FACTOR V LEIDEN AND PROTHROMBIN G20210A MUTATIONS IN THAI PATIENTS AWAITING KIDNEY TRANSPLANT

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Abstract. Renal transplantation provides the best long-term treatment for chronic renal failure, but thrombosis of the transplanted renal artery or renal vein is one of the causes of kidney failure in the early postoperative period. Factor V Leiden (FVL) and prothrombin G20210A mutation are the most frequent genetic abnormalities associated with venous thrombosis. We investigated the prevalence of FVL and prothrombin G20210A by polymerase chain reaction with restriction fragment length polymorphism in 75 Thai patients awaiting renal transplant, and a control group of 106 healthy blood donors. Of those awaiting renal transplant, none was found to carry FVL or prothrombin G20210A mutations. Neither the heterozygous nor the homozygous FVL mutation nor the prothrombin G20210A mutation was detected in the 106 healthy volunteers. Although we failed to detect FVL and prothrombin G20210A mutation among those waiting for a kidney transplant, the population size was small. Further studies need to be performed in order to ascertain if these coagulation mutations are of relevance in predicting patients at risk of early transplant failure.

INTRODUCTION

Kidney transplantation is the best treatment for end-stage renal disease. Factors that determine outcome include antigenic disparity, such as ABO blood group and major histocompatibility antigen or HLA, between donor and recipient, the type of immunologic response mounted by the host, and the immunosuppressive regimen used to prevent graft rejection. Renal allograft rejection may be due to hyperacute rejection from binding of cytotoxic antibodies and complement activation, acute rejection from cellular immune responses, or chronic rejection with characteristic vasculopathy and immune-mediated graft obliteration (Watnick et al, 2001).

Thrombosis of the transplanted renal artery or renal vein is one of the causes of kidney failure in the early postoperative period (Allen et al, 1987). Therefore, one might consider additional genetic risk factors for thrombosis, such as factor V Leiden (FVL) and prothrombin G20210A mutations. The FVL genetic mutation is a point mutation in coagulation factor V, which results in a shift in the hemostatic balance in favor of procoagulation and an increased risk of thrombosis (Bertina, 1999; Rosendaal, 1999). Additionally, the prothrombin G20210A mutation has recently been shown to be associated with prothrombin plasma levels and the risk of venous thrombosis (Poort et al, 1996). FVL and prothrombin G20210A mutations are common among Caucasian populations (3-7% and 2-5% respectively) but they are rare in Asian populations (Rees et al, 1995; Rosendaal et al, 1998). However, from our previous studies, the heterozygous allele FVL can be detected in 11.11% of Thai patients with venous thrombosis.
(Prayoonwiwat et al, 2000). The aim of this study is to determine the prevalence of FVL and prothrombin G20210A mutations in the Thai population and in patients awaiting renal transplant.

MATERIALS AND METHODS

Seventy-five patients awaiting renal transplant, 54 males and 21 females, of the Renal Unit, Phramongkutklao Hospital, Bangkok, Thailand, were enrolled in this study. Their ages ranged from 5 to 50 years; the mean age was 38 years. A control group of 106 healthy blood donors at the Blood Bank, Army Institute of Pathology, Bangkok, Thailand (78 males and 28 females) was included. The ages of the blood donors ranged from 23 to 52 years; the mean age was 36 years. Informed consent was obtained from all subjects. Polymerase chain reaction with restriction fragment length polymorphism (PCR-RFLP) was used in the detection of FVL and prothrombin G20210A mutations. The analysis of FVL was performed as described by Prayoonwiwat et al (2000).

For the detection of prothrombin G20210A, the PCR product was generated in 50 µl reaction mixtures that contained 100 to 200 ng of genomic DNA, 250 nmol/l of each primer (Rees et al, 1995), 200 mmol/l of each dNTP and 2.5 units of AmpliTaq DNA polymerase (Perkin Elmer, New Jersey, USA). The reaction mixture was placed in a PTC 200 Thermal Cycler (MJ Research, MA, USA) and subjected to 35 cycles of amplification (94°C for 45 seconds, 56°C for 45 seconds, and 72°C for 45 seconds). Ten microlitres of PCR product was digested overnight with Hind III and analyzed by agarose gel electrophoresis.

RESULTS

Seventy-five patients waiting for renal transplant and 106 healthy volunteers were studied. For FVL, the undigested PCR product showed 223 base pairs (bp) and after being cleaved with Mnl I, a normal allele, produced bands of 37, 82 and 104 bp, while the FVL allele produced the homozygous pattern bands of 82 and 141 bp; moreover, bands 37, 82, 104 and 141 bp were produced by the heterozygous allele. For the prothrombin G20210A mutation, the undigested PCR product showed 345 bp and after being cleaved with Hind III, a normal allele generated only 345 bp, while the prothrombin G20210A mutation produced the homozygous pattern bands of 322 and 23 bp. Furthermore, bands 345, 322 and 23 bp were produced by the heterozygous allele (Fig 1).

Of the 75 patients awaiting kidney transplant, none was found to carry FVL or prothrombin G20210A mutations. Neither the heterozygous nor the homozygous FVL allele nor the prothrombin G20210A mutation was detected in 106 healthy volunteers.

DISCUSSION

Renal transplantation provides the best long-term treatment for chronic renal failure, but early vascular thrombotic complications resulting in loss of the allograft and increased long-term incidence of venous and arterial thrombosis are recognized complications (Allen et al, 1987; Bertina, 1999; Rosendaal, 1999).
Renal vein thrombosis has been frequently associated with risk factors such as catheters, surgery or trauma, but it has also been demonstrated a pathogenetic role of genetic prothrombotic risk factors, such as FVL and prothrombin G20210A. Carriers of these genetic factors are at increased risk of venous thrombosis than healthy individuals (Irish et al., 1997; Oh et al., 1999; Wheeler et al., 2000).

The prevalence of genetic risk factors for thrombosis varies greatly in different parts of the world, both in patients with thrombosis and in the general population. FVL and prothrombin G20210A are the most common genetic defects leading to thrombosis (Rees et al., 1995; Poort et al., 1996).

Previous studies have shown an association of FVL and prothrombin G20210A mutations with thrombosis in patients who have undergone renal transplantation (Fischereder et al., 1998; Wuthrich, 2001; Wuthrich et al., 2001; Fischereder et al., 2001; Hocher et al., 2002). Although we failed to detect the FVL and prothrombin G20210A mutations in the patients waiting for renal transplant that we studied, the population size was small. Further studies need to be performed in order to ascertain whether these coagulation mutations are of relevance in predicting patients at risk of early transplant failure.

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REFERENCES


Poort SR, Rosendaal FR, Reitsma PH, Bertina RM. A common genetic variation in the 3′-untranslated region of the prothrombin gene is associated with elevated plasma prothrombin levels and an increase in venous thrombosis. Blood 1996; 88: 3698-703.


