Correlation between *ICAM1* and *VCAM1* gene polymorphisms and histopathological changes in kidney allograft biopsies

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Abstract

Introduction: The immunoglobulin-like molecules intercellular adhesion molecule-1 (ICAM-1) and vascular adhesion molecule-1 (VCAM-1) are responsible for endothelial cell-leukocyte adhesion followed by transmigration of leukocytes through the endothelial cell lining. The aim of this study was to examine the correlation between polymorphisms in *ICAM1* and *VCAM1* genes and histopathological changes in transplanted kidney biopsies.

Material and methods: The study enrolled 82 Caucasian renal transplant recipients (48 males, 34 females). Genotyping of the rs5498 *ICAM1* and the rs1041163 and rs3170794 *VCAM1* gene polymorphisms was performed using real-time polymerase chain reaction (PCR). Biopsies were performed in 82 patients and were reviewed by a renal pathologist and the Banff working classification criteria were used.

Results: There were no significant associations between *VCAM* gene polymorphisms and histopathological changes in kidney allograft biopsies. *ICAM1* gene polymorphism was associated with the grade of interstitial fibrosis. Interstitial fibrosis was more severe among individuals with the G allele than those with the A allele (AA vs. GG+AG, $p = 0.017$). There were no statistically significant associations between *ICAM1* gene polymorphism and other histopathological changes in kidney allograft biopsies.

Conclusions: The results of our study suggest that rs5498 *ICAM1* gene polymorphism is associated with the grade of interstitial fibrosis in kidney recipients and the changes are more severe in patients with the G allele.

Key words: biopsy, histopathology, intercellular adhesion molecule-1, kidney allograft, polymorphism, vascular adhesion molecule-1.

Introduction

From the beginning of the 1970s kidney biopsy became the gold standard for diagnosis of its disorders. This procedure allows one not only to evaluate the morphological structure of transplanted kidney, but also to identify the ongoing processes in renal tissue and their intensification. Therefore biopsy is performed in kidney graft recipients with delayed graft function, to confirm acute rejection and to assess the degree of chronic rejec-

tion. Moreover, allograft biopsy is performed according to the protocol for monitoring the status of the properly functioning transplanted kidney. In 1991 in Banff (Canada) a group of scientists determined the criteria for evaluating renal kidney biopsies, allowing for standardization of the rejection process. Over the next years, during subsequent meetings, the criteria were revised, taking into account the latest discoveries in the field of clinical immunology and transplantology. Currently the Banff working classification is standard for kidney transplant pathology assessment [1-4].

Transplantation is the preferred method of treatment in established kidney failure because it prolongs the life of the patient and improves quality of life. The pathogenesis of graft rejection includes allograft tissue and endothelial injury, which are the result of immune response activation [5-7]. Cytokines and reactive oxygen species released during reperfusion increase the expression of adhesion molecules on the surface of endothelial cells [8-11]. Intercellular adhesion molecule-1 (ICAM-1, CD54, *ICAM1* gene located on 19th chromosome, locus 19p13.3-p13.2) is an immunoglobulin expressed on endothelial cells, smooth muscle cells, macrophages and activated lymphocytes. Intercellular adhesion molecule-1 plays a crucial role in initiating the immunological response through the adhesion of circulating leukocytes to the blood vessel wall and transendothelial migration to tissue [12]. Vascular adhesion molecule-1 (VCAM-1, CD106, *VCAM1* gene located on primer of the 1st chromosome, *locus* 1p31-32) is present on the endothelium and antigen-presenting cells. This protein is an endothelial receptor for VLA-4 of the β1 subfamily of integrins and for integrin α4β7. Due to the interaction of these proteins, the T-cell response to alloantigens is initiated. It is significant for the early development of both acute and chronic rejection of the transplanted kidney [13]. This was confirmed in experimental studies evaluating VCAM-1 concentrations in rats undergoing renal graft chronic rejection. There was a correlation between the level of this adhesion molecule and histopathological changes in the transplanted organs. In addition, effective reduction of VCAM-1 expression in kidney allografts was linked to the reduction of the prevalence of chronic rejection [14].

Some reports suggest that ICAM-1 and VCAM-1 synthesis has a genetic background [15]. There are several polymorphisms among *ICAM1* and *VCAM1* genes, which are associated with changes in expression of these molecules and therefore may affect the function of the allograft and immune response after kidney transplantation. Previous studies indicated that polymorphisms rs5498:A>G in exon 6 of the *ICAM1* gene, and rs3170794:T>C and rs1041163:T>C in the *VCAM1* gene promoter correlated with ICAM1 and VCAM1 levels, as well as with various diseases [16-19].

The aim of this study was to examine the association between polymorphisms rs5498:A>G in exon 6 of the *ICAM1* gene, and rs3170794:T>C and rs1041163: T>C in the *VCAM1* gene promoter and histopathological changes in transplanted kidney biopsies.

Material and methods

The study enrolled 82 consecutive Caucasian renal transplant recipients (48 males, 34 females, mean age: 47.63 ±12.96 years) in whom a kidney biopsy was performed because of impaired graft function. The PAJUNK DeltaCut biopsy system was used. All biopsies were reviewed by a renal pathologist and the Banff working classification criteria were used [3]. All patients received the standard immunosuppressive protocol with triple drug therapy including a calcineurin inhibitor (cyclosporine A or tacrolimus), mycophenolate mofetil and steroids. The local ethics committee of the Pomeranian Medical University in Szczecin, Poland approved the protocol of the study.

Genotyping

Genomic DNA was extracted (precipitation with trimethyl ammonium bromide salts) from leukocytes contained in 450 µl whole blood samples with ethylenediaminetetraacetic acid (EDTA) as an anticoagulant, using a non-organic and non-enzymatic extraction method. DNA was then precipitated in 99.5% ethanol and dissolved in distilled water. The range of DNA concentrations varied from 100 ng/µl to 500 ng/µl. We standardized DNA with a Nanodrop ND-1000 spectrophotometer and equalized DNA concentrations to 20 ng/µl. This material was used as a matrix for the amplification in real time polymerase chain reaction (RT-PCR). We analysed three single nucleotide polymorphisms (SNPs): rs5498:A>G in exon 6 of the ICAM1 gene, and rs3170794:T>C and rs1041163:T>C in the *VCAM1* gene promoter with *TaqMan* probes in a 7500 Fast Real-Time PCR System machine (Applied Biosystems, USA). The substrates for the reaction were as follows: TaqMan® Genotyping Master Mix (Applied Biosystems, USA), two starters (900 µM), and two *TaqMan* probes with 5' reporter pigment and 3' non-fluorescent quencher

Table I. Clinical characteristics of patients

Age years	47.55 ±12.96
Sex [M/F]	48/34
Delayed graft function	31 patients
Time after transplantation [months]	31 ±9
Creatinine [mg/dl]	1.76 ±0.58
Cyclosporine A [ng/ml]	150-200
Tacrolimus [ng/ml]	7-12

Table II. Kidney allograft histopathological changes (i, t, v, ah, ci, cg, ct, cv, mm) in association with rs1041163 *VCAM1* gene polymorphism

Biopsy parameter and genotypes	Grade 0		Grade 1		Grade 2		Grade 3		Mean ± SD	M-W test *p* for comparison TT vs. CC+TC
	n	%	*n*	%	*n*	%	*n*	%		
(i) *n* = 72										
TT	16	32.00	15	30.00	10	20.00	9	18.00	1.24 ±1.10	0.58
TC	3	15.00	9	45.00	4	20.00	4	20.00	1.36 ±1.00	
CC	1	50.00	1	50.00	0	0.00	0	0.00		
(t) *n* = 64										
TT	13	30.23	18	41.86	8	18.60	4	9.30	1.07 ±0.94	0.29
TC	4	21.05	8	42.11	5	26.32	2	10.53	1.33 ±0.97	
CC	0	0.00	1	50.00	0	0.00	1	50.00		
(v) *n* = 57										
TT	36	92.31	2	5.13	0	0.00	1	2.56	0.13 ±0.52	0.67
TC	14	87.50	1	6.25	1	6.25	0	0.00	0.17 ±0.51	
CC	2	100.0	0	0.00	0	0.00	0	0.00		
(ah) *n* = 68										
TT	16	34.78	23	50.00	6	13.04	1	2.17	0.83 ±0.74	0.24
TC	6	30.00	8	40.00	6	30.00	0	0.00	1.05 ±0.79	
CC	0	0.00	1	50.00	1	50.00	0	0.00		
(ci) *n* = 68										
TT	25	54.35	14	30.43	5	10.87	2	4.35	0.65 ±0.85	0.44
TC	10	50.00	5	25.00	4	20.00	1	5.00	0.82 ±0.91	
CC	0	0.00	2	100.0	0	0.00	0	0.00		
(cg) *n* = 55										
TT	37	90.24	3	7.32	0	0.00	1	2.44	0.15 ±0.53	0.28
TC	10	83.33	2	16.67	0	0.00	0	0.00	0.21±0.43	
CC	1	50.00	1	50.00	0	0.00	0	0.00		
(ct) *n* = 68										
TT	8	17.39	24	52.17	10	21.74	4	8.70	1.22 ±0.84	0.99
TC	2	10.00	13	65.00	3	15.00	2	10.00	1.23 ±0.75	
CC	0	0.00	2	100.0	0	0.00	0	0.00		
(cv) *n* = 51										
TT	24	66.67	3	8.33	4	11.11	5	13.89	0.72 ±1.14	0.25
TC	6	46.15	3	23.08	1	7.69	3	23.08	1.07±1.22	
CC	1	50.00	0	0.00	1	50.00	0	0.00		
(mm) *n* = 60										
TT	25	59.52	10	23.81	1	2.38	6	14.29	0.71 ±1.07	0.34
TC	7	43.75	4	25.00	4	25.00	1	6.25	0.89 ±0.96	
CC	1	50.00	1	50.00	0	0.00	0	0.00		

p value calculated with the Mann-Whitney (M-W) test; i – interstitial infiltration, t – tubulitis, v – intimal arteritis, ah – arteriolar hyaline thickening, ci – interstitial fibrosis, cg – glomerulopathy, ct – tubular atrophy, cv – fibrous intimal thickening, mm – mesangial matrix increase

(200 µM). The probe specific for the more frequent gene allele was marked with 6-FAM reporter pigment and the probe specific for the less frequent gene allele was marked with VIC pigment. For the rs5498: A>G and rs3170794:T>C SNPs analysis two ready-made assays were used: No. C__8726337_40 and C__30900705_10. For the rs1041163:T>C SNP analysis we prepared a new assay with the pair of primers 5'-GAC CTC TGG GTT ACT TGT TTA TAA GCT-3', 5'-GAGATGCTGTTCTAGGGTGTGG-3' and the following probes: FAM – TAG GGA TCA GAA AAA TTG A, VIC – TAG GGA TCA GAG AAA TTG A. The reactions were

Table III. Kidney allograft histopathological changes (i, t, v, ah, ci, cg, ct, cv, mm) in association with rs3170794 *VCAM1* gene polymorphism

Biopsy parameter and genotypes	Grade 0		Grade 1		Grade 2		Grade 3		Mean ± SD	M-W test *p* for comparison TC vs. TT
	n	%	*n*	%	*n*	%	*n*	%		
(i) *n* = 72										
TT	19	27.54	24	34.78	14	20.29	12	17.39	1.28 ±1.06	1.00
TC	1	33.33	1	33.33	0	0.00	1	33.33	1.33 ±1.53	
(t) *n* = 64										
TT	17	27.87	25	40.98	13	21.31	6	9.84	1.13 ±0.94	0.39
TC	0	0.00	2	66.67	0	0.00	1	33.33	1.67 ±1.15	
(v) *n* = 57										
TT	51	91.07	3	5.36	1	1.79	1	1.79	0.14 ±0.52	1.00
TC	1	100.0	0	0.00	0	0.00	0	0.00	–	
(ah) *n* = 68										
TT	21	31.82	31	46.97	13	19.70	1	1.52	0.91 ±0.76	0.46
TC	1	50.00	1	50.00	0	0.00	0	0.00	0.50 ±0.71	
(ci) *n* = 68										
TT	33	50.77	20	30.77	9	13.85	3	4.62	0.72 ±0.88	0.48
TC	2	66.67	1	33.33	0	0.00	0	0.00	0.33 ±0.58	
(cg) *n* = 55										
TT	47	87.04	6	11.11	0	0.00	1	1.85	0.17 ±0.50	1.00
TC	1	100.0	0	0.00	0	0.00	0	0.00	–	
(ct) *n* = 68										
TT	9	13.85	37	56.92	13	20.00	6	9.23	1.25 ±0.81	0.20
TC	1	33.33	2	66.67	0	0.00	0	0.00	0.67 ±0.58	
(cv) *n* = 51										
TT	29	60.42	6	12.50	6	12.50	7	14.58	0.81 ±1.14	0.95
TC	2	66.67	0	0.00	0	0.00	1	33.33	1.00 ±1.73	
(mm) *n* = 60										
TT	32	55.17	14	24.14	5	8.62	7	12.07	0.78 ±1.04	0.89
TC	1	50.00	1	50.00	0	0.00	0	0.00	0.50 ±0.71	

p value calculated with the Mann-Whitney (M-W) test; i – interstitial infiltration, t – tubulitis, v – intimal arteritis, ah – arteriolar hyaline thickening, ci – interstitial fibrosis, cg – glomerulopathy, ct – tubular atrophy, cv – fibrous intimal thickening, mm – mesangial matrix increase

performed at 95°C for 10 min due to AmpliTaq Gold polymerase activation, then 40 cycles of 15 s at 92°C and 60 s at 60°C. The genotypes were determined automatically through the comparison of 6-FAM and VIC pigment fluorescence. Then the results were verified in the review of the amplification plot curves.

Statistical analysis

The association of histopathological changes in renal grafts with genotypes was analysed with Mann-Whitney test. Value of p below 0.05 was considered as statistically significant.

Results

The clinical characteristic of patients are presented in Table I. As shown in Tables II and III, there were no significant associations between *VCAM* gene polymorphisms and histopathological changes in kidney allograft biopsies.

With regard to the *ICAM1* gene polymorphism, there was a significant association with the grade of interstitial fibrosis. Interstitial fibrosis was more severe among individuals with the G allele than those with the A allele (AA vs GG+AG, p = 0.017) (Table IV). Moreover, mesangial matrix increase was more enhanced among individuals with the *ICAM1* G allele than among those with the A allele (AA vs. GG+AG, p = 0.06) (Table II). These differences were on the border of statistical significance. There were no statistically significant associations between *ICAM1* gene polymorphism and other histopathological changes in kidney allograft biopsies.

Additionally we analysed the creatinine concentrations in association with studied polymorphisms.

Table IV. Kidney allograft histopathological changes (i, t, v, ah, ci, cg, ct, cv, mm) in association with rs5498 *ICAM1* gene polymorphism

Biopsy parameter and genotypes	Grade 0		Grade 1		Grade 2		Grade 3		Mean ± SD	M-W test *p* for comparison AA vs. GG+AG
	n	%	*n*	%	*n*	%	*n*	%		
(i) *n* = 72										
AA	8	33.33	8	33.33	4	16.67	4	16.67	1.17 ±1.09	
AG	9	27.27	11	33.33	7	21.21	6	18.18	1.30 ±1.07	0.50
GG	3	20.00	6	40.00	3	20.00	3	20.00	1.40 ±1.06	
(t) *n* = 64										
AA	6	27.27	9	40.91	4	18.18	3	13.64	1.18 ±1.00	
AG	9	33.33	10	37.04	7	25.93	1	3.70	1.00 ±0.88	0.94
GG	2	13.33	8	53.33	2	13.33	3	20.00	1.40 ±0.99	
(v) *n* = 57										
AA	18	90.00	1	5.00	0	0.00	1	5.00	0.20 ±0.70	
AG	25	96.15	0	0.00	1	3.85	0	0.00	0.08 ±0.39	0.79
GG	9	81.82	2	18.18	0	0.00	0	0.00	0.18 ±0.40	
(ah) *n* = 68										
AA	9	40.91	10	45.45	3	13.64	0	0.00	0.73 ±0.70	
AG	7	20.59	17	50.00	10	29.41	0	0.00	1.09 ±0.71	0.21
GG	6	50.00	5	41.67	0	0.00	1	8.33	0.67 ±0.89	
(ci) *n* = 68										
AA	16	69.57	6	26.09	1	4.35	0	0.00	0.35 ±0.57	
AG	12	37.50	13	40.63	5	15.63	2	6.25	0.91 ±0.89	0.017
GG	7	53.85	2	15.38	3	23.08	1	7.69	0.85 ±1.07	
(cg) *n* = 55										
AA	15	100.0	0	0.00	0	0.00	0	0.00	0.00 ±0.00	
AG	23	79.31	5	17.24	0	0.00	1	3.45	0.28 ±0.65	0.086
GG	10	90.91	1	9.09	0	0.00	0	0.00	0.09 ±0.30	
(ct) *n* = 68										
AA	4	17.39	15	65.22	4	17.39	0	0.00	1.00 ±0.60	
AG	2	6.06	20	60.61	6	18.18	5	15.15	1.42 ±0.83	0.16
GG	4	33.33	4	33.33	3	25.00	1	8.33	1.08 ±1.00	
(cv) *n* = 51										
AA	8	53.33	1	6.67	3	20.00	3	20.00	1.07 ±1.28	
AG	18	69.23	3	11.54	3	11.54	2	7.69	0.58 ±0.99	0.39
GG	5	50.00	2	20.00	0	0.00	3	30.00	1.10 ±1.37	
(mm) *n* = 60										
AA	13	76.47	2	11.76	0	0.00	2	11.76	0.47 ±1.00	
AG	13	41.49	12	38.71	4	12.90	2	6.45	0.84 ±0.90	0.065
GG	7	58.33	1	8.33	1	8.33	3	25.00	1.00 ±1.35	

p value calculated with the Mann-Whitney (M-W) test; i – interstitial infiltration, t – tubulitis, v – intimal arteritis, ah – arteriolar hyaline thickening, ci – interstitial fibrosis, cg – glomerulopathy, ct – tubular atrophy, cv – fibrous intimal thickening, mm – mesangial matrix increase

ICAM1 rs5498 GG genotype was associated with increased creatinine concentrations (1.96 ±0.56 mg/dl) in comparison with AG (1.72 ±0.66 mg/dl) and AA genotypes (1.69 ±0.51 mg/dl) (AA+AG vs. GG, $p = 0.004$). There were no statistically significant associations between *VCAM* gene polymorphisms and creatinine concentrations.

Discussion

In our study we examined the association between polymorphisms in *ICAM1* and *VCAM1* genes and histopathological changes in transplanted kidney biopsies. We observed a statistically significant association between the grade of interstitial fibrosis

and rs5498 *ICAM1* gene polymorphism. Histopathological lesions were more severe among G allele carriers than among those with the A allele.

Intercellular adhesion molecule-1 is an adhesion receptor and is found on leukocytes and endothelial cells [20]. Increased expression of ICAM-1 has been found in biopsies of renal allograft with rejection [21]. Previous reports have shown the presence of VCAM-1 on parietal cells lining Bowman's capsule [22, 23]. In kidney allografts with rejection the increased expression of VCAM-1 was observed in muscular artery endothelium and in smooth muscle cells. It has been postulated that the upregulation of both ICAM-1 and VCAM-1 could facilitate the destruction of capillaries and tubular cells in kidney rejection [24, 25].

Adhesion molecules may mediate the infiltration process, but may also serve as a costimulatory signal for T-cell activation by antigen-presenting cells. Previous studies examined the expression of adhesion molecules in inflammatory renal diseases and revealed increased expression of adhesion molecules in biopsies with chronic histological damage. Increased expression of ICAM-1 and VCAM-1 was observed in different renal diseases and correlated with structural tubular damage and interstitial fibrosis [26].

In recent years, the frequency of acute rejection episodes after kidney transplantation has been decreasing, but interstitial fibrosis and tubular atrophy are still the main cause of kidney function loss. Infiltration of inflammatory cells into the allograft interstitium is the basis of the alloimmune response that leads to injury and subsequent interstitial fibrosis and chronic allograft failure [27]. Clinical factors that are correlated with interstitial fibrosis include older recipient age, greater human leukocyte antigen (HLA) mismatch and the use of calcineurin inhibitors [28, 29]. Kwiatkowski *et al*. described changes in allograft biopsies which where associated with preservation method during cold ischaemia time. Interstitial fibrosis and tubular atrophy were diagnosed more often in hypothermically stored allografts. Moreover, there was a higher frequency of interstitial fibrosis [30]. Heilman *et al*. analysed histopathological changes in allografts among patients with the steroid withdrawal protocol. Biopsies were performed during the reperfusion, and in the 1st, 4th and 12th month after transplantation. Interstitial fibrosis was diagnosed in 19% of kidney recipients 1 year after transplantation. It was shown that increased creatinine serum concentrations and recipient's age were the risk factors of interstitial fibrosis [31]. Servais *et al*. examined the effect of cyclosporine to sirolimus conversion on interstitial fibrosis. Biopsy results revealed no differences between cyclosporine and sirolimus treated patients in the percentage of interstitial fibrosis [32]. In another study the authors evaluated the genes associated with the onset of interstitial fibrosis and tubular atrophy. The 3-month biopsies of the kidneys with interstitial fibrosis and tubular atrophy showed overexpression of several genes that are important in T- and B-cell activation and the immune response and are involved in profibrotic processes. Furthermore, several genes with transporter and metabolic functions were underrepresented in these biopsies. The authors conclude that interstitial fibrosis and tubular atrophy occur already before they are detectable with conventional histopathological examination of renal allografts. Thus, microarray technology applied to protocol biopsies provides insight into the pathophysiology of these processes and might allow for a more timely therapeutic intervention [33].

In conclusion, the results of our study suggest that rs5498 *ICAM1* gene polymorphism is associated with the grade of interstitial fibrosis in kidney allograft recipients and the changes are more severe in patients with the G allele.

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