Nephrol Dial Transplant (2006) 21: 3555–3558
doi:10.1093/ndt/gfl400
Advance Access publication 12 September 2006

Original Article

NDT
Nephrology Dialysis Transplantation

Thrombophilia and avascular necrosis of femoral head in kidney allograft recipients

Yakup Ekmekci[1], Kenan Keven[1], Nejat Akar[2], Yonca Egin[2], Sule Sengul[1], Sim Kutlay[1], Sehsuvar Erturk[1] and Bulent Erbay[1]

[1]Department of Nephrology and [2]Department of Pediatric Molecular Genetic, Ankara University School of Medicine, Ankara, Turkey

Abstract

Background. Thrombophilia has been implicated in the development of avascular necrosis (AVN) in various diseases. We aimed to search for the relation of both prothrombin gene G20210A mutation and factor V G1691A (factor V Leiden) mutation with AVN among kidney transplant recipients.

Methods. Nineteen patients with AVN and 38 control patients without AVN were included. Clinical information was collected, and gender, age, type of renal allograft, duration and type of dialysis, presence of acute rejection, and cumulative doses of ciclosporin and corticosteroid administration were taken into consideration. Genotypes of factor V G1691A and prothrombin G20210A were determined by direct sequencing of genomic DNA.

Results. Factor V Leiden mutation was detected in six patients (31.6%) among patients with AVN and in only three patients (7.9%) in the control group ($P = 0.048$). Two patients (10.5%) in the AVN group were determined to have prothrombin G20210A mutation, while no prothrombin G20210A mutation was detected in the control group. When both of the mutations causing thrombophilia were considered, a total of eight patients (42.1%) in the AVN group and three patients (7.9%) in the control group were identified ($P = 0.004$).

Conclusion. Thrombophilia seems to be an important risk factor for development of AVN. More studies are needed to clarify the role of factor V G1691A and prothrombin G20210A mutation for AVN.

Keywords: avascular necrosis; factor V Leiden; kidney transplantation; prothrombin G20210A; thrombophilia

Introduction

Avascular necrosis (AVN) of the femoral head is a rare, debilitating complication seen after kidney transplantation and its aetiology remains unknown, although several mechanisms have been proposed. Impaired blood flow, caused by arterial ischaemia and/or venous thrombosis, leading to ischaemic necrosis in some parts of the femoral head contributes to the pathogenesis of AVN. Several studies have reported the role of heritable thrombophilia in the pathogenesis of AVN [1,2] in addition to the known risk factors such as steroid use, calcineurin dose and bone metabolism. These studies included systemic lupus erythematosus (SLE) and sickle cell anaemia, which are the most commonly associated diseases for the development of AVN [3]. In this respect, we aimed to study the frequency of the prothrombin gene G20210A mutation and factor V G1691A mutation (factor V Leiden) in our patients with AVN to determine whether or not these mutations can be the causative factor for the development of AVN in kidney allograft recipients.

Subjects and methods

Study design

From 1993 to the present, 243 patients have undergone renal transplantation at the Ankara University School of Medicine. Among these patients, 22 who were diagnosed to have post-transplant AVN at our clinic were selected as the study group. As three patients were lost to follow-up (missing completely at random) and excluded, a total of 19 patients were available for the study. The control group was comprised of patients who underwent transplantation just before and after each selected post-transplant AVN patient, for a total of 38 patients. AVN was diagnosed on the basis of symptoms and magnetic resonance imaging (MRI) study. All patients had symptoms (pain), they were on late stage of AVN (stage 3–4) and underwent to surgery.

Correspondence and offprint requests to: Kenan Keven, MD, Ankara University School of Medicine, Department of Nephrology, Ibni Sina Hospital, 06100, Ankara, Turkey.
Email: keven@medicine.ankara.edu.tr

© The Author [2006]. Published by Oxford University Press on behalf of ERA-EDTA. All rights reserved.
For Permissions, please email: journals.permissions@oxfordjournals.org

Downloaded from https://academic.oup.com/ndt/article/21/12/3555/1869174 by Ankara University Library (ANK) user on 11 December 2021

There was no occult AVN in the group. No MRI investigation was performed for the control group and they were all asymptomatic.

The local Ethics Committee of Ankara University School of Medicine approved the study and all patients gave informed consent. Gender, age at the time of transplantation, type of renal allograft (cadaveric or living), duration and type of dialysis, cause of renal disease, duration of post-operative care, presence of acute rejection, the immunosuppressive drug used, the number of pulse steroid therapies and comorbid diseases (hypertension or diabetes mellitus) were evaluated in all patients. Serial biochemical determinations were recorded for serum calcium, phosphate, alkaline phosphatase, haematocrit value, platelet count, cholesterol and triglyceride levels at post-operative 1, 3, 6 and 12 months, and then annually up till the point of AVN occurrence. The mean levels of these parameters were determined and used for the analyses. The mean dose of steroid and ciclosporin (CsA) for post-transplant 3 months and the mean cumulative dose of steroid and CsA from the beginning to time of AVN were included in the analyses in AVN patients. The same analyses were also conducted for the control group.

Assays for genotyping

For genetic analysis, we obtained venous blood in EDTA-treated sample tubes, from which cells were separated by centrifugation at 3000x*g* for 15 min. The buffy coat layer was then removed and stored at −70°C pending DNA extraction by standard techniques. Factor V G1691A and prothrombin G20210A polymorphisms were determined in patients by polymerase chain reaction (PCR) amplification and digestion with *Mn*II and *Hin*dIII, respectively [2–11].

Statistical analysis

SPSS version 11.5 for Windows (SPSS Inc., Chicago, IL) was used for data analysis. All numerical data were expressed as mean ± SD. Comparisons were performed between AVN group ($n = 19$) and their controls ($n = 38$) using unpaired *t*-test for quantitative variables, and Fisher's exact test for the frequencies of qualitative variables. Bonferroni correction was applied for multiple tests. $P < 0.05$ is considered significant. Power analyses were also performed. For prothrombin G20210A, group sample sizes of 19 and 38 achieve 81% power to detect a difference of 29% between the null hypothesis that both group proportions are 30% and the alternative hypothesis that the proportion in group 2 is 1% using a one-sided Fisher's exact test with a significance level of 2.5%. For factor V Leiden, group sample sizes of 19 and 38 achieve 81% power to detect a difference of 34% between the null hypothesis that both group proportions are 39% and the alternative hypothesis that the proportion in group 2 is 5% using a one-sided Fisher's exact test with a significance level of 2.5%. Serial biochemical determinations including serum calcium, phoshorus, alkaline phosphatase, haematocrit value, platelet count, cholesterol and triglyceride levels at post-operative 1, 3, 6 and 12 months, and then annually up till the point of AVN occurrence were compared with repeated measures ANOVA.

Table 1. Demographic data and biochemical parameters of patients with AVN and controls

	AVN (19)	Controls (38)	*P*
Age (years)	37.8 ± 9.7	34.2 ± 9.3	0.17
Males/females	16/3	32/6	1.0
Renal allograft (living/cadaveric)	16/3	32/6	1.0
Haemodialysis/peritoneal dialysis	18/1	31/7	0.17
Duration of dialysis (month)	18.2 ± 20.0	18.0 ± 18.5	0.96
Months to AVN	20.6 ± 12.9		
Calcium (mg/dl)	9.3 ± 0.6	9.2 ± 0.5	0.75
Phosphate (mg/dl)	3.5 ± 0.6	3.8 ± 1.1	0.74
Alkaline phosphatase (IU/l)	93.3 ± 42.4	89.6 ± 36.1	0.74
Creatinine (mg/dl)	1.49 ± 0.51	1.55 ± 0.64	0.19
Haematocrit (%)	36.7 ± 3.9	35.2 ± 3.9	0.20
Platelet count (mm^3)	219 433 ± 52 680	245 008 ± 48 217	0.10
Triglycerides (mg/dl)	194.8 ± 72.4	185.0 ± 68.7	0.65
Cholesterol (mg/dl)	208.8 ± 35.2	199.8 ± 29.1	0.36
BMI (body weight/height^2)	24.2 ± 2.0	24.3 ± 3.1	0.98
Diabetes mellitus	1 (5.3%)	3 (7.9%)	0.59

Data: Mean ± SD.
AVN, avascular necrosis; BMI, body mass index.

Results

Demographic features of the study population and controls are given in Table 1. There were no statistically significant differences in any of these variables between the two groups.

The causes of end-stage renal failure were unknown in nine (47.4%) and 21 (55.3%), chronic glomerulonephritis in four (21.1%) and six (21.1%), chronic interstitial nephritis in three (15.8%) and nine (23.7%), polycystic kidney disease in two (10.5%) and one (2.6%), and hereditary nephritis in one (5.3%) and renal amyloidosis in one (2.6%), in patients with AVN and the control group, respectively. The aetiologies of renal diseases were not different between transplant patients with AVN and without AVN ($P > 0.05$). No patient had a history of SLE, sickle cell disease, previous history of bone fracture, venous thromboembolism and alcohol abuse in both AVN and control groups.

The mean biochemical parameters in the study groups following transplantation are listed in Table 1. No statistically significant difference was present in these variables at the time intervals examined. Number of human leukocyte antigen (HLA) mismatches (1.5 ± 1.4 *vs* 1.8 ± 1.1) and presence of acute rejection (26.3% *vs* 28.9%) were also similar. We did not evaluate the protein C, S and antithrombin III levels of our patients.

Two patients in the AVN group and six patients in the control group were on tacrolimus treatment. Table 2 summarizes the cumulative doses of steroids and CsA in the AVN group and controls. No difference was observed in these parameters between the two groups.

When thrombophilia was evaluated, in the patients with AVN, a total of eight patients (42.1%) were

Downloaded from https://academic.oup.com/ndt/article/21/12/3555/1869174 by Ankara University Library (ANK) user on 11 December 2021

Table 2. Immunosuppressive therapy in patients with AVN and controls

	AVN (19)	Control (38)	*P*
Number of pulse steroid	0.4 ± 0.7	0.2 ± 0.6	0.77
Steroid (mg/kg)			
3 months	80.8 ± 30.1	73.6 ± 19.8	0.28
Total dose	137.4 ± 54.6	138.8 ± 55.4	0.92
CsA (mg/kg)			
3 months	347.3 ± 169.3	416.1 ± 227.1	0.25
Total dose	1461.7 ± 1052.8	1755 ± 997.6	0.31

Table 3. Factor V G1691A and prothrombin G20210A mutations in patients with AVN and controls

	AVN ($n=19$) *n* (%)	Control ($n=38$) *n* (%)	*P**
Factor V Leiden	6 (31.6)	3 (7.9)	0.048
Prothrombin G20210A	2 (10.5)	0	>0.05
Factor V Leiden or Prothrombin G20210A	8 (42.1)	3 (7.9)	0.004

*Because of Bonferroni correction, $P < 0.0167$ was considered significant.

identified to have factor V Leiden (six patients) or prothrombin G20210A mutation (two patients). In the control group, there were three patients with factor V Leiden (7.9%), while no patient had prothrombin G20210A mutation (Table 3).

Discussion

In this study, in order to investigate whether thrombophilia can be a risk factor for development of AVN after kidney transplantation, we tested factor V Leiden and prothrombin gene mutations in our patients. We found that these mutations seem to be an important risk factor for the development of AVN of the femoral head in kidney allograft recipients.

AVN is a rare complication in kidney allograft recipients; the reported incidence in recent series ranges from 3 to 16% [4,5]. The incidence in our patients was 7.8%. Corticosteroids probably play a central role in AVN of bone in transplant recipients, but how they exert this effect remains uncertain. It has been suggested that the fat emboli secondary to hypercortisolism lodge in subchondral bone, causing vascular occlusion and bone death. Conflicting reports have been published of correlations between the total dose of steroid administered and the development of AVN, with positive correlations found by some investigators [6,7] but not by others [8]. Although the pathology of steroid-induced AVN is controversial and has not been clarified, it is believed that rapid ischaemic change occurs ultimately in the femoral bone, which is followed by AVN. Some studies have reported a decrease in the incidence of AVN after the introduction of CsA [9,10]. This might be due to decreased rejection episodes and lower steroid administration under the CsA treatment. In our study, the total dose of steroid and CsA at 3 months post transplantation and at the time of AVN did not differ between the groups. Also, the mean number of pulse steroid therapies received was not higher in patients with AVN. The potential association of immunosuppressive medications other than steroids with AVN is more controversial. In particular, CsA and tacrolimus have been identified to be associated with a higher risk of AVN in some [11,12], but not all, reports from the literature [13]. Abbott *et al.* [12] using the US Renal Data System database, studied 27 772 kidney allograft recipients to assess the risk of AVN under the different maintenance immunosuppressive medications. In this study, AVN was significantly more common in the CsA group in comparison with other drug groups. Sakai *et al.* [11] in their study reported that AVN was significantly higher in kidney allograft recipients who were treated with CsA in comparison with those treated with tacrolimus. The dose of steroid and calcineurin inhibitors were similar between the groups; however, it is obvious that this cannot exclude the pathophysiological importance of both the drugs in development of AVN in this study. We suggest that genetic thrombophilia can act together with acquired risk factors, such as steroids and calcineurin inhibitors, to induce AVN.

Anomalies in the coagulation and fibrinolytic systems at the onset of AVN have been evaluated previously [14,15]. Recent reports by Glueck *et al.* [2] have suggested that inherited thrombophilia (activated protein C resistance, deficiency of proteins C and S) may be associated with thrombosis of the vessels of the proximal femur and lead to necrosis of the epiphysis. In one report, the heterozygosity for factor V Leiden mutation (4.9% in patients, 0.7% in controls) seemed to be associated with the development of Perthes disease (AVN of the femoral head in childhood) [1]. However, other investigators could not prove the role of thrombophilia in the aetiology in the development of AVN [16,17]. SLE and sickle cell disease are the reported diseases in which AVN seems to be associated with thrombophilia including factor V Leiden and prothrombin gene mutations. Although both mutations have been evaluated as risk factors for early and long-term allograft function in kidney recipients [18,19], no association with AVN has been studied in these patients. Heterozygosity for the factor V Leiden mutation predisposed renal allograft recipients to venous thromboembolic complications, graft perfusion defects, acute rejection and early transplant loss [18,19]. The prevalence of factor V Leiden mutation was not different in end-stage renal failure patients and a normal population. It was found to be 4% in kidney allograft recipients [19,20]. Similarly, the prothrombin gene mutation was found to be a risk factor for thrombotic complications both on haemodialysis and after renal transplantation; however, these were case reports and there was insufficient data. In our study, while the patients with AVN had significantly higher

Downloaded from https://academic.oup.com/ndt/article/21/12/3555/1869174 by Ankara University Library (ANK) user on 11 December 2021

frequency of thrombophilia, acute rejection episodes and allograft function appeared to be the same in both groups. Furthermore, we did not find any previous history of venous thromboembolism in our patients with or without thrombophilia. However, it should be borne in mind that the patients with thrombophilia might develop early allograft loss due to thromboembolism or severe acute rejection. These patients naturally could not develop AVN and they were not involved in the study.

The importance of abnormal mineral metabolism in the pathogenesis of AVN is based on the observation of patients undergoing dialysis, mostly with hyperparathyroidism, who develop AVN in the absence of steroids. Although duration of dialysis, serum calcium, phosphorus and alkaline phosphatase levels were not different between the groups in our study, lack of parathyroid hormone level monitoring and the relatively small number of patients were the limitations of our study.

Further studies should focus on proving the role of haemostatic alterations in the pathogenesis of AVN and, especially, the role of thrombophilia in AVN. The rationale and potential for using antithrombotic and anticoagulant drugs in order to prevent or slow the process of AVN have not been clarified and this is an issue to be addressed in future trials.

Acknowledgements. The authors deeply appreciate the statistical assistance of Assoc. Prof. A.H Elhan, from Biostatistical Department of Ankara University School of Medicine. This study was supported by The Scientific and Technological Research Council of Turkey (Project: SBAG-HD-47).

Conflict of interest statement. None declared.

References

1. Arruda VR, Belangero WD, Ozelo MC *et al*. Inherited risk factor for thrombophilia among children with Legg-Calve-Perthes disease. *J Ped Orthop* 1999; 19: 84–87
2. Glueck CJ, Crawford A, Roy D, Freiberg R, Glueck H, Stroop D. Association of antithrombotic deficiencies and hypofibrinolysis with Legg-Calve-Perthes disease. *J Bone Joint Surg* 1996; 78: 3–13
3. Andrade Fl, Annichino-Bizzacchi JM, Saad ST, Costa FF, Arruda VR. Prothrombin mutant, factor V Leiden, and thermolabile variant of methylenetetrahydrofolate reductase among patients with sickle cell disease in Brazil. *Am J Hematol* 1998; 1: 46–50
4. Elmstedt E, Svahn T. Skeletal complications following renal transplantation. *Acta Orthop Scand* 1981; 52: 279–286
5. Nielson HE, Melson F, Christensen MS. Aseptic necrosis of bone following renal transplantation. *Acta Med Scand* 1977; 202: 27–32
6. Patton PR, Pfaff WW. Aseptic bone necrosis after renal transplantation. *Surgery* 1988; 103: 63–68
7. Hawking KM, Van Den Bosch BF, Wilmink JM. Avascular necrosis of bone after renal transplantation. *N Eng J Med* 1976; 294: 397
8. Ibels LS, Alfrey AC, Muffer WE, Weil R. Avascular necrosis of bone following renal transplantation. Experience in 194 transplant recipients and review of the literature. *Medicine* 1978; 57: 25
9. Landmann J, Renner R, Gachter A, Thiel G, Harder F. Cyclosporin A and osteonecrosis of the femoral head. *J Bone Joint Surg* 1987; 69: 1226–1228
10. Lausten GS, Lemser T, Jensen PK, Egfjord M. Necrosis of the femoral head after kidney transplantation. *Clin Transplantation* 1998; 12: 572–574
11. Sakai T, Sugano N, Kakado Y, Takahara S, Ohzono K, Yoshikawa H. Tacrolimus may be associated with lower osteonecrosis rates after renal transplantation. *Clin Orthop* 2003; 415: 163–170
12. Abbott KC, Koff J, Bohen EM *et al*. Maintenance immunosuppression use and the associated risk of avascular necrosis after kidney transplantation in the United States. *Transplantation* 2005; 3: 330–336
13. Thang S, Chan TM, Lui SL, Li FK, Lo WK, Lai KN. Risk factors for avascular bone necrosis after renal transplantation. *Transplant Proc* 2000; 32: 1873–1875
14. Glueck CJ, Freiberg R, Tracy T, Stroop D, Wang P. Thrombophilia and hypofibrinolysis: pathophysiologies of osteonecrosis. *Clin Orthop Rel Res* 1997; 334: 43–56
15. Balasa VV, Gruppo RA, Glueck CJ *et al*. Legg-Calve–Perthes disease and thrombophilia. *J Bone Joint Surg* 2004; 12: 2642–2647
16. Hayek S, Kenet G, Lubetsky A, Rosenberg N, Gitel S, Wientroub S. Does thrombophilia play an aetiological role in Legg-Calve-Perthes disease? *J Bone Joint Surg* 1999; 4: 686–690
17. Kealey WD, Mayne EE, McDonald W, Murray P, Cosgrove AP. The role of coagulation abnormalities in the development of Perthes disease. *J Bone Joint Surg* 2000; 5: 744–746
18. Fischereder M, Schneeberger H, Lohse P, Kramer BK, Schlondorff D, Land W. Increased rate of renal transplant failure in patients with the G20210A mutation of the prothrombin gene. *Am J Kidney Dis* 2001; 5: 1061–1064
19. Wuthrich RP, Civara-Muzar S, Booy C, Maly FE. Heterozygosity for the factor V Leiden (G1691A) mutation predisposes renal transplant recipients to thrombotic complications and graft loss. *Transplantation* 2001; 3: 549–550
20. Kujovich JL. Thrombophilia and thrombotic problems in renal transplant patients. *Transplantation* 2004; 7: 959–964

Received for publication: 27.12.05
Accepted in revised form: 13.6.06

Downloaded from https://academic.oup.com/ndt/article/21/12/3555/1869174 by Ankara University Library (ANK) user on 11 December 2021