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Interleukin-17 gene polymorphisms in patients with post-transplant diabetes mellitus

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Abstract. – OBJECTIVE: Post-transplant diabetes mellitus (PTDM) is a common complication after organ transplantation which leads to impaired graft function. Various factors may increase the risk of the development of PTDM. It has been reported that cytokines and genetic variations of inflammatory cytokines were associated with glucose homeostasis or diabetes. The pro-inflammatory cytokine IL-17, which is produced by T-helper 17 (Th17) cells, has been reported to be involved in the glucose metabolism and pathogenesis of diabetes via the induction of low-grade inflammation. The aim of this study was to examine the association between polymorphisms in the IL17A (rs2275913) and IL17F (rs11465553, rs2397084, rs763780) genes with post-transplant diabetes mellitus.

PATIENTS AND METHODS: The study included 169 patients of Caucasian origin who received kidney transplants. For the purpose of the study, the patients were subdivided into two subgroups: patients with PTDM (n = 23) and patients without PTDM (n = 146). Standard immunosuppression consisted of tacrolimus, mycophenolate mofetil, and steroids.

RESULTS: Post-transplant diabetes was diagnosed in 10.97% of the carriers of the IL17F rs763780 TT genotype and 42.86% of those with the TC genotype (TC vs TT: OR = 6.09, 95% CI 1.89-19.66, p = 0.0048). In multivariate analysis, older recipient age and the presence of the TC genotype were independent significant predictors of higher risk of post-transplant diabetes.

CONCLUSIONS: The results of this study suggest an association between the IL17F rs763780 polymorphism and post-transplant diabetes.

Key Words:
IL-17, Gene, Polymorphism, Renal transplant, Diabetes mellitus.

Introduction

Post-transplant diabetes mellitus (PTDM) is a metabolic disorder that develops in response to a relative insulin deficiency in patients after organ transplantation[1]. Transplant recipients are at a particularly high risk of developing PTDM as a consequence of factors in addition to those that affect the general population, including the immunosuppressive agents used in transplant management protocols. PTDM is not a separate entity, but a symptom of the metabolic disorder, which is induced by immunosuppression.

Several studies confirmed, that calcineurin inhibitors and glucosteroids are associated with the development of PTDM. Both tacrolimus and CsA reduce insulin synthesis by inhibiting insulin gene transcription[2,3]. There is also increasing evidence that persistent low-grade inflammation could result in insulin resistance in target tissues, which plays a major role in the pathogenesis and progression of type 2 diabetes mellitus (T2DM) and subsequent cardiovascular disease. Studies have demonstrated a link between the chronic activation of pro-inflammatory signalling pathways and decreased insulin sensivity[4]. It has been reported that cytokines and genetic variations of inflammatory cytokines were associated with glucose homeostasis or diabetes[5]. The pro-inflammatory cytokine, IL-17 is produced by T-helper 17 (Th17) cells[6,7]. This cytokine is involved in the pathogenesis of diabetes and glucose metabolism via the induction of low-grade inflammation[8].

In the *IL17* gene, several polymorphisms have been detected. Some of them may regulate the expression of messenger ribonucleic acid (mRNA) and IL-17 synthesis[9-11]. The aim of this study was to examine the association between polymorphisms in the *IL17A* and *IL17F* genes and post-transplant diabetes mellitus.

Patients and Methods

The study included 169 patients of Caucasian origin who received kidney transplants. Partici-

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pants were included consecutively from those that underwent renal transplantation in the Department of Nephrology, Transplantology and Internal Medicine at the Pomeranian Medical University in Szczecin, Poland. For the purpose of this study, the patients were subdivided into two subgroups: patients with PTDM (n = 23) and patients without PTDM (n = 146). Patients who had a diagnosis of diabetes mellitus prior to transplant (either as the cause of kidney disease or comorbidity) and patients with graft failure or death within one month post-transplant were excluded. Patients with haemoglobin A1c continuously over 6.5%, fasting blood glucose ≥ 7.0 mmol/l, or requiring treatment with oral hypoglycaemic agents or insulin for more than 3 months after transplantation were diagnosed as having PTDM[12].

Standard immunosuppression consisted of tacrolimus, mycophenolate mofetil, and steroids. Tacrolimus was initiated at 0.1 mg/kg, with doses adjusted to maintain serum levels between 10 and 12 ng/ml in the first month after transplantation and then between 8 and 10 ng/ml. Mycophenolate mofetil was given in doses of 2 g per day, while prednisolone was given in doses of 10-20 mg per day. The study was approved by the local Ethics Committee and written informed consent was obtained from all subjects.

Genotyping

DNA was extracted from 200 µL of whole blood samples using a GeneMATRIX Quick Blood DNA Purification Kit (EURx, Gdansk, Poland). SNPs within the *IL17A* and *IL17F* genes were genotyped using TaqMan genotyping assays from Life Technologies (Assay ID: C__15879983_10 for rs2275913, C__25765149_10 for rs11465553, C___2488913_10 for rs2397084 and C___2234166_10 for rs763780). Genomic Fluorescence data were captured using a 7500 FAST Real-Time PCR System (Applied Biosystems, Warsaw, Poland).

Statistical Analysis

The consistency of genotype distribution with Hardy-Weinberg equilibrium (HWE) was assessed with the use of the exact test. Frequencies of genotypes and alleles were compared with the chi-square or Fisher exact test. Multivariate logistic regression analysis was performed to find independent predictors of PTDM. $p<0.05$ was considered statistically significant.

Results

The *IL17A* rs2275913 GG genotype was detected in 41.9%, GA in 47.0% and AA in 11.1% of the recipients. The distribution of genotypes studied was in Hardy-Weinberg equilibrium (HWE p-value = 0.59). The *IL17F* rs11465553 GG genotype was detected in 90.4% and GA in 9.6% of the recipients (HWE p-value = 1.00). The *IL17F* rs2397084 TT genotype was detected in 80.4%, TC in 18.1% and CC in 1.5% of the recipients (HWE p-value = 0.51). The *IL17F* rs763780 TT genotype was detected in 93.7% and TC in 6.3% of the recipients, but no CC homozygote was found (HWE p-value = 1.0).

Post-transplant diabetes was not statistically significantly associated with genotypes and alleles of the *IL17A* rs2275913, *IL17F* rs11465553 and rs2397084 polymorphisms (Table I). Post-transplant diabetes was diagnosed in 10.97% of carriers of the *IL17F* rs763780 TT genotype and 42.86% of those with TC (TC vs TT: OR = 6.09, 95% CI 1.89-19.66, p = 0.0048, Table I).

Multivariate analysis was performed to check whether the *IL17F* rs763780 polymorphism is an independent risk factor for post-transplant diabetes. In this analysis, older recipient age and presence of the TC genotype were independent significant predictors of a higher risk of post-transplant diabetes (Table II).

Discussion

Post-transplant diabetes is a significant complication after renal transplantation and has shown an increasing incidence in recent years. The post-transplant progression from normoglycaemia through glucose intolerance to diabetes in a susceptible patient is generally rapid, occurring within the first 3 months[13]. In PTDM both insulin secretion and peripheral insulin action appear to be perturbed[14]. Inflammatory cytokines and chemokines appear to be centrally involved in this process. Previous studies have revealed that the development of PTDM is related to the genotypes of several genes. Clinical and experimental studies have identified interleukins (IL) associated with diabetes. It has been reported that genetic variations of inflammatory cytokines were associated with glucose homeostasis or diabetes. Kim et al[15] examined the association between PTDM and 18 single nucleotide polymorphisms (SNPs) located within the 10 genes of interleukins or

Table I. Association between *IL17A* and *IL17F* gene polymorphisms and post-transplant diabetes mellitus.

	PTDM absent		PTDM present					
	n	%	n	%	*p* value^		*p* value*	OR (95% CI)
IL17A rs2275913 genotype								
GG	59	84.29%	11	15.71%	0.79	AA+GA vs GG	0.50	0.74 (0.31-1.79)
GA	71	87.65%	10	12.35%		AA vs GA+GG	1.00	0.77 (0.17-3.61)
AA	16	88.89%	2	11.11%		AA vs GG	1.00	0.67 (0.13-3.34)
						GA vs GG	0.64	0.76 (0.30-1.90)
						AA vs GA	1.00	0.89 (0.18-4.45)
IL17A rs2275913 allele								
G	189	64.73%	32	69.57%		A vs G	0.62	0.80 (0.41-1.57)
A	103	35.27%	14	30.43%				
IL17F rs11465553 genotype								
GG	131	86.75%	20	13.25%	0.72	AA+GA vs GG	0.72	1.31 (0.35-4.93)
GA	15	83.33%	3	16.67%		AA vs GA+GG	1.00	-
AA	0	0.00%	0	0.00%		AA vs GG	1.00	-
						GA vs GG	0.72	1.31 (0.35-4.93)
						AA vs GA	1.00	-
IL17F rs11465553 allele								
G	277	94.86%	43	93.48%		A vs G	0.72	1.29 (0.36-4.64)
A	15	5.14%	3	6.52%				
IL17F rs2397084 genotype								
TT	123	87.23%	18	12.77%	0.58	CC+TC vs TT	0.55	1.49 (0.50-4.40)
TC	21	80.77%	5	19.23%		CC vs TC+TT	1.00	-
CC	2	100.00%	0	0.00%		CC vs TT	1.00	-
						TC vs TT	0.36	1.63 (0.55-4.86)
						CC vs TC	1.00	-
IL17F rs2397084 allele								
T	267	91.44%	41	89.13%		C vs T	0.58	1.30 (0.47-3.59)
C	25	8.56%	5	10.87%				
IL17F rs763780 genotype								
TT	138	89.03%	17	10.97%	0.0048	TC vs TT	0.0048	6.09 (1.89-19.66)
TC	8	57.14%	6	42.86%				
IL17F rs763780 allele								
T	284	97.26%	40	86.96%		C vs T	0.0059	5.33 (1.76-16.14)
C	8	2.74%	6	13.04%				

^Test χ^2; * Fisher's exact test.

their receptors, which might be related to β-cell dysfunction after kidney transplantation. Eleven SNPs among the 18 studied (61.1%) were significantly associated with PTDM development after adjusting for age, sex, and tacrolimus usage. In particular, significant variations of IL-7R, IL-17E, IL-17R, and IL-17RB, which was recently reported to be associated with type 1 diabetes mellitus, could be associated with the pathogenesis of PTDM in renal transplant recipients[15].

In our study, post-transplant diabetes was significantly associated with the *IL17F* T/C

Table II. Multivariate logistic regression analysis with PTDM as the dependent variable.

Independent variables	OR (95% CI)	*p*
Age (years)	1.06 (1.01-1.11)	0.015
Sex (male vs female)	1.38 (0.49-3.85)	0.53
IL17F rs763780 (TC vs TT)	6.27 (1.80-21.89)	0.0037

(rs763780) polymorphism. Kawaguchi et al[10] reported that the *IL-17F T/C* (rs763780) polymorphism causes a histidine (His)-to-arginine (Arg) substitution at amino acid 161 (H161R) and may have functional consequences regulating synthesis of IL-17.

The main function of IL-17-secreting T cells is to mediate inflammation, by stimulating the production of inflammatory cytokines, such as TNF-α, IL-1B and IL-6, and inflammatory chemokines that promote the recruitment of neutrophils and macrophages. The strong inflammatory response promoted by T_H-17 cells has been associated with the pathogenesis of a number of autoimmune and inflammatory disorders previously attributed to TH-1 or TH-2[16]. These so-called Th17 T cells produce IL-17A and IL-17F; IL-17 mediates inflammation through a receptor (IL-17R) composed of two subunits: IL-17RA and IL-17RC[17].

Emamaullee et al[18] reported that Th17 cells are involved in the pathogenesis of autoimmune diabetes; therefore, the development of Th17 targeted therapeutic agents may be of benefit in this disease. Arif et al[19] indicated that Th17 cells produced by patients type 1 diabetes secrete IL-17 in response to β-cell autoantigens; they also revealed that this cytokine is actively produced in inflamed islets close to the onset of disease. This provides evidence for the existence of a multistep pathway for β-cell destruction in which IL-17 has a key role.

It has been reported that plasma IL-17 concentration is higher in patients with insulin resistance[20]. Peripheral blood mononuclear cells from T2DM patients also secreted higher levels of IL-17 in response to T-cell stimuli, compared with those from non-diabetes subjects. These results suggest that IL-17 could play a pivotal role in the pathogenesis of insulin resistance in T2DM. It is well known that chronic inflammation impairs glucose uptake in peripheral tissues, such as skeletal muscle, and plays an important role in the pathogenesis of insulin resistance. Recently, several groups have confirmed the potential involvement of Th17 cells in diabetogenesis[21].

Conclusions

The above-mentioned studies indicate the significant role of IL-17 in the pathogenesis of diabetes, whereas the results of this study suggest an association between the *IL17F* rs763780 polymorphism and post-transplant diabetes. Nevertheless, this hypothesis requires further investigation.

Conflict of Interest

The Authors declare that there are no conflicts of interest.

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