Original Article

Association between the Three Polymorphisms of the Glucocorticoid Receptor Gene and the Early Clinical Outcome in Kidney Transplantation Patients

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What's Known

- Glucocorticoids, the mainstay of immunosuppressive therapies, may prevent acute graft rejection and delayed graft function.
- Some patients show glucocorticoid resistance.
- Glucocorticoid receptor gene polymorphisms, namely N363S, Bcl1, and ER22/23EK, are associated with glucocorticoid sensitivity in various populations.

What's New

- N363S, Bcl1, and ER22/23EK polymorphisms had significant associations with acute rejection and delayed graft function after renal transplantation, as the clinical outcomes of glucocorticoid resistance.
- N363S, Bcl1, and ER22/23EK polymorphisms showed no significant associations with the length of hospital stay after transplantation.

Abstract

Background: Glucocorticoids are pivotal components of immunosuppressive regimens in solid organ transplantations. This study aimed to assess the possible association between the ER22/23EK, N363S, and Bcl1 polymorphisms, and short-term clinical outcomes, including acute rejection and delayed graft function (DGF), in kidney transplantation recipients.

Methods: A case-control study was conducted in a 2-year period on adults with transplanted kidneys, comprised of subjects without rejection (n=50, control) and those with documented rejection within one year after transplantation (n=50, case), between April 2017 and September 2018, in Shiraz, Iran. Demographic characteristics and clinical and paraclinical findings were gathered. The genotyping of the ER22/23EK, N363S, and Bcl1 polymorphisms was carried out via polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). The association between the genotypes and DGF as well as rejection types was evaluated using either the chi-square or Fisher exact test. A stepwise logistic regression analysis was conducted to determine the independent factors of acute rejection within the first year after transplantation.

Results: The study population consisted of 64 men and 36 women. The frequency of mutated alleles was 0.32 for G (Bcl1), 0.02 for S (N363S), and 0.065 for A (ER22/23EK). There was no significant association either between the studied polymorphisms and acute rejection or between the Bcl1 (P=0.17), N363S (P=0.99), and ER22/23EK (P=0.99) genotypes and DGF. The length of hospital stay after kidney transplantation was slightly more in N363N and ER22/23EK wild allele carriers. However, this difference was not statistically significant.

Conclusion: Our data suggested no statistically significant association between the genotypes of the studied polymorphisms and early clinical outcomes after kidney transplantation.

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Keywords ● Kidney transplantation ● Receptors ● Glucocorticoids ● Polymorphism ● Genetic

Introduction

End-stage renal disease (ESRD), with its poor prognosis,

usually necessitates renal replacement therapy, including dialysis or renal transplantation.[1] Renal transplantation, rather than dialysis, is now deemed the treatment of choice for ESRD because it improves the quality of life and survival.[2, 3]

The United Network for Organ Sharing (UNOS) recorded 21,167 kidney transplantations in the United States, from January 1, 1998, to August 31, 2019.[4] Iran is the leading country in the Middle East in the field of kidney transplantation. As a case in point, over 2,263 and 2,101 renal transplantations were done in the country in 2018 and 2019, respectively.[5]

Acute rejection is a common and critical complication of renal transplantation.[6] In the United States, one year after surgery, 10% of deceased-donor kidney transplantations in 2009 to 2010 led to at least one episode of acute rejection. Nonetheless, this rate of incidence decreased to 8% in the years 2013 to 2014.[7] According to a retrospective study spanning 37 years (1998–2015) on 2557 kidney transplantation recipients in the Urmia Kidney Transplantation Center in Iran, graft loss was developed in just 86 (3.36%) patients within the first month of surgery.[8]

Delayed graft function (DGF) is an acute kidney injury and a state of renal suboptimal function within the first week after transplantation, which leads to dialysis requirement and may be associated with shorter graft survival. DGF duration implies the number of days before an estimated glomerular filtration rate of 10 mL/min or greater is attained by the transplanted kidney.[9] A research conducted in 2013 in the United Kingdom reported a DGF incidence rate of 24.3% to 70% in brain-death-donor transplantations and 41.2% to 90% in circulatory-death-donor transplantations.[10]

Glucocorticoids, the mainstay of immunosuppressive therapy in both induction and maintenance phases after solid organ transplantation, may affect the immune system through different mechanisms. Altering the expression of some genes, either directly or indirectly, and exerting anti-inflammatory and immunomodulatory effects are a number of functions served by glucocorticoids.[11] Resistance to glucocorticoids is a major challenge with unknown molecular mechanisms.

N363S, a point mutation in exon 2, causes the substitution of AAT by AGT in codon 363, presenting with asparagine change to serine.[12] Bcl1 mutation consists of C-to-G change at 646 nucleotides downstream from exon 2.[13] Two contiguous codons, 22 and 23, show alterations in sequence as a result of ER22/23EK mutation. The change from GAGAGG to GAAAAG causes the substitution of glutamic acid-arginine (E-R) with glutamic acid-lysine (E-K) in produced proteins.[13] Mutation in codon 22 does not lead to a change in amino acid sequence, whereas mutated codon 23 produces a different amino acid. Generally, the N363S and Bcl1 polymorphisms have been observed to increase glucocorticoid sensitivity, while the ER22/23EK polymorphism is often associated with relative resistance.[14, 15]

This study aimed to assess the possible association between the ER22/23EK, N363S, and Bcl1 polymorphisms, and short-term clinical outcomes, including acute rejection and DGF, in kidney transplantation recipients at a referral center in Iran.

Patients and Methods

Study Setting

The present study enrolled 100 patients, who received renal transplantation from living donors or cadavers between April 2017 and September 2018 in Shiraz, Iran. The inclusion criteria were comprised of being at least 18 years old, undergoing first-time renal transplantation, having available data and blood samples, and consenting to participate in the study. The subjects were classified into two groups of control and case, both comprising 50 patients. The former group consisted of patients, who had maintained graft function within the first year after transplantation, with no acute rejection, whereas the latter group encompassed patients, who experienced at least one documented episode of acute rejection (cellular, humoral, or mixed) during the first year following transplantation, regardless of its type and mechanism. The study protocol was approved by the Medical Ethics Committee of Shiraz University of Medical Sciences (IR.SUMS.REC.1397.1032), and written informed consent forms were taken from each patient at the time of study entry. The parents and grandparents of each patient were of Iranian descent.

Data Gathering

Required information from each recruited patient was obtained through a review of medical charts and face-to-face interviews. The data included the recipient's age; gender; etiology of chronic kidney disease; dialysis duration and type prior to transplantation; donor type (deceased vs. living), age, and gender; cold ischemia time; length of hospital stay after transplantation; immunosuppressive regimen in induction and maintenance phases; other co-administered

medications; underlying diseases; and rejection type (only in the case group). DGF was defined as the requirement for dialysis in the first week after transplantation.[10]

Sampling and Preparation

Blood samples were provided from the Sample Bank of the Shiraz Transplant Research Center. Through the use of 2 mL of the Ficoll solution for 10 mL of a whole blood sample, followed by centrifuge, buffy coats were extracted. The buffy coats contained monocytes and lymphocytes. Thus, five-to-ten times higher DNA concentrations were obtained.[16] The buffy coats were stored at -20 ºC.

DNA Extraction

DNA extraction from 200 µL of the buffy coats was carried out using the FavorPrep Blood Genomic Extraction Mini KIT (Favorgen Biotech, Taiwan) in accordance with the manufacturer's protocol. With a NanoDrop, DNA quantity was measured using 5 µL of DNA and 45 µL of double-distilled water (DDW). Given that the optimum DNA quantity for polymerase chain reaction (PCR) was considered 100 ng, individual volumes of extracted DNA for each sample were calculated.

DNA Amplification

The ER22/23EK, N363S, and Bcl1 polymorphisms were evaluated. The genotypes of both groups were determined via the amplification of genomic fragments through PCR. Particular reaction mixtures were prepared for each polymorphism. The mixtures contained 0.5 µL of 10 µM working solutions of forward and reverse primers (all from 100 µM stock solutions) (Eurofins Scientific, Belgium), 2.5 µL PCR 10X buffer (CinnaGen, Iran), 0.75 µL of 10 mM dNTPs (CinnaGen, Iran), 0.75 µL of 50 mM $MgCl_2$ (CinnaGen, Iran), and 0.5 µL Taq DNA polymerase (CinnaGen, Iran). For the evaluation of the N363S polymorphism, 0.5 µL tween was added in order to eliminate the undesirable bands. The primer sequences were as follows:
ER22/23EK forward primer:
5'-GATTCGGAGTTAACTAAAAG-3'
ER22/23EK reverse primer:
5'-ATCCCAGGTCATTTCCCATC-3'
N363S forward primer:
5'-AGTACCTCTGGAGGACAGAT-3'
N363S reverse primer:
5'-GTCCATTCTTAAGAAACAGG-3'
Bcl1forward primer:
5'-TGCTGCCTTATTTGTAAATTCGT-3'
Bcl1 reverse primer:
5'-AAGCTTAACAATTTTGGCCATC-3'

The PCR process, carried out with a thermal cycler (Eppendorf, Germany), involved five minutes of denaturation at 95 ºC, followed by 45 seconds of denaturation at 94 ºC, one minute of annealing at an optimum annealing temperature, and eventually one minute of DNA extension at 72 ºC. The last three steps were repeated for 30 cycles. The annealing temperatures were set at 55.2 ºC, 51.2 ºC, and 55.2 ºC for ER22/23EK, N363S, and Bcl1, respectively. The PCR products were examined randomly via electrophoresis on 1.5% agarose gel and 0.588% buffer.

Bcl1 Genotyping with the Bcl1 Restriction Enzyme

As in polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP), the amplified fragments were digested using the Bcl1 restriction enzyme (New England Biolabs Inc., USA) in order to determine the patients' genotypes. Through the addition of 2.5 µL buffer, 0.5 µL enzyme, and 3 µL DDW to 20 µL PCR product, digestion mixtures were prepared. Digestion was completed in two hours of incubation at 50 ºC.

The restriction enzyme causes the fraction of the 335 bp DNA fragment into two fragments of 117 and 222 bp in the case of wild (CC) genotypes. This enzyme results in three fragments (117, 222, and 335 bp) and just one fragment (undigested, 335 bp) for the CG and GG genotypes, respectively.[17]

N363S Genotyping with the MluCI Restriction Enzyme

For the determination of the N363S genotypes, MluCI (New England Biolabs Inc., USA) was added to relevant PCR products. Each digestion mixture was prepared by adding 2.5 µL buffer, 0.5 µL endonuclease enzyme, and 3 µL DDW to 20 µL PCR product. The mixtures were incubated at 37 ºC for two hours, while inactivation was not required. MluCI is an isoschizomer of Tsp5091; in other words, it recognizes the same sequence.[18] MluCI acts at its restriction site and produces three fragments (19, 95, and 134 bp) if the wild genotype (NN) is present at codon number 363. Enzyme function on the N363S and S363S genotypes results in two bands (95 and 153 bp) and one band (153 bp), respectively.[19, 20]

ER22/23EK Genotyping with the MnlI Restriction Enzyme

The genotypes of the ER22/23EK polymorphism via PCR-RFLP were determined through the use of MnlI endonuclease (New England Biolabs Inc., USA).

Through the addition of 2.5 µL buffer, 0.5 µL enzyme, and 3 µL DDW to 20 µL PCR product, digestion mixtures were prepared subsequent to 40 minutes of incubation at 50 ºC. The enzymatic compartments of the preparations were inactivated via 20 minutes of incubation at 65 ºC, if immediate electrophoresis was not available. Finally, the bands, which indicated fragments from the three restriction processes, were separated on an agarose gel using electrophoresis. They were observed under ultraviolet light.

Statistical Analysis

The statistical analyses were performed using the SPSS software, version 20, (IBM Company, N.Y., U.S.A.). The categorical data were reported in percentage terms. The normality of the distribution of the continuous data was evaluated using the Kolmogorov–Smirnov test. In the case of normal and abnormal distributions, the data were reported as mean±SD, and median+interquartile ranges, respectively. A stepwise logistic regression analysis with odds ratios (ORs) and 95% confidence intervals (CI) was applied to determine the associated factors of acute rejection within the first year after transplantation. In the univariate model, each demographic, clinical, and laboratory datum was entered separately. Those with a P value of less than 0.05 were selected and then entered simultaneously into the final multivariate model. The association between the genotypes and the patients' length of hospital stay after transplantation was evaluated using the independent-sample *t* test. The association between the genotypes and DGF as well as rejection types was evaluated using either the chi-square or Fisher exact test. A P value of less than 0.05 was considered statistically significant for all the above analyses. The Hardy–Weinberg test was applied using the Arlequin software, version 3.11, (Bern, Swiss) to determine the distribution of the alleles. A P value of less than 0.05 in this test indicated no deviation in the distribution of the genotypes and alleles over different generations in a population.

Results

During the study period, 250 kidney transplantation recipients were screened primarily. A total of 150 patients were excluded from the study due to incomplete data and medical charts (n=58), lack of access to blood samples for genetic analysis (n=42), second transplantation (n=35), and spontaneous pancreas and kidney transplantation (n=15). Finally, 100 patients were enrolled in the study.

The mean±SD age of the patients was 41.25±13.52 years. The most common etiology of ESRD was hypertension (39%). Most of the patients (91%) were on hemodialysis before transplantation. The median time of dialysis before transplantation was 12 months. Almost all the patients (97%) received kidney allografts from deceased donors, and 91% of the study population had negative panel-reactive antibodies. The median cold ischemia time of the transplanted kidneys was 3.5 hours. Eighty percent of the patients received anti-thymocyte globulin during the induction phase of immunosuppression early after transplantation. Forty-six percent of the immunosuppressive regimen within the maintenance phase was prednisolone (Nisopred, Iran Hormone Pharmaceutical Company, Iran)+tacrolimus (Prograf, Astellas Pharma Inc., the Netherlands)+mycophenolate mofetil (Cellcept, Zahravi Pharmaceutical Company, Iran).

According to the univariate analysis, the trough level of tacrolimus (OR: 0.76, 95% CI: 0.61 to 0.95; P=0.01) and the administration of anti-thymocyte globulin as induction of immunosuppression (OR: 2.85, 95% CI: 0.99 to 8.17; P=0.05) were selected (table 1). Nevertheless, after these variables were fitted into the final model of logistic regression. Only the trough level of tacrolimus was significantly associated with rejection (OR: 0.76, 95% CI: 0.61 to 0.95; P=0.02) (table 2). In other words, with each 1 ng/dL increase in the tacrolimus trough level, the average risk of allograft rejection decreased by about 24%.

The genotypes of the N363S, Bcl1, and ER22/23EK polymorphisms were evaluated via PCR-RFLP. According to the bands, which appeared on the electrophoresis gel, the genotypes were defined. (Their distribution is reported in table 3.) The Hardy–Weinberg test was conducted; it showed no deviation in the distribution of the genotypes in the subjects regarding the Bcl1 (P=0.60), N363S (P=0.90), and ER22/23EK (P=0.70) polymorphisms. The frequency of mutated alleles was 0.32 for G (Bcl1 polymorphism), 0.02 for S (N363S polymorphism), and 0.06 for A (ER22/23EK polymorphism). None of the subjects was S363S (i.e., homozygous for the mutated allele). The other genotypes of the N363S polymorphism and the different genotypes of the Bcl1 and ER22/23EK polymorphisms had no significant association with acute graft rejection.

The chi-square test showed no statistically significant associations between the Bcl1 (P=0.17), N363S (P=0.99), and ER22/23EK (P=0.99) genotypes and DGF (table 4).

Table 1: Comparisons of different demographic, clinical, and paraclinical data of the subjects between the case and control groups in the univariate model

Variables		Case Group (n=50)	Control Group (n=50)	Univariate	
				P value	OR (95% confidence interval)
Age (year) (mean±SD)		40.7±13.58	41.8±13.56	0.69	1.006 (0.97-1.03)
Sex (%)	Male	32 (64)	32 (64)	-	1
	Female	18 (36)	18 (36)	1	1 (0.44-2.26)
Etiology (%)	Others	32 (64)	29 (58)		1
	Hypertension	18 (36)	21 (42)	0.54	0.78 (0.35-1.74)
Length of pre-transplantation dialysis (month, mean±SD)		15.0±12	17.5±12	0.56	0.99 (0.96-1.02)
Donor type (%)	Cadaver	48 (96)	49 (98)	-	1
	Living	2 (4)	1 (2)	0.56	0.49 (0.04-5.58)
Donor age (year) (mean±SD)		36.38±14.18	36.14±15.19	0.34	1.01 (0.98-1.04)
Donor sex (%)	Male	41 (82)	36 (72)	-	1
	Female	9 (18)	14 (28)	0.24	0.56 (0.22-1.46)
Cold ischemia time (hour), median (interquartile range(		5.13 (4.5)	7.7 (3.0)	0.98	0.99 (0.89-1.11)
Panel reactive antibody (%)	Negative	44 (88)	47 (94)		1
	Positive	6 (12)	3 (6)	0.303	2.14 (0.50-9.07)
Receiving anti-thymocyte globulin as an induction of immunosuppression (%)	Yes	44 (88)	36 (72)	-	1
	No	6 (12)	14 (28)	0.051	2.85 (0.995-8.173)
Anti-thymocyte globulin dose (mg), median (interquartile range)		375 (300)	310 (300)	0.39	0.99 (0.99-1.001)
Immunosuppressive maintenance regimen (%)	Tacrolimus+Mycophenolate+Prednisolone	42 (84)	4 (72)	---	1
	Others	8 (16)	46 (28)	0.152	2.042 (0.77-5.42)
Glomerular filtration rate at hospital admission (mL/min, median+interquartile range)		7.5±0.0	8.4±0.25	0.19	1.06 (0.97-1.16)
Cyclosporine trough level (ng/dL, mean±SD)		711.873±184.1	422.41±149.62	0.37	0.99 (0.98-1.007)
Tacrolimus trough level (ng/dL, mean±SD)		7.45±3.18	5.56±2.12	0.01	0.76 (0.61-0.95)
Delayed graft function (%)	Yes	7 (14)	4 (8)	---	1
	No	43 (86)	46 (92)	-0.343	1.87 (0.51-6.85)

SD: Standard deviation; OR: Odds ratio; A univariate logistic regression analysis was used.

Table 2: Comparisons of receiving anti-thymocyte globulin as induction of immunosuppression and the tacrolimus trough level between the case and control groups in the multivariate model

Variables		Case Group (n=50)	Control Group (n=50)	Multivariate	
				P value	OR (95% confidence interval)
Receiving anti-thymocyte globulin as induction of immunosuppression (%)	Yes	44 (88)	36 (72)		1
	No	6 (12)	14 (28)	0.91	0.90 (0.16-4.97)
Tacrolimus trough level (ng/dL, mean±SD)		7.45±3.18	5.56±2.12	0.02	0.76 (0.61-0.95)

SD: Standard deviation; OR: Odds ratio; A multivariate logistic regression analysis was used.

Table 3: Comparisons of the different genotypes of the Bcl1, N363S, and ER22/23EK polymorphisms between the case and control groups

Polymorphism		Case Group (n=50)	Control Group (n=50)	P value
Bcl1 (%)	CC (Wild type)	20 (40)	24 (48)	0.15
	CG (Heterozygous mutant)	26 (52)	22 (44)	
	GG (Homozygous mutant)	4 (8)	4 (8)	
N363S (%)	N363N (Wild type)	49 (98)	47 (94)	ND
	N363S (Heterozygous mutant)	1 (2)	3 (6)	
	S363S (Homozygous mutant)	0 (0)	0 (0)	
ER22/23EK (%)	GG (Wild type)	45 (90)	46 (92)	0.15
	GA (Heterozygous mutant)	1 (2)	4 (8)	
	AA (Homozygous mutant)	4 (8)	0 (0)	

ND: Not done (because of the inadequate number of heterozygous and homozygous mutant carriers); The chi-square test or the Fisher exact test was used.

Table 4: Comparisons of the genotypes of the Bcl1, N363S, and ER22/23EK polymorphisms between the patients with and without DGF

Polymorphism		Patients with DGF (n=11)	Patients without DGF (n=89)	P value
Bcl1 (%)	CC (wild)	7 (63.36)	37 (41.57)	0.17
	GC/GG (mutated)	4 (36.36)	52 (58.42)	
N363S (%)	N363N (wild)	11 (100)	85 (95.50)	0.99
	N363S/S363S (mutated)	0 (0)	4 (4.49)	
ER22/23EK (%)	GG (wild)	10 (90.90)	81 (91.01)	0.99
	AG/AA (mutated)	1 (9.1)	8 (8.99)	

DGF: Delayed graft function; The chi-square test or the Fisher exact test was used.

Table 5: Comparisons of the Bcl1, N363S, and ER22/23EK genotypes and the length of hospitalization after kidney transplantation

Polymorphism		Length of Hospital Stay, d (mean±SD)	P value
Bcl1	CC (wild)	17.52±9.10	0.68
	GG/GC (mutated)	18.25±8.68	
N363S	N363N (wild)	17.96±8.99	0.87
	N363S/S363S (mutated)	17.25±3.4	
ER22/23EK	GG (wild)	17.97±8.82	0.89
	AG/AA (mutated)	17.56±9.53	

SD: Standard deviation; The independent-sample *t* test was used.

As is shown in table 5, the length of hospitalization after kidney transplantation was comparable between the wild and mutant allele carriers of the studied polymorphisms.

Discussion

In the present study, the N363S, Bcl1, and ER22/23EK polymorphisms of the glucocorticoid receptor had no significant effect either on acute rejection or DGF, as the clinical outcomes of glucocorticoid resistance, or on the length of hospitalization following transplantation. To the best of our knowledge, this is the first study to evaluate the effects of these polymorphisms on early clinical outcomes in renal transplantation recipients.

Glucocorticoid resistance has been reported in some patients with chronic diseases, chronic stress, cardiovascular risk factors, and major depressive disorders, as well as elderly patients.[21] It has been observed that ER22/23EK mutation in exon 2 and A3669G in exon 9β can enhance the expression of the β subtype of the receptor, rather than α, leading to glucocorticoid resistance.[21, 22] Lower cortisol levels in Bcl1 carriers after the dexamethasone suppression test led to the conclusion that it might increase glucocorticoid sensitivity due to its linkage to functional polymorphisms. It is supposed that this polymorphism plays a role in obesity with visceral adiposity, depression, inflammatory, and autoimmune diseases.[13] Men aged between 55 and 67 years with the GG genotypes of the Bcl1 polymorphism showed significantly lower bone mineral density in the femoral neck and the trochanter. However, the finding was not significant anymore after the correction for the body mass index.[23] The N363S polymorphism seems to enhance the receptor capacity and glucocorticoid sensitivity by the poly-phosphorylation of serine and threonine in the protein N-terminal.[24] N363S-mutated patients, who carry the serine allele, may have an increased body mass index and cortisol sensitivity in the dexamethasone suppression test.[20] In some studies, higher levels of cholesterol were observed among patients with N363S variant carriers.[13]

The carriers of the ER22/23EK polymorphism experienced less tendency to adrenal insufficiency, and homozygous and heterozygous Bcl1 genotype carriers developed a longer duration of this and more frequent adverse effects after the corticosteroid induction phase, respectively.[25] On the other hand, some studies have pointed out the age-[26] and gender-specific effects of the ER22/23EK polymorphism.[13] Assessing this issue was not statistically feasible in our study due to the low number of mutant allele carriers of the ER22/23EK polymorphism. This polymorphism may advocate the translation initiation shift from methionine 27 to methionine one and, thus, an increase in glucocorticoid receptor-A production.[26] Furthermore, no association was found between glucocorticoid-induced avascular osteonecrosis of the femoral head and the N363S and ER22/23EK polymorphisms.[27]

Currently, there are only three published articles and one congress abstract considering

the different clinical aspects of NR3C1 polymorphisms in kidney transplantation. In this regard, among 65 Japanese renal transplantation patients receiving prednisolone (between 2.5 and 15 mg/d) as a part of their maintenance immunosuppressive regimen, a significant difference was seen between the CC and GG genotypes of the Bcl1 polymorphisms concerning the prednisolone area under the curve for 12 hours (AUC_{0-12}). Prednisolone AUC was significantly higher in Bcl1 G carriers one year after transplantation. Still, there was no significant difference between half-life or time to reach the maximum plasma concentration of prednisolone and the different genotypes of the Bcl1 polymorphisms. The authors suggested that prednisolone might transactivate the CYP3A4 promoter via the Bcl1 polymorphism, which could enhance its own intestinal clearance and decrease its plasma level.[28] In a study on 126 Japanese kidney transplantation recipients receiving a tacrolimus-based immunosuppressive regimen, it was demonstrated that the risk of dyslipidemia was significantly higher in G-allele carriers of the Bcl1 polymorphism in comparison with the CC genotype.[29] In contrast, there was no significant association between two polymorphisms of NR3C1 (JST006606 and JST032069) and the risk of osteonecrosis secondary to corticosteroids in the Japanese kidney transplantation recipients.[30] Finally, Michetti and colleagues in a congress abstract reported that there were no significant associations between the different genotypes of Bcl1, as well as the N363S polymorphisms, and new-onset diabetes after transplantation among 96 Italian renal transplantation recipients.[31]

In the current study, none of the patients had the homozygous mutated N363S genotype, and the frequency of the mutated allele was 0.02. Interestingly, another Iranian research reported the same frequency of the mutant allele of this polymorphism in pediatric patients with acute lymphoblastic leukemia (ALL).[32] This value was 0.06 in patients with major depressive disorders in Iran.[33] In contrast, a study on a sample of the Turkish population, divided into an ALL group and a control group, revealed that there was no N363S mutation in the subjects.[34] Even some studies declared that the N363S and ER22/23EK polymorphisms were not observed in a sample of the Asian population.[35]

The frequency of the Bcl1 mutant allele in our study was 0.32. Similarly, the frequency of the G allele was 0.215 in healthy subjects and 0.33 in patients with major depressive disorders in a study on a sample of the Iranian population.[33] Namazi and colleagues reported a frequency rate of 0.195 for the Bcl1 mutant allele among a sample of Iranian pediatrics with ALL.[32] This rate was 0.202 in a study on a sample of Japanese kidney transplantation recipients.[29] The Bcl1 and N363S polymorphisms may have synergistic effects on blood pressure.[36] Since the N363S mutated allele had a low frequency in our subjects, evaluating the probable synergistic effects of the Bcl1 and N363S polymorphisms on the risk of relapse or DGF was not statistically feasible.

We found that the ER22/23EK mutant allele had a frequency rate of 0.065 in this research. In addition, all the patients in the case group were homozygous carriers of the mutant allele. The frequency was much higher than that reported in another study in the Middle East (Turkish population), which reported that patients and controls had the frequency rate of 0.01 and 0.02 of the mutated allele, respectively.[37] The frequency of the mutant allele of this polymorphism in a sample of Iranian pediatric patients with ALL was 0.005.[32] Nouraei and colleagues reported that the frequency rate of the mutant allele of the ER22/23EK polymorphism in their group of Iranian patients with major depressive disorders under fluoxetine treatment was 0.67.[38]

Among the different studied demographic, clinical, and paraclinical characteristics, only the tacrolimus trough level, which correlates well with its total body exposure, was significantly associated with the risk of acute rejection in that with each 1 ng/dL increase in the trough level of tacrolimus, the average risk of allograft rejection dropped by about 24%. According to the literature, there is a relationship between the concentration, efficacy, and toxicity of tacrolimus. Subtherapeutic concentrations are associated with an increased risk of allograft rejection, and supratherapeutic levels can increase the risk of its adverse effects (e.g., renal dysfunction, neurotoxicity, and hypertension).[39]

The major drawback of the current study is its relatively small sample size due to both financial and time constraints, which could partially justify the lack of a significant association between the studied polymorphisms and the clinical outcome indices. The low frequency of the mutated alleles of the studied polymorphisms, especially N363S and ER22/23EK, can give further prominence to this issue. However, the statistical power, calculated at the end of the study, was 93.11%. We recommend that the effects of these polymorphisms on glucocorticoid resistance be evaluated in a larger sample with the same methodology. We also suggest investigating exon and intron sequences responsible and encoding genes involved other than NRC31. It may be prudent

to assess the effect of these polymorphisms on prednisolone pharmacokinetic indices such as AUC, C_{max}, and T_{max}. Furthermore, the possible effects of some other confounding factors such as donor-specific antibodies and underlying diseases other than those causing ESRD could also be considered in future studies.

Conclusion

Our data indicated no statistically significant association between the genotypes of the Bcl1, N363S, and ER22/23EK polymorphisms of NR3C1 and early clinical outcomes after kidney transplantation, including acute rejection, DGF, and duration of postoperative hospital stay. Among the studied variables, only the tacrolimus trough level was significantly associated with the risk of acute rejection.

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