Thrombophilic Abnormalities in Patients With or Without Pulmonary Embolism Following Elective Spinal Surgery

A Pilot Study

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Abstract *Background:* Significance of the thrombophilic abnormalities in development of venous thromboembolism (VTE) has been studied with total hip arthroplasty and acute traumatic spinal cord injury. However, their role as risk factors for VTE in elective spinal surgery remains to be determined. *Questions/Purposes:* To determine the role of thrombophilic abnormalities in the development of pulmonary embolism (PE) following elective spine surgery. *Methods:* Case and control groups were created in patients who had undergone elective spinal surgery for degenerative conditions. The PE group comprised 12 patients whose post-operative course was complicated by development of PE. The control group included 12 patients

with an uneventful post-operative course. Demographic data including age, gender and surgical procedures were matched between the PE group and the control group. Both groups were evaluated for thrombophilic and hypofibrinolytic risk factors at 3 months post-operatively or later. Blood tests were performed to measure fasting serum homocysteine, antithrombin III, and protein C. Molecular genetic testing was conducted for detection of the plasminogen activator inhibitor-1 4G/4G, and prothrombin 3' UTR gene mutations. *Results:* Heterozygous mutation (G20210A) of prothrombin was detected in two patients (16.7%) in the PE group, whereas no such mutation was noted in the control group. Plasminogen activator inhibitor-1 4G/4G homozygous mutation was seen in three in the PE group and two in the control group. Of homocysteine, antithrombin III and protein C, only one patient in each group showed abnormal levels of homocysteine. In total, half of the patients in the PE group had at least one thrombophilic abnormality, whereas three (25%) patients showed such abnormality in the control group. *Conclusion:* These findings suggest the involvement of thrombophilic abnormalities, especially the heterozygous G20210A mutation, in the development of PE in patients undergoing elective spinal surgery.

Level of evidence: Level III Prognostic Study.

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Introduction

Venous thromboembolic disease, including deep vein thrombosis (DVT) and pulmonary embolism (PE), continues to pose significant morbidity and mortality following orthopedic surgery [3, 10, 11, 13]. In the current practice, patients at high risk for venous thromboembolism (VTE), such as those undergoing arthroplasty or hip fracture surgery, are routinely treated with a pharmacological thromboprophylactic

measure [2]. In contrast, no pharmacological prophylaxis is recommended in patients without additional risk factors who undergo elective spinal surgery [2, 6]. This is due to the concern for resulting bleeding complications including epidural hematoma [4] and also the thought that the risk of VTE in elective spinal surgery is relatively low [3, 6, 9, 13]. Therefore, understanding and evaluation of risk factors for VTE are important in decision making of thromboprophylaxis treatment in cases with elective spinal surgery.

Inherited thrombophilias serve as a group of risk factors predisposing to VTE [5, 8, 17]. They are caused by deficiencies or dysfunction of proteins of the clotting cascade, including antithrombin III, protein C, prothrombin and plasminogen activator inhibitor-1. Significance of such thrombophilic abnormalities in development of VTE has been studied with total hip arthroplasty [18] and acute traumatic spinal cord injury [12]. However, their role as risk factors for VTE in elective spinal surgery remains to be determined.

In the present study, we created case and control groups in patients who had undergone elective spinal surgery for degenerative conditions and comparatively analyzed the deficiency or mutation of thrombophilic proteins.

Our aim was to determine the role of thrombophilic abnormalities, including that of serum homocysteine, antithrombin III, protein C and molecular genetic abnormalities, in the development of PE following elective spine surgery.

Methods

Following approval from Institutional Review Board, case (PE) and control groups of 12 patients each, from a single institution, were created. Inclusion criteria for case group were patients who underwent major elective spinal surgery for lumbar fusion, and developed PE within 2 weeks of the procedure. This was diagnosed and documented by pulmonary computerized tomography. Informed consents were obtained from each patient. Inclusion criteria for control group were patients, with matched demographics, who underwent same or similar elective spinal surgery for fusion, and did not develop the above complication. The same protocol for post-operative prophylaxis was applied to all patients, consisting of Pneumatic Sequential Compression Device (PSCD) and early mobilization with physical therapy. PSCD was applied during the operation and post-operatively in the hospital. All patients were interviewed for known thromboembolic risk factors.

To determine whether thrombophilic abnormalities are associated with development of PE following elective spine surgery, we created two groups and compared clinical thromboembolic risk factors and thrombophilic abnormalities. The case group included 12 patients whose post-operative course was complicated by development of PE. CT scans of the chest were used to diagnose PE in 11 patients and ventilation–perfusion scan in the remaining one patient. The diagnosis was made at an average of 9.3 days (range, 5–15 days) following surgery. Patients in control group had an uneventful post-operative course. DVT was ruled out by using ultrasound (US) Doppler examination as a screening test, of the lower extremity veins at post-operative day 15, or earlier if indicated on clinical

Table 1 Patient demographics

Parameter	PE group	Control group
Age (years) ^a	61.6±10.9	61.6±10.0
Male	5 (41.6%)	6 (50%)
Body mass index (kg/m ²) ^a	28.6±4.9	27.6±7.5
Cervical spine surgery	1 (8.3%)	1 (8.3%)
Lumbar spine surgery	11 (91.7%)	11 (91.7%)

^a Mean ± standard deviation

exam. Demographic data including age, gender and surgical procedures were well matched between the PE group and the control group (Table 1). Number of patients who had thromboembolic risk factors did not differ between the groups, including advanced age, active cancer, lower extremity paresis, previous VTE, and anterior or anterior–posterior spinal surgery (Table 2).

Both groups were evaluated for thrombophilic and hypofibrinolytic risk factors at a mean of 13.2 weeks (range, 11–15.2) post-operatively. Blood tests were performed to measure fasting serum homocysteine, antithrombin III, and protein C. In addition, molecular genetic testing was carried out for detection of the plasminogen activator inhibitor-1 4G/4G, and prothrombin 3 UTR gene mutation. Molecular analyses of the above-listed genes were performed by single polymerase chain reaction with the use of the patient genomic DNA isolated from the peripheral blood leukocytes. The polymerase chain reaction amplification, performed twice for each DNA sample, was carried out as previously reported [18]. Polymerase chain reaction products were detected by ethidium bromide staining after digestion with specific restriction enzymes and electrophoresis in 1.5% agarose gels.

Statistical analysis was performed under guidance of a statistical consultant with a commercial statistical software package (PRISM Version 4.0 for Macintosh; Graphpad). Grouped data was tested for normal distribution using the Kolmogorov–Smirnov test. Two-tailed Student's *t*-test and Mann–Whitney test was used for normally and not-normally distributed data for inter-group comparison, respectively. The Fisher's exact probability test was used for the analysis

Table 2 Frequency of patients with thromboembolic risk factors

Parameter	PE group	Control group	<i>p</i> value
Advanced age (over 65 years)	4 (33.3%)	5 (41.6%)	0.67
Active cancer	0 (0%)	0 (0%)	1.00
Lower extremity paresis	0 (0%)	0 (0%)	1.00
Previous VTE	0 (0%)	0 (0%)	1.00
Anterior or antero-posterior spinal surgery	4 (33.3%)	4 (33.3%)	1.00
Obesity (BMI >30 kg/m ²)	4 (33.3%)	5 (41.6%)	0.67
Estrogen-containing oral contraceptives	0 (0%)	0 (0%)	1.00
Hormone replacement therapy	0 (0%)	0 (0%)	1.00
Inflammatory bowel disease	0 (0%)	0 (0%)	1.00
Nephrotic syndrome	0 (0%)	0 (0%)	1.00
Myeloproliferative disorder	0 (0%)	1 (8.3%)	0.31

BMI body mass index, *PE* pulmonary embolism, *VTE* venous thromboembolism

Table 3 Frequency of thrombophilic abnormalities

Parameter	PE group	Control group	<i>p</i> value
Prothrombin G20210A mutation (heterozygous)	2 (16.7%)	0 (0%)	0.14
Plasminogen activator inhibitor-1 mutation (4G/4G homozygous)	3 (25%)	2 (16.7%)	0.62
Homocysteine level (>15 $\mu\text{mol/l}$)	1 (8.3%)	1 (8.3%)	1.00
Antithrombin-III level (<75% of normal)	0 (0%)	0 (0%)	1.00
Protein C level (<70% of normal)	0 (0%)	0 (0%)	1.00
At least one abnormality	6 (50%)	3 (25%)	0.21

PE pulmonary embolism

of data in a 2×2 cross contingency table. The level of significance was set at $p < 0.05$.

Results

In regard to thrombophilic abnormalities, there was a difference in the genetic testing compared between our study groups. Of two genetic abnormalities examined, heterozygous mutation (G2021A) of prothrombin was detected in two patients (16.7%) in the PE group (Table 3). In contrast, no such mutation was noted in the control group. Plasminogen activator inhibitor-1 4G/4G homozygous mutation was seen in three in the PE group and two in the control group. Serum levels of homocysteine, antithrombin III and protein C were not significantly different between the PE group and the control group (Fig. 1). None of the patients exhibited homozygous G2021A mutation. Of homocysteine,

antithrombin III and protein C, only one patient in each group showed abnormal levels of homocysteine. In total, half of the patients in the PE group had at least one thrombophilic abnormality, whereas three (25%) patients showed such abnormality in the control group. The differences were not statistically significant. None of the patients had two or more thrombophilic abnormalities.

Discussion

In this pilot study, we analyzed thrombophilic abnormalities in two groups of patients who had undergone elective spinal surgery. We found no difference in the incidence of thrombophilic abnormalities comparing the PE and control groups. However, heterozygous mutation (G2021A) of prothrombin was detected in two patients (16.7%) in the PE group in contrast to no detection of this mutation in the controls.

The pilot study is preliminary in nature and has its limitations, including the small number of participants leading to the underpowered analysis. Also, our study lacked comprehensive analysis, that included the factor V Leiden mutation and ethylenetetrahydrofolate reductase mutation. Our study also faced the inadequacy of verification or work-up bias where we depended on only one laboratory for results.

The role of thrombophilic abnormalities on VTE has been analyzed by a number of case control studies [1, 5, 7, 14, 16]. However, in these studies, the case groups comprised patients with DVT or those with VTE (DVT patients and PE patients). The present study is strict in design where the patients with PE, but not those with DVT, constituted the case group. Although, this is a minor drawback of our study, the authors

Fig. 1. Serum levels of homocysteine, antithrombin III, and protein C in the PE group and the control group. Levels of serum homocysteine were $11.6 \pm 2.4 \mu\text{mol/l}$ (mean $\pm$ SD) in the PE group and $11.4 \pm 2.3 \mu\text{mol/l}$ in the control group (a). Levels of antithrombin III were $104.7 \pm 11.6\%$ in the PE group and $103.1 \pm 11.7\%$ in the control group (b). Levels of protein C were $131.9 \pm 25.0\%$ in the PE group and $128.6 \pm 34.7\%$ in the control group (c). There were no statistically significant differences between the groups.

chose to focus on PE alone as it may be fatal in several cases and no clear guidelines exists in its pre-operative identification of risk factors or protocol for prophylaxis. Future studies of this nature, with larger sample size are indicated.

The heterozygous G2021A mutation of prothrombin gene was detected in two patients (16.7%) in the PE group, and none in the control group. In our previous study [18] analyzing patients undergoing total hip arthroplasty, four of the 14 patients (28.6%) in the PE groups showed the G2021A mutation of prothrombin. None of the 14 patients in the control group experienced such mutation. These findings correctively support the high specificity (100%) of this mutation in association with PE. A meta-analysis by Gohil et al. [5], revealed that 1,859 out of 21,605 patients (8.7%) in the case group with VTE (DVT or DVT + PE) and 849 of 27,947 (3.0%) in the control groups showed prothrombin G2021A mutation, respectively. None of the 79 articles analyzed had the frequency of this mutation over 5% in the control group [5]. Accordingly, the mutation is expected in less than one patient in a cohort of 12 patients. The presence of two patients with the prothrombin G2021A mutation (16.7%) is much higher percentage than expected.

Plasminogen activator inhibitor-1 4G/4G homozygous mutation was seen in three (25%) in the PE group and two (16.7%) in the control group. In the literature, a meta-analysis with 22 articles by Tsantes et al. [15], disclosed the frequency of the 4G/4G homozygous mutation in 29.0% (767 of 2,644 patients) in the case group (DVT or DVT + PE) and 24.0% (897 of 3,739 patients) in the control group, indicating the lack of striking difference. Plasminogen activator inhibitor-1 4G/5G heterozygous mutation was detected, in the present study, in eight (66.7%) in the PE group and six (50%) in the control group. In the meta-analysis the frequency of the 4G/5G heterozygous mutation was 52.2% in the case group and 47.9 in the control group [15].

In conclusion, this pilot study provides compelling evidence of an association of thrombophilic abnormalities and PE in patients undergoing elective spinal surgery, especially with respect to the association of the heterozygous G2021A mutation with PE risk. The heterozygous G2021A mutation represented a highly specific thrombophilic abnormality (100% specificity in both the present study and our previous study) for PE, suggesting an important role in the development of PE following elective spinal surgery.

Disclosures Each author certifies that he or she has no commercial associations (e.g., consultancies, stock ownership, equity interest, patent/licensing arrangements) that might pose a conflict of interest in connection with the submitted article. One or more of authors has or may receive payments or benefits from a commercial entity that may be perceived as a potential conflict of interest.

Each author certifies that his or her institution has approved the reporting of these cases, that all investigations were conducted in conformity with ethical principles of research, and that informed consent for participating in the study was obtained.

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