pre and post-transplant patients' sera using the Luminex™ pooled HLA antigen (LMX), the phenotype bead assay (LMID)(Immucor-Lifecodes, Stamford, CT) and a single antigen panel (One Lambda, Canoga Park, CA).

[f]Antibody mediated rejection

[g]Cell mediated rejection

Data are presented as proportions followed by percentage(%)

Table 3

	FSGS n = 49	No FSGS n = 51	OR (95% CI)	*p* value
Renal allograft loss	31/49 (63%)	20/51 (39%)	2.6694 (1.1895 to 5.9905)	*0.0173*
Death censored allograft loss	29/49 (59%)	13/51 (25.5%)	4.2385 (1.8130 to 9.9086)	*0.0009*
All cause mortality	8/49 (16.3%)	13/51 (25.4%)	0.5704 (0.2130 to 1.5275)	0.2639
Mean graft survival time **95% Confidence Interval**	52.48 months (38.94 – 66.02)	54.20 months (37.51 – 70.88)	Not applicable	0.8702

Secondary outcomes. FSGS group showed increased renal allograft loss compared to the comparison group with statistically significant odds ratio as shown. This was more evident in the death censored renal allograft loss with higher odds ratio and statistical significance. All cause mortality was lower in FSGS group compared to the comparison group; however the difference was not statistically significant. Mean allograft survival was almost similar in both groups without statistically significant differences.

Data are presented as percentage (%) unless otherwise mentioned.

Table 4

AT1R-Abs Levels (units/ml)	***FSGS** **n = 49**	**No FSGS** **n = 51**	*p value*
9-17	29/49 (59%)	25/51 (49%)	
17-40	13/49 (27%)	16/51 (31%)	
>40	7/49 (14%)	10/51 (20%)	*p*= 0.5779
*FSGS, focal segmental glomerulosclerosis			

Figures

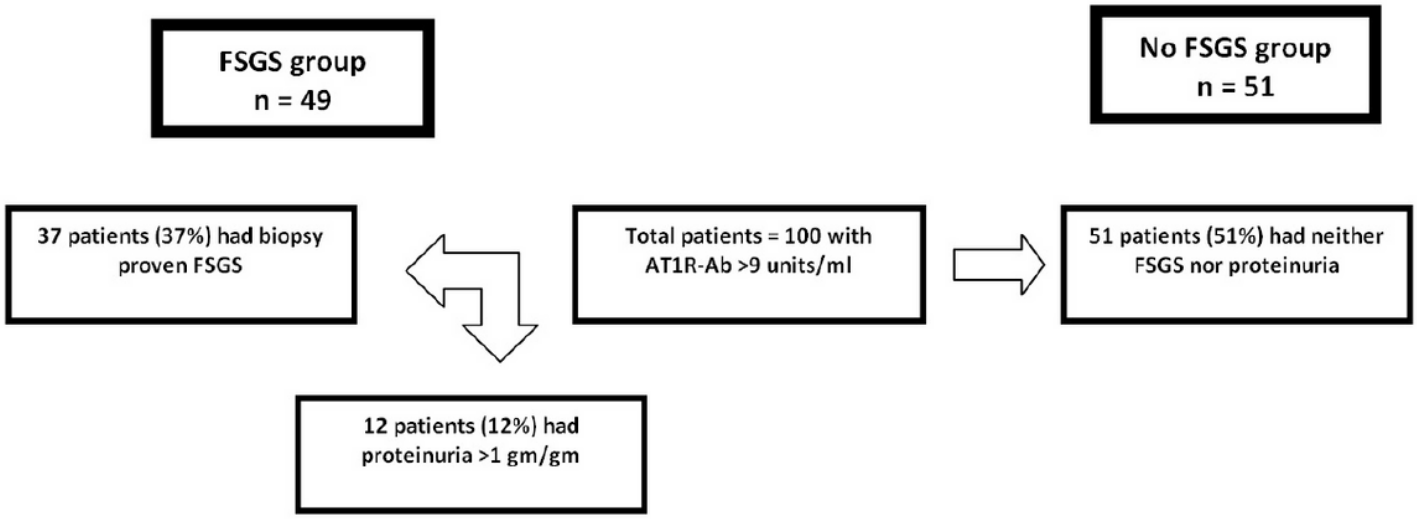

Figure 1

primary outcome.

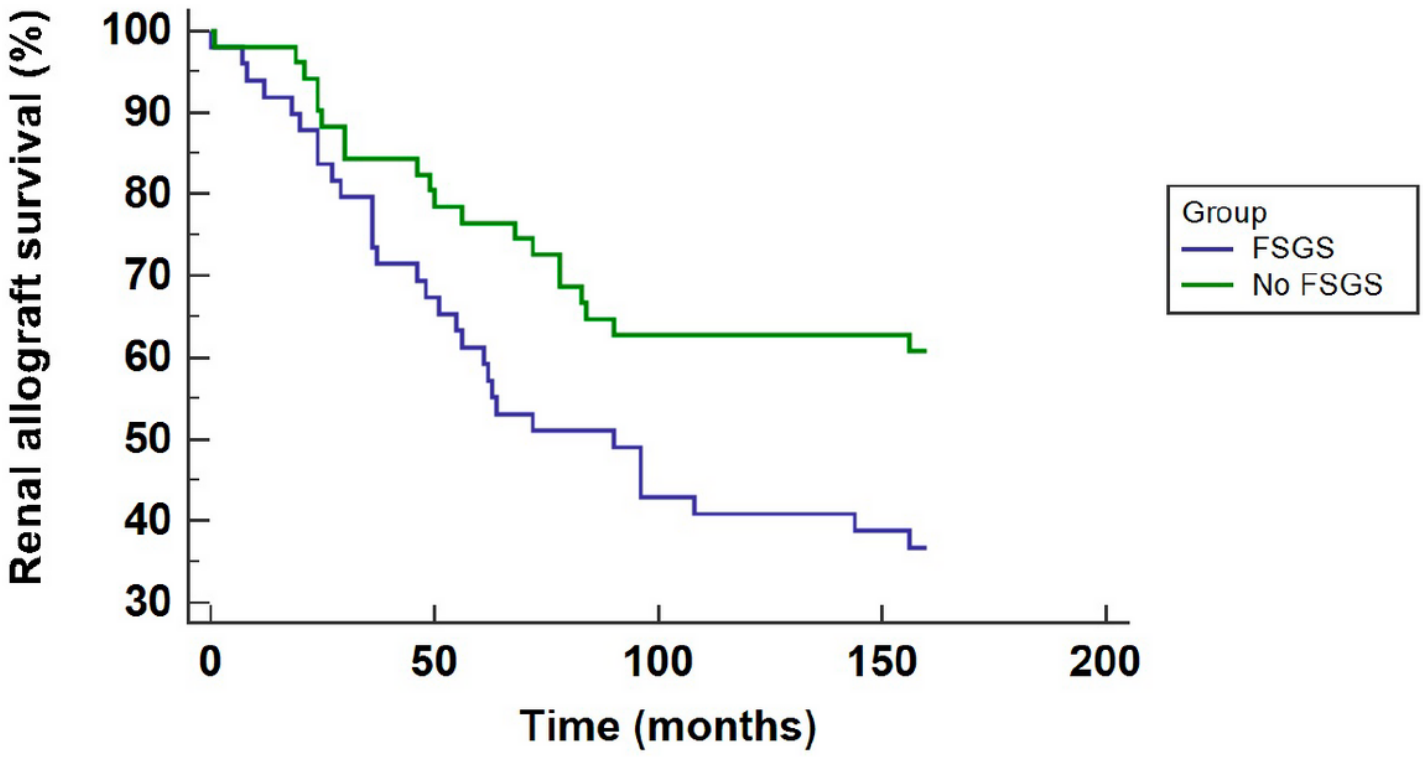

Figure 2

Kaplan-Meier curve for renal allograft survival. Renal allograft survival was 37% in FSGS group, compared to 61% in the comparison group; p = 0.017. Mean allograft survival time (not shown) was comparable in FSGS group (54.3 months) and in comparison group (54.15); p = 0.99.

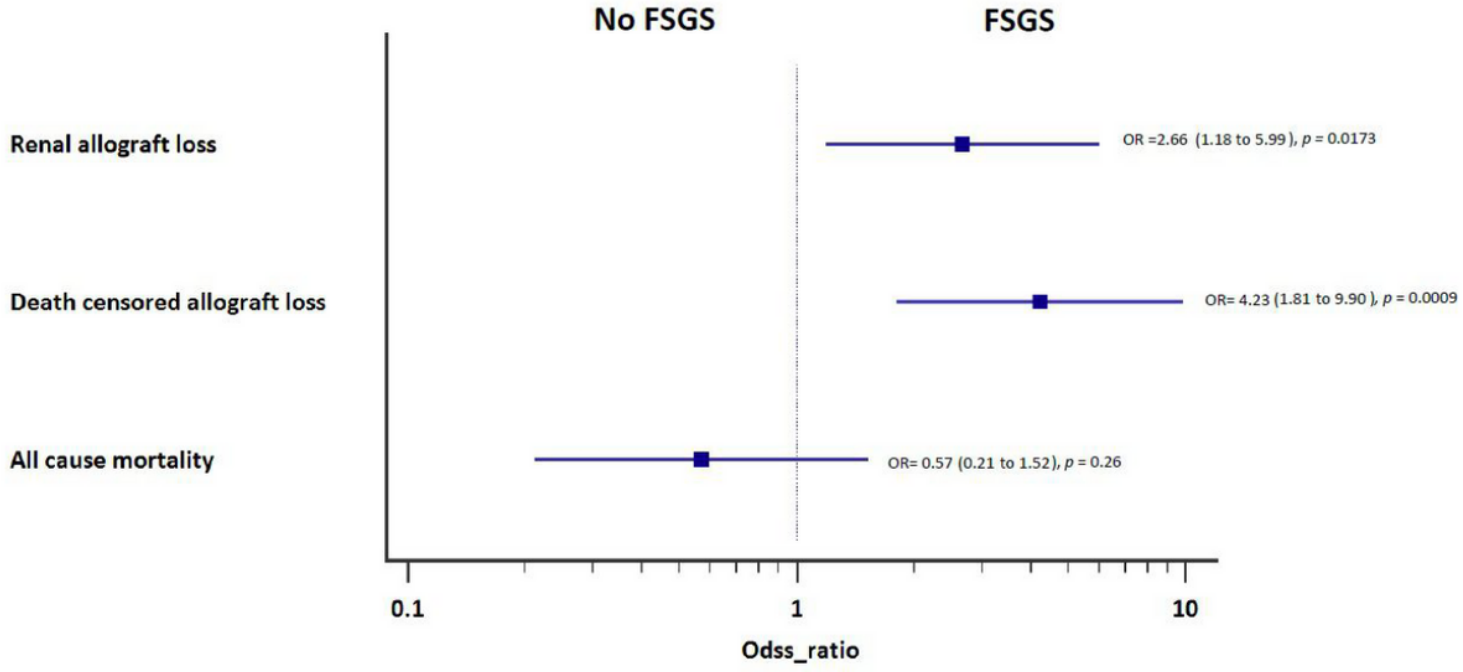

Figure 3

forest plot for secondary outcomes. Renal allograft loss showing statistically significant odds ratio of 2.66 (1.18-5.99) in FSGS group compared to comparison group. Death censored allograft loss showing even more significant difference in FSGS group in comparison to other group, with odds ratio of 4.23 (1.81 - 9.90). All cause mortality was less in FSGS group than in comparison group, however the difference is not statistically significant.