The Frequency of Factor V Leiden Mutation in Patients with Lung Cancer

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Abstract

Factor V (FV) Leiden mutation is responsible for the development of inherited activated protein C (APC) resistance in the majority of cases and cancer is an acquired risk factor for thrombosis. The aim of this sudy is to investigate the factor V Leiden mutation frequency in patients with lung cancer. Screening for the FV Leiden mutation in 44 patients with lung cancer was performed using polymerase chain reaction amplification of exon 10 followed by Mnl I restriction enzyme digestion and agarose gel electrophoresis. Results are as follows: 44 lung cancer patients were included in the study (40 males, 4 females, age range 42-87, median age 60); 34 (77%) had non-small cell lung cancer (NSCLC), 9 (21%) had small cell lung cancer (SCLC) and one had a combined carcinoma. Six of them were nonsmokers while the others were heavy smokers and they had no history of previous thromboembolic events. Only one patient was heterozygous and none of them was homozygous for FV Leiden mutation. Factor V Leiden mutation frequency was not increased in patients with lung cancer. However further investigations should be undertaken to assess the interaction of genetic risk factors and cancer. It would be beneficial to define the risk rates for cancer patients receiving anticoagulant therapy who are carriers of a genetic risk factor and have no genetic risk factor for thrombosis.

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Introduction

Thromboembolism is one of the common complications in patients with malignancies. Thrombosis occurs when physiological antithrombotic systems are defective or when prothrombotic activity overwhelms the normal physiological antithrombotic mechanism in cancer patients (1,2). Tumor cells may directly activate the blood clotting system or indirectly stimulate mononuclear cells to synthesize and express various procoagulants (2-4) and other risk factors that would enhance the risk of thrombosis, including surgery, bed rest, chemotherapy, stroke previous venous thromboembolism, infection, advanced age (1,3,5,6). Protein C, the key component of the natural anticoagulant pathway, inhibits coagulation by degrading Factors Va and VIIIa when activated. Normal balance of procoagulant and anticoagulant mechanisms favor anticoagulation. When a defect occurs in this system, there will be an increased risk of thrombosis (7). Inherited activated protein C (APC) resistance, which is found in 20 to 60% of patients with venous thrombosis is due to a single point mutation in the FV gene-substitution of arginin (R) at position 506 with a glutamin (Q), named FV Leiden in at least

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Table 1. Patient characteristics	
Histopath cell type	**No of patients**
NSCLC	34
-squamous cell	22
-adenocancer	9
-large cell	1
-unclassified	2
SCLC	9
Combined carcinoma (squamous-small cell)	1
Stages	
IA	1
IB	1
IIB	1
IIIA	4
IIIB	17
IV	11
Limited disease	7
Extensive disease	2
Smoking habit	
Smoker	38
Nonsmoker	6

95% of cases (7). Risk factors known to increase the risk of thrombosis may be either genetic or acquired or of combined origin and attention has focused recently on the role of inherited and acquired molecular factors in determining thromboembolic risk (6,8). It can be suggested that alterations in the coagulation system of cancer patients may interfere with existing activated protein C resistance caused by FV Leiden mutation which would increase the risk of thrombosis for these patients. Therefore, we have investigated the FV Leiden mutation frequency in our patients with lung cancer.

Material and Methods

The study group consisted of patients diagnosed with lung cancer in our department, from different regions of our country. In staging evaluation thoracoabdominal and cranial CT scans and whole body bone scans were used. Staging was done by using TNM staging for NSCLC patients (9). Patients with SCLC were grouped as having limited or extensive disease [10]. Their smoking habits and previous thromboembolic events were ascertained. Blood samples were taken before they received any treatment and were stored at -20°C. Screening for the FV gene 1691 G—A mutation was performed using polymerase chain reaction amplification of exon 10 using primers 5' TGCCCAGTGCTTAACAAGACCA 3' and 5' CTTGAAG-GAAATGCCCCATTA3' followed by Mnl I restriction enzyme digestion and agarose gel electrophoresis (11).

Results

Forty four lung cancer patients (40 males and 4 females) were studied. Ages ranged between 42 and 87, and the median age was 60. The characteristics of the patients are shown in Table 1. They had no thromboembolic event history. Six of the 44

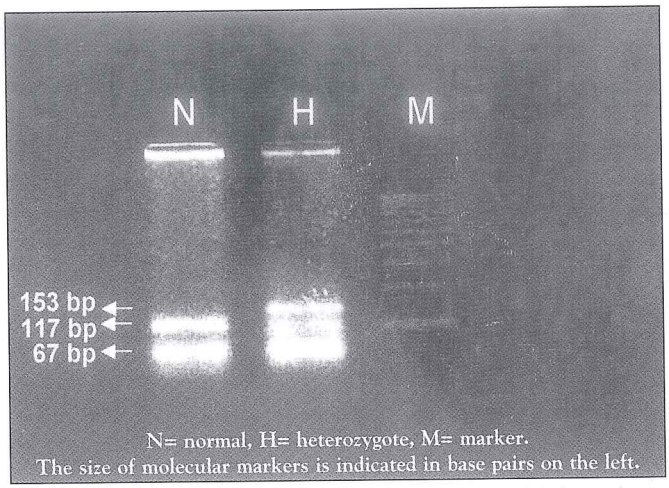

Figure 1. *Agarose gel electrophoresis photograph.*

patients had no history of smoking (4 females and 2 males), while the others were heavy smokers. According to haplotype analysis, only one patient was heterozygous and none was homozygous for FV Leiden mutation. A photograph from Agarose gel electrophoresis is seen in Figure 1.

Discussion

The pathogenesis of venous thrombosis is complex, involving the interaction of acquired risk factors with genetic predispositions (12). Van Boven et al (13), recently reported that the incidence of thrombosis in the context of inherited antithrombin deficiency combined with an acquired risk factor was increased 20-fold, but they did not include malignancy among the acquired risk factors. The prevalence of venous thrombosis in cancer patients has been estimated at up to 15% antemortem and higher (50%) postmortem (14). The incidence of newly diagnosed malignancy is increased among patients with unexplained venous thromboembolism during the first 6 to 12 months after the thromboembolic event (15). Twenty percent of lung cancer patients develop venous thrombosis during the course of the disease (16). De Lucia et al (17), investigated activated protein C pathway in patients with advanced gastrointestinal cancer and genetic analyses showed that only one patient had a FV Leiden mutation. They suggested that the high prevalence of APC resistance in cancer is due to an acquired defect in the APC pathway. Green et al. (18) studied activated protein C resistance in advanced cancer patients and they concluded that APC resistance is not due to FV Leiden but is associated with elevated levels of factor VIII and fibrinogen; they evaluated 39 patients who had different types of solid tumors; they had advanced metastatic cancer and most were on chemotherapy, radiotherapy or hormonal therapy.

Haim et al (19), reported recently that the prevalence of FV Leiden mutation in cancer patients with thromboembolism (2%) was not significantly different from that in cancer patients without thromboembolism, but was significantly lower than that of patients with thromboembolism without cancer (33%). Sifontes et al (20), found that FV Leiden mutation does

not play a significant role in the overall incidence of thrombosis in children with cancer, but they also recommend that children with cancer and a family history of venous thromboembolism should be evaluated for FV Leiden mutation. In another study, it is reported that among patients with malignancies, carriers of FV Leiden mutation had a five-fold increased risk of thromboembolism (8). Our study is somewhat different from other studies, because we enrolled only lung cancer patients with different stages (IA-IV, limited-extensive disease), who had not received any treatment, and had no history of previous thromboembolic events; and this is the first study performed for the factor V Leiden mutation frequency in patients, having the same kind of solid tumor. Our primary aim was to find the frequency of FV Leiden mutation in lung cancer patients under well known increased risk of thrombosis before they exposed to additional acquired risk factors due to their malignancy such as chemotherapeutic agents, surgical procedures, bed rest and infections.

Factor V Leiden mutation is common in Caucasians with a prevalence of 1% to 15% in Western societies. Heterozygotes for this mutation have a seven fold increased risk of venous thrombosis and homozygotes are eigthy times more susceptible [12]. For homozygous conditions the mutation rate was reported as 7% by Özbek et al., and 10% by Akar et al., 10% by Gül et al. in different studies (21,22,23).

These data reflect the frequency of the mutation in healthy individuals living in Ankara and İstanbul, the two largest cities, representative of the Turkish population. These results are as high as those in Europe. In our study, we found the heterozygocity rate as 2% and there was no patient homozygote for the mutation. Compared with the other study groups from different regions of Turkey the heterozygocity is relatively lower in our study group of lung cancer patients. The low heterozygocity rate may be due to the small number of patients. The presence of heterozygosity may influence and have a synergistic effect on the increased risk of thrombosis in these selected groups of cancer patients.

The question is: Should the lung cancer patients be screened for this mutation? What will be the rationale for screening? The patients with malignancies who are carriers of FV Leiden mutation would be under an increased risk for thrombosis due to the presence of both genetic and acquired risk factors, in spite of the reports that there is no strict relation between the FV Leiden mutation and increased risk of thrombosis, apart from one study which reported a five-fold increase in risk (8,17,18,20). Our study also shows that the frequency of FV Leiden mutation is not increased in cancer patients. Hence, it is not easy to assess the risk of thrombosis for individual cancer patients due to the existence of many factors. We can suggest that improvements in prevention and management of thromboembolism would reduce morbidity and mortality.

Therefore, after a careful evaluation of previous thrombosis, family history, ethnic origin and other individual acquired risk factors, laboratory tests including clotting factors, fibrinogen, fibrinogen/fibrinogen degradation products, thrombocytosis, (24) factor V Leiden can be investigated in the high risk patients.

We believe that interaction of genetic risk factors and cancer in the occurence of thrombosis should be investigated further. It would be beneficial to define the risk rates for cancer patients receiving anticoagulant therapy who are carriers of a genetic risk factor and have no genetic risk factor for thrombosis.

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