

Heritable Thrombophilia-Hypofibrinolysis and Osteonecrosis of the Femoral Head

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Abstract We hypothesized that inherited thrombophilia and hypofibrinolysis were risk factors for osteonecrosis of the femoral head. We compared measures of thrombophilia and hypofibrinolysis in referred new adult patients with idiopathic osteonecrosis ($n = 71$) or secondary osteonecrosis ($n = 62$) with the same measures in sex- and race-matched healthy control subjects. Heritable thrombophilic Factor VIII and hypofibrinolytic Lp(a) were more frequently high in the 71 patients with idiopathic osteonecrosis than in control subjects. High Factor VIII, Factor V Leiden heterozygosity, and resistance to activated protein C, all heritable thrombophilias, were more frequently present in the 62 patients with secondary osteonecrosis than in control subjects. Our data suggest inherited thrombophilia and hypofibrinolysis are risk factors for both idiopathic and secondary osteonecrosis of the head of the femur.

Level of Evidence: Level IV, prognostic study. See the Guidelines for Authors for a complete description of levels of evidence.

Introduction

Osteonecrosis (ON) of the femoral head is either secondary, associated with various factors such as corticosteroids, alcoholism, lupus erythematosus, hip trauma (dislocation, fracture), chemotherapy, HIV-AIDS, dysbaria, and others, or considered idiopathic when there is no known etiology or risk factors [3, 20, 58]. In 1993, we reported two brothers with idiopathic bilateral hip ON who were homozygous for the hypofibrinolytic 4G/4G polymorphism of the plasminogen activator inhibitor-1 gene (PAI-1 gene) and had very high levels of the PAI-1 gene product, hypofibrinolytic plasminogen activator inhibitor (PAI-Fx) [34]. This kindred [34] and four similar patients reported by Van Veldhuisen in 1993 [76] led us to speculate that some cases of ON of the hip are caused by familial or acquired hypofibrinolysis-thrombophilia with resultant pathoetiologic venous thrombosis in the femoral head [5, 24, 32].

Initially we [31], and subsequently others [5, 12, 19, 41, 44, 46, 49, 51, 59, 65, 76, 79, 80], reported data suggesting venous thrombosis in the femoral head, mediated in many cases by thrombophilia and hypofibrinolysis, leads to increased intraosseous venous pressure and thence to impaired arterial flow, osseous hypoxia, and bone death. Beyond hip ON, thrombophilia-hypofibrinolysis also appears associated with some cases of Legg-Calve-Perthes disease [5, 19, 23, 28, 33, 41, 72].

To confirm and extend previous data, we asked whether thrombophilia and hypofibrinolysis were risk factors for ON of the femoral head in patients with idiopathic ON or ON associated with high-dose, long-term corticosteroids. We further asked whether age, race, diabetes, hypertension, and cigarette smoking influenced any association between Factor VIII levels and ON.

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Each author certifies that his or her institution has approved the human protocol for this investigation, that all investigations were conducted in conformity with ethical principles of research, and that informed consent was obtained.

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Materials and Methods

We compared measures of thrombophilia and hypofibrinolysis in 133 previously unreported patients with idiopathic and secondary (corticosteroid-acquired) ON of the head of the femur with healthy race- and sex-matched normal control subjects. Alcoholism, postchemotherapy, HIV-AIDS, and/or a history of fracture or dislocation of the hip excluded patients from the study. After excluding patients for these reasons, we identified 133 previously unreported patients who had measures of thrombophilia and hypofibrinolysis in the course of their evaluations. All patients meeting these criteria were included in the study. The research protocol was approved by the FDA and the Institutional Review Board at the Jewish Hospital; signed informed consent was obtained.

ON was documented by anteroposterior and frog-leg lateral radiographs of both hips and by MRI evaluation [31]. MRI was used to confirm the clinical diagnosis of ON [31]. We made no attempt to quantify the extent of femoral head involvement by MRI. A consensus diagnosis was made from the imaging studies by a four-person committee of radiologists-orthopaedists blinded to patients' clinical status, age, and hip symptoms [31]. No selection bias was used beyond these exclusion criteria. This evaluation provided a new, previously unreported cohort (Table 1) of 71 patients with idiopathic ON and 62 with ON associated with corticosteroids (approximately 3000–4000 mg prednisone or its equivalent) [20, 58]. Seven of 25 women (28%) with idiopathic ON and three of 30 (10%) women with secondary ON developed ON while taking exogenous estrogens.

We estimated sample size based on patient-control differences in key measures of thrombophilia (Factor V

Leiden mutation [10], resistance to activated protein C [22], Factor VIII [38]) and hypofibrinolysis (Lp[a]) [64]. Sample size analyses revealed that with $\alpha = 0.05$ and power 80%, based on patient-control differences in Factor V Leiden [10], Factor VIII [38], resistance to activated protein C [22], and Lp(a) [64], there should be 33 or more, 32 or more, 25 or more, and 32 or more subjects in both patient and control groups, respectively. Our sample size was adequate to ascertain patient-control differences in our key measures of thrombophilia and hypofibrinolysis with $\alpha = 0.05$ and $\beta = 0.20$, because there were 71 patients with idiopathic ON versus 69 control subjects and 62 patients with secondary ON versus 62 control subjects.

The 71 patients with idiopathic ON included 46 men (42 white, four black) and 25 women (23 white, two black). The 62 patients with secondary ON included 32 men (26 white, six black) and 30 women (26 white, four black). As control subjects, 44 adult men were available, including 17 previously described healthy male hospital personnel [5] and 27 new healthy men from family studies (39 white, one black, and four Asian). Fifty-seven healthy adult female control subjects included 23 previously described healthy female hospital personnel [5] and 34 new healthy women from family studies (49 white, five black, and three Asian). Subjects were excluded from the control groups if they were pregnant or taking estrogens, raloxifene, tamoxifen, corticosteroids, or anticoagulants, all of which might affect serologic measures of thrombophilia-hypofibrinolysis [4, 30].

We compared thrombophilia and hypofibrinolysis in patients with 1:1 race- and sex-matched healthy normal control subjects. Mean age in the 46 men and 25 women with idiopathic ON (48 ± 12 years and 49 ± 9 years, respectively) was greater than the race- and sex-matched

Table 1. Differences between patients with idiopathic and secondary osteonecrosis and race- and sex-matched healthy normal control subjects for heritable thrombophilia and hypofibrinolysis

Variable	High factor VIII (greater than 150%)	High Lp(a) (35 mg/dL or greater)	
Idiopathic osteonecrosis (n = 71; 46 men, 25 women)	19/71 (27%)	25/69 (36%)	
Race- and sex-matched control subjects (n = 69; 44 men, 25 women)	3/66 (5%)	12/67 (18%)	
p	0.0004	0.016	
Variable	High factor VIII (greater than 150%)	Factor V Leiden heterozygosity	Resistance to activated protein C
Secondary osteonecrosis (n = 62; 32 men, 30 women)	16/62 (26%)	6/61 (10%)	8/51 (16%)
Race- and sex-matched control subjects (n = 62; 32 men, 30 women)	5/60 (8%)	0/61 (0%)	2/59 (3%)
p	0.011	0.028	0.042

adult male and female control subjects (43 ± 12 years and 43 ± 9 years, respectively). Mean age in the 32 men and in the 30 women with secondary ON was 46 ± 10 years and 44 ± 11 years, respectively, similar to the race- and sex-matched male and female control subjects (41 ± 10 years and 45 ± 14 years, respectively).

Of the 71 patients with idiopathic ON, 19 (27%) smoked, similar to 11 of 69 (16%) race- and sex-matched control subjects ($p = 0.12$). Of the 62 patients with secondary ON, 13 (21%) smoked, similar to eight of 62 (13%) race- and sex-matched control subjects ($p = 0.23$).

As previously described [4, 5], blood was collected in 3.2% buffered sodium citrate (one part citrate:nine parts blood). The samples were immediately transported and centrifuged at $2600 \times g$ for 15 minutes to obtain platelet-poor plasma. The samples were run in batches. The plasma was frozen in aliquots and stored at -70°C . Blood for polymerase chain reaction (PCR) analysis was drawn in tubes containing the appropriate anticoagulant (ethylene diamine tetra-acetic acid).

PCR analysis was used to study four heritable thrombophilic gene mutations: heterozygosity for the G1691A Factor V Leiden, G20210A prothrombin gene, the platelet glycoprotein PL A1/A2 mutation, homozygosity for the C677T MTHFR mutation [4, 5, 24, 30, 35], and the heritable hypofibrinolytic 4G/4G mutation of the PAI-1 gene [4].

Serologic tests used to study thrombophilia were anticardiolipin antibodies IgG and IgM, the lupus anticoagulant, deficiency in proteins C and S (total and free), antithrombin III, homocysteine, and Factors VIII and XI [4, 5, 29, 30, 35]. Protein C, total and free protein S, and antithrombin III levels below the fifth percentile for normal control subjects were considered abnormal [30]. Homocysteine, anticardiolipin antibodies IgG and IgM, Factor VIII, and Factor XI equal to or over the ninety-fifth percentile for normal control subjects were considered abnormal [30, 35].

Hypofibrinolysis studied by serologic tests included PAI-Fx and Lp(a) [4, 5, 29, 30, 35]. Plasma PAI-Fx and Lp(a) levels equal to or over the ninety-fifth percentile for normal control subjects were considered abnormal [30].

Differences in PCR and serologic measures of thrombophilia-hypofibrinolysis between patients with idiopathic or secondary ON and control subjects were assessed using chi square tests (Table 1). Wilcoxon tests were used to compare age in patients versus control subjects. Because Factor VIII might be influenced by age, race, diabetes, hypertension, and cigarette smoking [17], stepwise logistic regression analysis was used with the dependent variable being Factor VIII (level high/normal) and explanatory variables group, age, race, diabetes, hypertension, and cigarette smoking. These models were run separately for

idiopathic and secondary ON. All statistical evaluations were performed using SAS (SAS/STAT software, 9.1.3, 2002; SAS Institute, Cary, NC).

Results

Heritable thrombophilia and hypofibrinolysis were more common in patients with ON than in control subjects (Table 1). Patients with idiopathic ON were more likely ($p = 0.0004$) than sex- and race-matched healthy control subjects to have high (greater than 150%) levels of heritable thrombophilic Factor VIII and were also more likely ($p = 0.016$) to have inherited high levels of hypofibrinolytic Lp(a) (Table 1). Patients with secondary ON were more likely than sex- and race-matched healthy control subjects to have high (greater than 150%) levels of Factor VIII ($p = 0.011$), to be heterozygous for the Factor V Leiden mutation ($p = 0.028$), and to have heritable thrombophilic resistance to activated protein C ($p = 0.042$) (Table 1).

Factor VIII levels in patients with idiopathic and secondary ON were higher than in control subjects ($p = 0.0017$, $p = 0.02$, respectively) independent of age, race, hypertension, diabetes, and cigarette smoking.

Discussion

We [31] and others [5, 12, 19, 41, 44, 46, 49, 51, 59, 65, 76, 79, 80] have suggested venous thrombosis in the femoral head, mediated in many cases by thrombophilia and hypofibrinolysis, leads to increased intraosseous venous pressure and subsequent impaired arterial flow, osseous hypoxia, and bone death. To confirm these suggestions we asked whether thrombophilia and hypofibrinolysis increased the risk for ON of the femoral head in patients with idiopathic ON or ON associated with high-dose, long-term corticosteroids.

Our study had the following limitations. We did not have a second control group who received comparable doses of corticosteroids but who did not develop ON of the femoral head on prospective followup. We did not measure intraosseous pressure or do intramedullary venography [55, 73] to document reduction in venous return, venous stasis, intraosseous hypertension, or decreased arterial inflow. To optimally further explore the hypothesis that inherited or acquired thrombophilia-hypofibrinolysis mediates osseous venous thrombosis [24, 25, 27, 29–34, 39, 43, 44, 51, 59, 62, 65, 72, 76, 79, 80], a placebo-controlled, double-blind clinical trial [31] would be needed.

We found heritable, thrombophilic high Factor VIII was much more common in both idiopathic and

secondary ON than in healthy control subjects. Heritable, hypofibrinolytic high Lp(a) was more common in idiopathic ON than in control subjects. In addition, heterozygosity for the thrombophilic Factor V Leiden mutation and thrombophilic resistance to activated protein C were more common in ON associated with corticosteroids than in normal control subjects. These findings, congruent with the amelioration of idiopathic ON with low-molecular-weight heparin therapy [31], suggested to us and other authors [1, 5, 8, 11, 41, 49, 50, 62, 65, 76, 79, 80] that thrombophilia-hypofibrinolysis-mediated thrombosis is a potentially reversible cause of ON of the head of the femur. Experimental models of ON [12, 13, 52, 63] and Legg-Calve-Perthes disease [57] implicate venous occlusion as a precipitating event with subsequent increased intraosseous pressure, reduced arterial inflow, ischemia, and infarction. We believe thrombophilia-hypofibrinolysis, promoting deep osseous venous thrombosis, initiates this cascade [5, 12, 29, 31, 32, 44, 46, 57, 63].

There are many associations between genetic mutations-polymorphisms and ON. Single nucleotide polymorphisms in the multidrug resistance gene have been associated with corticosteroid-induced ON [2]. Genetic variation in alcohol-metabolizing enzyme genes is related to alcoholism-induced ON [15]. In two families with autosomal-dominant multigenerational idiopathic ON, Chen et al. [16] mapped a candidate gene to a 15cM region between D12S1663 and D12S1632 on chromosome 12q13. Another genetic mechanism for development of idiopathic ON appears to involve mutations in the endothelial nitric oxide synthase gene that controls nitric oxide release [26, 48]. Endothelial nitric oxide synthase polymorphisms can act alone, synergistically with cigarette smoking as a genetic risk factor for idiopathic ON, or in concert with thrombophilia-hypofibrinolysis [26].

In the current report, high levels of thrombophilic Factor VIII [6, 53, 54] were more common in patients with idiopathic ON or secondary ON than in control subjects (27% versus 5%, 26% versus 8%). High Factor VIII can be inherited [6, 53, 54, 69] or acquired related to smoking-diabetes-hypertension-mediated inflammation [17]. In the current study, the higher Factor VIII in patients with idiopathic and secondary ON versus control subjects could not be attributed to race, age, smoking, diabetes, or hypertension, suggesting high Factor VIII in subjects with ON is not an acute phase reactant, but a contributor to thrombosis [45]. Our finding of high Factor VIII associated with idiopathic and secondary ON is congruent with associations of familial thrombophilias (V Leiden, prothrombin gene, low protein S) [39, 65] with idiopathic ON in adults and with Legg-Calve-Perthes disease (V Leiden [5], low protein S [23, 33]).

Familial hypofibrinolytic high Lp(a) was associated with idiopathic ON in the current study and has previously been reported as a risk factor for idiopathic ON [66]. High Lp(a) has been associated with familial clustering of bone marrow edema of the hip in three sisters [7].

The G1691A Factor V Leiden mutation [75] was more common in patients with secondary ON than in healthy control subjects (10% versus 0%). Bjorkman et al. [10] reported a higher prevalence of the V Leiden mutation in patients with idiopathic (but not secondary) ON than in the Swedish population. Zalavras et al. [80] reported that the V Leiden mutation was present in 18% of 72 patients (23 idiopathic, 49 secondary ON) versus 4.6% of control subjects. Bjorkman et al. [9] also reported that the V Leiden mutation was associated with ON of the knee. Celik et al. [14] did not find an excess of the V Leiden mutation in patients who developed corticosteroid-associated secondary ON after renal transplant. The V Leiden mutation and/or thrombophilic resistance to activated protein C are also risk factors for Legg-Calve-Perthes disease [5, 19, 22, 41].

Resistance to activated protein C, a heritable risk factor for venous thrombosis with and without the V Leiden mutation [74], was more commonly present in patients in the current report with secondary ON than in control subjects (16% versus 3%). In secondary ON occurring after corticosteroid-treated severe acute respiratory syndrome, resistance to activated protein C was more common in patients than in control subjects [71].

We found endogenous and exogenous hyperestrogenemia were common in female patients with ON (28% idiopathic, 10% secondary) consistent with our previous studies [27, 30, 37, 39] and those of Montella et al. [61] When estrogen-induced thrombophilia [40] is superimposed on familial thrombophilia-hypofibrinolysis, intraosseous thrombosis is promoted, facilitating development of ON [30, 32, 36, 37, 39].

Preservation of the femoral head is the ultimate goal of treatment of ON, but, as summarized by Lieberman [56], "...development of successful strategies to treat this disease has been difficult to do because ON is associated with numerous different diseases and neither the etiology nor the natural history have been delineated clearly." Assouline-Dayana et al. [3] concluded "...management of osteonecrosis is primarily palliative and does not necessarily halt or retard the progression of the disease. Treatment options focus on repairing the secondary changes that develop in the femoral head and not on reversing the idiopathic pathology." Currently, strategies for treatment of ON are difficult to develop [56], do not reverse ON pathologies [3], do not halt progression to segmental collapse [3], and all have certain limitations [18, 21, 42, 47, 70, 71]. Having outlined the strong association between thrombophilia-hypofibrinolysis with ON of the hip here and elsewhere [24, 25, 27, 29–34, 39,

43, 44, 51, 72, 79, 80], we have studied the use of 3 months of low-molecular-weight heparin in patients with Ficat Stage I or II ON of the hip and one or more thrombophilias or hypofibrinolyses [31]. Anticoagulation [29, 31, 32] with low-molecular-weight heparin [67] can stop the progression of idiopathic hip ON [31] in patients with thrombophilia-hypofibrinolysis, decreasing the frequency of THA [31].

The diagnosis of thrombophilia-hypofibrinolysis is also important in patients with ON because of associations with other venous thromboses, as a stimulus for coagulation screening in first-degree relatives of affected probands, and in identifying patients at high risk for deep venous thrombosis- pulmonary emboli after hip-knee arthroplasty for whom longer-term postoperative thromboprophylaxis may be warranted [60, 68, 77, 78].

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