

Vitamin D Receptor Genotype in Pancreas Allograft: A Pilot Study

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Abstract

Objectives: Transplanting of pancreatic grafts is an established treatment for diabetes mellitus. Polymorphisms in genes, coding for proteins involved in an immune response, may influence immunologic and nonimmunologic mechanisms that lead to allograft loss. Vitamin D receptor agonists have been shown to increase long-term allograft survival in humans.

Materials and Methods: Twenty-one pancreatic recipients transplanted in the Transplantation center of Shiraz University of Medical Sciences were selected and genotyped for the polymorphism of the vitamin D receptor genes (*FokI*), and the association of each genotype with acute rejection was evaluated. A control group of 100 unrelated otherwise healthy individuals, from the Iranian Blood Transfusion Organization were enrolled. The individuals were selected from Shiraz (a city located in Southern Iran), and the genotype frequency was compared with control group.

Results: The overall prevalence acute rejection was 28% (6/21). In the genotype study, homozygous FF presented in 15 patients (71%), heterozygous Ff presented in 6 patients (29%), and no homozygous ff was identified. In the control group, there were 50% with FF, 48% with Ff, and 2% with the ff genotype identified. The only genotype that was detected in rejection group was FF, while the frequency of FF in the nonrejection group was 60%.

Conclusions: This study examined several patients to determine whether the vitamin D receptor (*FokI*) genotype is involved in acute allograft rejection and requires deeper investigation.

Key words: *FokI*, polymorphism, Transplant, Rejection

Introduction

The prevalence of diabetes in Iran is approximately 7.9%.¹ It is estimated that the diabetic population is rapidly increasing by 10% each year. The presence of defects in insulin secretion and/or insulin action is the main cause leading to hyperglycemia.¹ Type 1 diabetes occurs when the pancreatic beta cells are destroyed by autoimmune origin, and the patient develops profound or absolute insulin deficiency. This form of diabetes accounts for approximately 8% to 10% of diabetes. The disease most often appears in childhood, but it may present at any age.² Mixtures of genetic and environmental factors are responsible for the autoimmune destruction of pancreatic beta cells. Over the past 10 years, the incidence of type 1 diabetes has increased.³

Type 2 diabetes occurs as the result of defects in both insulin secretion and peripheral resistance of insulin action. This form of the disease represents about 90% of diabetes cases. In recent years, the incidence of type 2 diabetes in children has increased dramatically.^{4, 5} Diabetes mellitus is associated with devastating complications including heart disease, retinopathy and blindness, nephropathy, end-stage renal disease, foot ulcers, and lower limb amputation.^{4, 5} Although a new insulin regimen improves glycosylated hemoglobin concentrations and reduces the rate of long-term complications, it does not completely prevent them.^{6, 7}

The goal of pancreas transplant is to restore normoglycemia with sufficient β -cell mass. Because

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of the high immunogenicity of pancreatic transplants, organ rejection may occur. Only 5% to 20% of patients with pancreatic graft rejections have clinical symptoms, the remaining have clinical symptoms that are subtle or nonexistent.⁶⁻¹⁰

Allograft rejection can be secondary to a variety of immune and nonimmune mechanisms.¹¹ Polymorphisms in genes, coding for proteins involved in immune response may influence immunologic and nonimmunologic mechanisms that lead to allograft loss. In transplant patients, immunomodulatory functions of vitamin D was emphasized.¹¹ Recent studies demonstrate a role for vitamin D in regulating immune cell proliferation, differentiation, and responsiveness.¹² Evidence from animal studies suggests that administration of 1,25-dihydroxyvitamin D can prevent acute allograft rejection after liver,¹³ kidney,^{14, 15} and heart transplant.¹⁶

The active metabolite of vitamin D, 1,25-dihydroxyvitamin D₃ (calcitriol), has a ligand, vitamin D receptor (VDR), which is found in various organs and tissues of the body. In the immune system, monocytes, macrophages, dendritic cells, and T cells not only express the VDR and respond to 1,25 OH-vitamin D, but they also can generate 1,25 OH-vitamin D from its circulating precursor.¹⁷⁻²³ Vitamin D inhibits dendritic cell differentiation and maturation, limits Th1 immunity,²⁴ promotes Th2 immunity,^{25, 26} and enhances regulatory T-cell pathways.²⁷

The VDR gene is located on chromosome 12q12-q14. More than 100 single nucleotide polymorphisms have been described within its 67076-bp sequence. Eight exons and 6 alternatively spliced regions (including the promoter region) are distributed in functionally relevant areas. Vitamin D receptor polymorphisms are associated with numerous diseases, such as osteoporosis,^{28, 29} diabetes mellitus type one³⁰ and type two,³¹ and organ graft survival.³² The start codon polymorphism *FokI* leads to a 424 amino acid protein instead of 427 amino acids.³²

Our study was designed to see whether there is an association between VDR (*FokI*) (rs10735810) genotype and the incidence of early acute rejection after pancreas transplant.

Materials and Methods

A consecutive series of 21 first pancreas recipients transplanted at the Transplantation Center of Nemazi

Hospital affiliated with the Shiraz University of Medical sciences between January 2009 and January 2011 were enrolled. The organs (pancreas and kidney in cases of simultaneous pancreas-kidney transplant operations) were procured from heart-beating deceased donors. Recipients were selected for transplant based on ABO compatibility and a negative T lymphocytotoxic crossmatch.¹⁰ A control group of 100 unrelated otherwise healthy volunteer individuals, aged 35 to 60 years, from the Iranian Blood Transfusion Organization were enrolled. The individuals selected from Shiraz (ie, a city in Southern Iran). They were selected according to the World Health Organization protocol for blood donation including all laboratory and clinical tests with normal parameters. The Ethics Committee of Shiraz University of Medical Sciences approved the protocol, which conformed to the ethical guidelines of the 1975 Helsinki Declaration; and a written, informed consent form was obtained from all subjects. An episode of acute rejection during postoperative hospital course was recorded. An acute rejection episode was defined based on clinical or biopsy findings according to Banff criteria.^{33, 34} Clinical rejection was identified as *an unexplained elevation in serum amylase, lipase, or glucose in the absence of infection, obstruction or evidence of drug toxicity*. Pancreatic grafts were considered as functioning, as long as the recipients were off insulin.

Detailed data of patients' demographics and pretransplant status were retrieved from our Transplant Database. Routine immunosuppression consisted of tacrolimus (started at 1 mg twice per day at day 1; increased gradually to a serum target level of 10 to 15 ng/dL), mycophenolate mofetil (started at 500 mg per day at day 1 and increased to 1000 mg per day adjusted to the white blood count level), and a corticosteroid.¹⁰ For the first-line treatment of acute rejection (either biopsy-proven or clinically treated), patients received methylprednisolone pulse therapy (500 mg/day, 4 times for 5 days). If there was no response to initial antirejection therapy, antilymphocyte antibody was used as a second-line treatment.

Genotype analyses

Genomic DNA was extracted from a peripheral blood sample using a commercial extraction kit (DNG plus DNA Extraction Kit, Sinagene Company, Tehran, Iran) according to the

manufacturer's protocol. The primers VDR1F (5'-agctggccctggcactgactctgctct-3') and VDR1R (5'-atggaaacacctgtcttctctcctc-3') were used in a polymerase chain reaction to amplify a 265-bp fragment containing the start codon polymorphism FokI T>C (rs10735810).^{13, 14, 35} The polymerase chain reaction products were digested with FokI (New England Biolabs, Ipswich, MA, USA) at 37°C for 3 hours, followed by electrophoresis in a 1.5% agarose-gel containing ethidium bromide. Homozygous cleavage by FokI generates 2 fragments, 69 and 196 bp (ff genotype), whereas the heterozygotes display all 3 bands (Ff genotype). Thus, the genotypes FF, Ff, and ff could be identified.

Statistical analyses

Differences in donor and recipients' sex, type of surgery, and genotype distributions between acute rejection (AR) and nonacute rejection groups (non-AR) and comparison with normal controls were analyzed with the chi-square test. Differences in age were analyzed using the *t* test. The level of significance was set at $P < .05$. Statistical analyses were performed with SPSS software (SPSS: An IBM Company, version 16.0, IBM Corporation, Armonk, New York, USA).

Results

Patients included 14 men and 7 women (mean age, 35.47 ± 9.5 y). Demographic and clinical characteristics are shown in Table 1. The overall incidence of rejection was 28.5% (6/21; 4 confirmed by biopsy). Statistical analyses of recipients' findings including donor and recipient age, sex, cold ischemic time, and type of transplant, showed no differences between the ARs and non-ARs (P value $> .05$). The distribution of genotypes frequency between recipients and controls are shown in Table 2. The genotype determined at position FokI T>C (rs10735810) was as follows: homozygous FF presented in 15 patients (71%), heterozygous Ff was present in 6 patients (29%), and no homozygous ff was identified.

In the control group, 50% with FF, 48% with Ff, and 2% with the ff genotype were identified. The only genotype that was detected in the rejection group was FF; neither Ff nor ff was identified. The frequency of FF in nonrejection group was 60% (Table 2). There was no significant correlation

between rejection episode and FokI genotypes ($P = .12$).

The 1-year patient survival for the pancreas transplant alone (PTA) group ($n=8/11$) was 73%, and 100% for simultaneous pancreas-kidney transplantation (SPK) ($n=7/7$), and pancreas after kidney (PAK) ($n=3/3$). The overall patient survival was 86% in this pilot study. With respect to graft survival, pancreatectomy was done in 3 patients (3/11, 37.5%) in the PTA group and 1 recipient in the PAK (33%, 1/3) who developed renal failure. The overall pancreas graft survival was 81% in this study.

Table 1. Demographics of Pancreas Graft Recipients

Parameter	ARs (%)	Non-ARs (%)
Number of patients	6 (29)	15 (61)
Recipient sex		
Male	2 (33)	10 (67)
Female	4 (67)	5 (33)
Cold ischemic time (h)	11.2 ± 3.1	10.3 ± 2.2
Recipient age (y, mean $\pm$ SD)	25.76 ± 8.2	27.1 ± 9.9
Donor age (y, mean $\pm$ SD)	30.9 ± 5.3	28.44 ± 5.1
Type of transplant		
SPK	2 (33)	5 (33)
PAK	-	3 (20)
PTA	4 (67)	7 (47)
Immunosuppression		
Tacrolimus and MMF + steroid	-	15 (100)
Tacrolimus and MMF + steroid + ATG	15 (100)	-

Abbreviations: ATG, antithymocyte globulin; MMF, mycophenolate mofetil; PAK, pancreas after kidney transplant; PTA, pancreas transplantations alone; SPK, simultaneous pancreas kidney transplant

Table 2. Distribution of VDR (FokI) Genotypes in Acute Rejection (ARs), Nonacute Rejections (non-ARs), and Controls

VDR Genotype (FokI)	ARs n=6 (%)	non-ARs n=15 (%)	Controls n=100 (%)
FF	6 (100)	9 (60)	50 (50)
Ff	0 (0)	6 (40)	48 (48)
ff	0 (0)	0 (0)	2 (2)

Discussion

Vitamin D affects the innate immune system and has a protective role against bacterial infections and tuberculosis.³⁶ The 1,25(OH)₂D is produced by monocytes and macrophages and do intracellular antimicrobial effects.³⁷

In transplant recipients, the antimicrobial actions of vitamin D also occur in barrier epithelial cells of the skin,^{38, 39} gut,⁴⁰ and lungs.⁴¹ Ginde and associates found that the prevalence of upper respiratory tract infections was greater in patients with lower 25-OHD levels.⁴²

Animals treated with calcitriol were resistant to *Candida albicans* and herpes simplex virus-1 infection.⁴³ It is believed that transplant recipients are at risk for vitamin D deficiency because of poor health after transplant, low dietary intake, and avoidance of sun exposure (because of an increased risk of skin cancer).^{44, 45} This may result in secondary hyperparathyroidism, bone loss, and fracture.⁴⁶ In a population study, patients treated with calcitriol during a heart transplant had a reduction in their cyclosporine requirement.⁴⁷

Calcitriol supplementation in kidney transplant recipients was associated with fewer episodes of acute cellular rejection,⁴⁸ decreased expression of costimulatory, and HLA-DR molecules than transplant recipients without calcitriol supplement.⁴⁵ In heart transplant recipients, those patients who were supplemented with cholecalciferol, had fewer rejection episodes.⁴⁹ The above-mentioned studies suggested a possible mechanism of vitamin D for allograft survival. The vitamin D receptor is the ligand of calcitriol, which is found in various tissues such as those in the immune system.^{50, 51}

Lavin and associates demonstrated that the FokI T allele is associated with improved renal allograft survival. They found no association between acute rejection rates and any VDR FokI genotypes.³⁴ Azarpira and associates evaluated the effect of the VDR gene polymorphism on 75 renal allograft recipients with at least 2 years' follow-up in an Iranian population.¹⁴ The frequency of FF was 40.9% in rejection versus 52.8% in the nonrejection group. The Ff genotype was detected in 50.1% of the rejection and 43.4% of the nonrejection group. Of the ff genotype, it was detected in only nonrejection recipients (3.8%). They found no significant association between VDR FokI genotypes and kidney allograft rejection. Few published studies have emphasized the role of VDR polymorphisms in liver transplant^{13, 52} and hematopoietic stem cell transplant outcomes.^{53, 54}

In pancreas transplant, graft survival rates have improved over time owing to refined surgical techniques and better immunosuppression.^{8, 10} However, there is no documented study in the literature that considers vitamin D receptor polymorphism in pancreatic transplant recipients. In this study, the only genotype that was found in the rejection group was FF genotype (100%), and its

frequency in the nonrejection group was 60%. This finding is different from our experience in renal transplant recipients in that FF was detected in only 40.9% of the rejection group.

This pilot study failed to demonstrate differences sufficient to warrant a fully powered study that would require 185 patients to demonstrate differences seen in renal allograft recipients. Further prospective human studies are required to explore the role of vitamin D and the VDR polymorphism in pancreas graft survival.

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