Glucocorticoid Receptor Polymorphisms and Avascular Osteonecrosis After Kidney Transplantation

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Keywords. kidney transplantation, osteonecrosis, glucocorticoid receptors, *NR3C1* gene, polymorphism

Original Paper

Introduction. Glucocorticoids (GCs) are commonly prescribed as immunosuppressive agents after kidney transplantation and their most common non-traumatic adverse effect is Avascular Necrosis (AVN) of the femoral head. In this regard, this study aimed to evaluate the glucocorticoid receptor (GR) polymorphisms among kidney transplant recipients and their potential role as a risk factor for the incidence of AVN.

Methods. In this study, 99 renal transplant recipients were evaluated for the correlations of GR polymorphisms including *N363S* (rs6195), *BclI* (rs41423247), *ER22/23EK* (rs6189/rs6190), and *A3669G* (rs6198) with AVN after renal transplantation.

Results. Results showed that none of the renal-transplanted patients neither with GC hypersensitive polymorphisms (*N363S* and *BclI*) nor with GC-resistant polymorphisms (*A3669G* and *ER22/23EK*) developed AVN ($P > .05$). In addition, the medications of the renal recipients with AVN were significantly different from the non-AVN patients ($P < .001$).

Conclusion. The study results indicate that the GR polymorphisms have no critical roles in the susceptibility to AVN after renal transplantation. However, further studies to confirm the results are recommended.

IJKD 2023;17:86-91
www.ijkd.org
DOI: 10.52547/ijkd.7221

INTRODUCTION

Immunosuppressive agents such as glucocorticoids (GCs) are widely used after kidney transplantation. Beyond their immunosuppressive effects, GCs have many adverse effects varying from simple acne to more complex side effects like cushingoid syndrome, diabetes mellitus,[1] and avascular necrosis (AVN).[2]

Avascular necrosis is defined as the death of bone cells[3] and up to 40% of patients receiving long-term GCs might develop AVN.[2] Although the exact mechanism of developing AVN is unknown, metabolic, genetic, and local factors such as changes in local blood flow and increased intravenous pressure have been reported to play essential roles in AVN development.[4-6] Glucocorticoids generally make their effects through the glucocorticoid receptor (GR) which belongs to the nuclear receptor family. The GR is encoded by the *NR3C1* gene that is located on chromosome 5.[7] It has been noticed that not all patients receiving GCs under identical conditions develop AVN; giving rise to this hypothesis that different individuals may have different sensitivity to GCs which is likely attributable to GR polymorphisms. A plenty of polymorphisms were found in the GR gene including *GR-9β* (rs6198), *N363S* (rs6195),

BclI (rs41423247), *ER22/23EK* (rs6189/rs6190), and *Tth111I* (rs10052957). These variants lead to differences in GC sensitivity, cortisol levels, body composition, metabolic features, autoimmune disorders, and cardiovascular diseases.[8]

Weaver *et al.* found a correlation between GR polymorphisms and an increased rate of metabolic syndrome for the first time in 1992.[9] It has been observed in many studies that some single nucleotide polymorphisms (SNPs) including *GR-9β*, *ER22/23EK*, and *Tth111I* are related to decreased levels of sensitivity to exogenous and endogenous GCs, which is a sign of a better metabolic profile.[10,11] Some other polymorphisms (*N363S* and *BclI*) are associated with a hypersensitivity to GCs.

Given the role of GCs in the development of AVN and the diverse effects of GR polymorphisms on GCs function, this study aimed to identify if GR SNPs could be involved in the pathogenesis of post-kidney transplant AVN.

MATERIALS AND METHODS

Ninety-nine kidney transplant recipients who were admitted to the kidney transplantation ward of Imam Reza Hospital, Tabriz, Iran, from July 2013 to September 2019 were enrolled in this retrospective study. Recipients more than 18 years old who receive the same treatment protocol entered this study. Patients with BK virus or cytomegalovirus (CMV) infections were excluded from the study. Sixteen patients with AVN were identified among the patients evaluated during the study period. In our transplant center, higher doses of GCs are administered both before surgery and right after. This regimen consists of a pulse dose of 5 to 10 mg/kg methylprednisolone, given intraoperatively, followed by a daily dose of 1 mg/kg prednisone that is gradually tapered 0.05 to 0.1 mg/kg by one year or less.

Symptomatic AVN cases were diagnosed based on hypodensity obtained from magnetic resonance imaging (MRI) results. The recipient's clinical characteristics, demographic characteristics, donor type (cadaver or live), the type and duration of dialysis before transplantation, post-transplantation body mass index (BMI), immunosuppressive protocol, GC dose, rejection history, family history of AVN, and diabetes mellitus were all recorded. Moreover, serum creatinine, triglyceride, and cholesterol levels were measured at the time of sampling. The results were compared between kidney recipients with and without AVN.

Genetic Study

GC-hypersensitive (*BclI* and *N363*S) and GC-resistant polymorphisms (rs6189/rs6190 and rs6198) were studied using specific primers (Table 1). The collected samples underwent the following PCR steps: 95 °C for 5 minutes, followed by 34 cycles at 95 °C for 30 minutes, 45 seconds at 59 °C and 40 seconds at 72 °C. Finally, the samples were incubated for 72 minutes at 72 °C for the final extension. The amplified fragments were directly sequenced.

Ethical Approval

All the study participants signed the informed consent form. This study was approved by the institutional ethics committee of Tabriz University of Medical Sciences (Ethics code: IR.TBZMED.REC.1400.957).

Statistical Analysis

Variables with a normal distribution were

Table 1. Primer Sequences of the Studied Polymorphisms

Gene Polymorphism	Type	Sequence (5' to 3')
GC- Hypersensitive Polymorphisms		
BclI (rs41423247)	Forward	TCTGGAGGACAGATGTACCA
	Reverse	CTTGCAGGAACATTTGAACGTA
N363S (rs6195)	Forward	TCTGGAGGACAGATGTACCA
	Reverse	CTTGCAGGAACATTTGAACGTA
GC- Resistant Polymorphisms		
ER22/23EK (rs6189/rs6190)	Forward	AATGTGGCATGCTGAATGGG
	Reverse	ACCCAGAAGAAAACTCCAAATCC
A3669G (rs6198)	Forward	TTCCAGTTTCACCTAAGTCTCAT
	Reverse	TGATGTTTCTCCATATTTGGCATTG

Abbreviation: GC, glucocorticoid.

expressed as means ± SD (standard deviations) while variables with a skewed distribution were presented as median [the first quartile (Q1) and the third quartile (Q3)]. Student *t*-test and chi-square test were measured for continuous and categorical variables, respectively. Statistical analysis was performed using SPSS version 22. $P < .05$ was reflected to be statistically significant.

RESULTS

The study participants (n = 99) received kidney transplants between July 2013 and September 2019, 16 of whom had AVN (8 men / 8 women) with a mean age of 43.63 ± 12.91. There was no significant difference between the family history of diabetes mellitus and AVN ($P > .05$). Moreover, no family history of AVN was observed in the study participants. The mean level of total cholesterol was 169.87 ± 40.92 mg/dL and the median value of triglyceride was 138 (106 to 232.5) mg/dL. Furthermore, 85.85% of the study participants (n = 85) received allografts from living donors and there was no significant correlation between the type of allografts and the risk of AVN (Fisher's exact Test, $P > .05$) (Table 2).

Causes of kidney failure in recipients with

Table 2. Baseline Characteristics of Renal Allograft Recipients

Characteristics	Kidney Recipients (n = 99)	Without AVN (n = 83)	With AVN (n = 16)	*P*
Gender (%)				
Man	56 (56.65)	92.3	7.7	> .05
Woman	39 (43.43)	89.7	10.3	
Medication (%)				
Tacrolimus	41 (44.44)	97.6	2.4	< .001
Cyclosporine	47 (51.51)	89.4	10.6	
Sirolimus	4 (4.04)	33.3	66.7	
Steroid Pulse (%)				
Yes	12 (12.12)	80	20	> .05
No	87 (87.87)	92.6	7.4	
Diabetes Mellitus (%)				
Yes	36 (36.36)	96.9	3.1	> .05
No	63 (63.63)	88.1	11.9	
Family History of Diabetes Mellitus (%)				
Yes	22 (22.22)	95	5	> .05
No	75 (75.75)	89.9	10.1	
Anti-thymocyte Globulin (%)				
Yes	16 (16.16)	78.6	21.4	> .05
No	83 (83.83)	93.5	6.5	
Type of Dialysis (%)				
HD	79 (79.79)	93.2	6.8	> .05
PD	20 (20.20)	83.3	16.7	
Type of Donor (%)				
Live	85 (85.85)	92.4	7.6	> .05
Cadaver	14 (14.14)	83.3	16.7	
Acute Rejection, TCMR[a] (%)				
Yes	4 (4.04)	100	0	> .05
No	95 (95.95)	90.9	9.1	
Age, y	44.36 ± 12.01	44.43 ± 12	43.63 ± 12.91	> .05
BMI, kg/m^2	25.6 (23.4 to 29.5)	25.6 (16.7 to 261)	24.65 (21.7 to 36.4)	> .05
On Dialysis Time, mo	18 (10.5 to 18)	18 (0 to 94)	23 (6 to 112)	> .05
Creatinine, mg/dL	1.2 (1.07 to 1.44)	1.2 ± 0.32	1.4 ± 0.35	> .05
Cholesterol, mg/dL	169.87 ± 40.92	168.46 ± 41.45	184.5 ± 33.78	> .05
Triglyceride, mg/dL	138 (106 to 232.5)	138 (51 to 684)	164.5 (125 to 306)	> .05

[a] Most of rejections were T-cell mediated rejection (TCMR).
Quantitative variables were reported as mean ± SD or median (quartile 1 to quartile 3) based on the normality of data distribution.
P value < .05 was considered significant.
Abbreviations: AVN, avascular necrosis; BMI, body mass index; HD, hemodialysis; PD, peritoneal dialysis.

AVN were glomerulonephritis (37.5%), urological problems (25.5%), and unknown (37.5%). There was no difference between AVN and the causes of kidney failure ($P > .05$). About 6.8% of recipients with AVN underwent hemodialysis and 16.7% underwent peritoneal dialysis prior to transplantation, although there was no significant association between AVN and types of dialysis ($P > .05$). About 51.51% of all kidney recipients received cyclosporine, 4.04% of recipients were treated with Sirolimus, and 44.44% received tacrolimus as maintenance immunosuppression, with a statistically significant difference between patients with and without AVN ($P < .001$). No significant association was observed between the AVN and steroid pulse therapy ($P > .05$) and anti-thymocyte globulin (ATG) ($P > .05$) in the participant kidney recipients.

Correlation Between Avascular Osteonecrosis and the Glucocorticoid Receptor Polymorphisms

In carriers of the *A3669G* polymorphism (n = 7), avascular osteonecrosis was negative (100%); no statistically significant correlation was observed between them ($P > .05$). Moreover, none of the nine patients with the *N363S* polymorphism experienced AVN (100%). There was no statistically significant correlation between the *BclI* allele and AVN ($P > .05$). Patients with the *ER22/23EK* polymorphism (rs6189/ rs6190) did not develop AVN and no significant correlation was observed between AVN and the *ER22/23EK* (rs6189/ rs6190) polymorphism ($P > .05$) (Table 3).

DISCUSSION

In this study, sixteen patients with kidney transplants developed AVN, although there was no significant correlation between AVN and GR SNPs. There were no significant differences between patients with or without AVN in terms of duration and types of dialysis, age, family history of AVN and diabetes mellitus, total cholesterol and triglyceride levels, type of donor, and causes of kidney failure.

Osteoporosis and AVN are the two most severe conditions that frequently occur following transplantation, and treatment with corticosteroids is considered the main risk factor.[12,13] Tang *et al.* found that there is a positive association between the incidence of AVN and the cumulative dose of intravenous GCs one year after renal transplantation.[14] Cumulative dose of corticosteroid could increase the incidence of AVN after renal transplantation and a higher rejection rate was observed among patients suffering from AVN.[15] However, other studies did not show any correlation between the corticosteroid dose and the development of AVN.[16,17]

Recent studies suggest that osteonecrosis could

Table 3. The Correlation Between AVN and *NR3C1* Gene Polymorphisms Among Renal Allograft Recipients

Gene polymorphisms	Without AVN (n = 83)	With AVN (n = 16)	*P**
ER22/23EK (rs6189/rs6190) (%)			
Yes	1 (100)	0 (0)	1
No	74 (82.22)	16 (17.88)	
A3669G (rs6198) (%)			
Yes	7 (100)	0 (0)	1
No	68 (80.95)	16 (19.5)	
BclI (rs41423247) (%)			
Yes	9 (100)	0 (0)	.597
No	66 (80.48)	16 (19.52)	
GC- Resistant Polymorphisms (%)			
Yes	8 (100)	0 (0)	1
No	68 (80.95)	16 (19.5)	
GC- Hypersensitive Polymorphisms (%)			
Yes	9 (100)	0 (0)	1
No	66 (80.48)	16 (19.52)	

*The frequencies were represented as number (percentage).
P value < .05 was considered significant.
Abbreviations: AVN, avascular necrosis; GC, glucocorticoid.

develop in some individuals due to genetic variations in the enzymes responsible for the metabolism of GCs and GRs, and protein transportation. There were no significant associations between *N363S*, *Tth111I*, *BclI*, *ER22/23EK*, and *A3669G* SNPs and the development of femoral head AVN in Chinese patients receiving GCs [18,19] Similarly, in the present study, we did not find any association between glucocorticoid receptor gene SNPs and AVN in population of kidney transplant recipients in the Northwest of Iran.

In a study on the Turkish population, Çakmak *et al.* showed that the prevalence of osteoporosis was significantly associated with *BclI* C/G polymorphism of *NR3C*1 gene.[20] In another study, Nowak *et al.* investigated the association of GR polymorphisms and side effects of GCs. They found no statistically significant correlation between *BclI* and *N363S* polymorphisms and GC side effects including arterial hypertension, cardiovascular diseases, diabetes mellitus, osteopenia/osteoporosis, and Cushingoid appearance. In addition, this study showed that patients with *GR-9β* and *ER22/23EK* polymorphisms needed higher doses of GC, therefore, they were probably more susceptible to osteopenia or osteoporosis.[21] In a study by Mottaghi *et al.* on kidney allograft recipients, there was no statistically significant relationship between acute rejection or delayed graft function and GR polymorphisms including *BclI*, *N363S*, and *ER22/23EK*.[22] In the present study, we did not find any significant correlation between GR polymorphisms and AVN in kidney allograft recipients.

The main limitation of this study was the small sample size. Therefore, to generalize the results of this study further investigations are required.

CONCLUSION

Our findings indicate that GC-resistant polymorphisms (*BclI* and N363*S*) and GC hypersensitive polymorphisms (rs6189/rs6190 and rs6198) did not increase the risk of AVN in the renal-transplanted recipients receiving GCs.

CONFLICT OF INTEREST

The authors have not disclosed any conflicts of interest.

ACKNOWLEDGMENT

We would like to appreciate cooperation of the Clinical Research Development unit of Imam Reza General Hospital, Tabriz, Iran in conducting this research.

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Received October 2022
Revised December 2022
Accepted February 2023