Factor V Leiden in Patients with Venous Thrombosis in Slovak Population

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Abstract. Resistance to activated protein C determined by factor V Leiden (FVL) is the most frequent inherited risk factor of venous thrombosis. The purpose of our work was to reveal the frequency of FVL in Slovak patients with venous thromboses, to characterise the nature of venous thromboses in this inherited thrombophilia, and to consider the screening approach to investigation of FVL in patients with venous thromboses.

350 patients with a diagnosis of venous thromboembolic disease from various regions of Slovakia were investigated. FVL, detected by polymerase chain reaction, was found in 128/350 (37%) patients with venous thromboses. 118/128 (92%) patients were heterozygous and 10/128 (8%) were homozygous carriers.

In 108/128 (84%) patients with FVL the thromboembolic disease occurred spontaneously. Phlebothrombosis occurred predominantly in the lower limbs – 117/128 (91%) patients, atypical localisations were rare. The first thromboembolic event was manifested before 40 years of age in 69% of patients. The family history was positive in 60/128 (47%) FVL carriers with thromboembolic disease. Recurrent thrombosis occurred in 30% of patients with FVL.

In agreement with findings in other European countries, the prevalence of FVL was high in Slovak patients with thromboembolic disease. The investigation of FVL seems to be justified in patients before 40 years of age with venous thrombosis of lower limbs, in the absence of triggering factors and with a family history of venous thromboembolic disease.

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Key words: Venous thrombosis — Thromboembolic disease — APCR — Factor V Leiden — Prevalence

Introduction

Thromboembolic disease is the third most frequent cause of morbidity and mortality in civilised countries and represents a serious health and social problem (Murray and Lopez 1997; Federman et al. 2002; Press et al. 2002).

Beside external risk factors a number of inherited thombophilic states can participate on thrombosis development (Lane et al. 1996; Simkova et al. 2001). Deficiency of the antithrombin III, protein C, protein S and resistance to activated protein C (APCR), along with a newly discovered mutation of prothrombin G20210A are the most important genetic determinants of venous thrombosis (Bertina et al. 1994; Arnaldi et al. 2001; Lane et al. 1996; Poort et al. 1996).

Although a deficit of antithrombin III, protein C and protein S are abnormalities associated with high incidence of thrombosis, they are relatively rare, participating in only 5–10 % of venous thromboses (Pabinger et al. 1992; Lane et al. 1996). The relatively recently discovered APCR, which is primarily based on genetical disorder known as factor V Leiden (FVL), occurring in white race in 3–13 % (De Stefano et al. 1996), has a high prevalence among patients with venous thromboses (20–60 %) (Bertina et al. 1994; Svenson and Dahlbck 1994; Bucciarelli et al. 1999) and attracts enormous interest at present.

In about 95 % of cases, the APCR is determined genetically by an abnormality of the factor V (i.e. factor V Leiden) (Ridker et al. 1995; Rosendaal et al. 1995; Bucciarelli et al. 1999). The point mutation of factor V in the position of nucleotide 1691, where guanine is replaced by adenine, provokes a structural change on the splitting place of the factor V, where the splitting by activated protein C takes place most quickly under physiological conditions. The disorder in splitting and persistence of active FVL in the circulation increases the potential for a pathologic thrombosis (Bertina et al. 1994). FVL is inherited by an authosomal dominant way. Heterozygous carriers have a 3–7 times increased risk of venous thrombotisation, homozygous even 80 times (Rosendaal et al. 1995; Gardner 2003).

Although there is enough data with respect to the prevalence of FVL in different parts of the world under various pathologic conditions, data of this kind involving Central and Eastern Europe are scarce. The aim of this work was to reveal the frequency of FVL in the set of Slovak patients with venous thrombosis and to characterise the nature of thrombosis in patients with FVL.

Materials and Methods

The study consisted of 350 patients, 216 women and 134 men, being the indoor-patients hospitalised at clinics of internal medicine or outpatients followed up in haematological ambulances since 1998 till 2002 with the diagnosis of venous thrombosis.

We received from each patient, beside a blood sample for genetical analysis, a questionnaire about the nature of venous thrombosis, the age when the first thrombosis occurred, concomitant occurrence of other prothrombotic risk factors, and result of APCR investigated by hemocoagulation method (ProC Global or ProC Global with factor V-deficit plasma). The preliminary investigation of APCR by coagulation method, however, was not necessary. The sampling was performed with the patient's consent.

Blood samples for genetical investigation (5 ml blood with 0.5 ml of 0.5 molar solution of EDTA) were stored by the temperature of −20 °C. Subsequently the DNA was isolated and FVL was investigated by polymerase chain reaction (PCR). 5′TGC CCA CTG CTT AAC AAG ACC A-3′a 5′-TGT TAT CAC ACT GGT GCT AA-3′oligonucleids as primers and Taq polymerase in 35 cycle PCR with temperatures 94 °C/60 °C/72 °C in individual steps were used for PCR analysis. The specific enzyme Mnl1 (Biolabs, MA, USA) was used for a restriction analysis. PCR analysis involved multiple amplification of 267 base pairs of the long DNA sequence of factor V and splitting by Mnl1. This splitting procedure revealed whether the allele in the position 1691 was normal (guanine) or mutated. The fragments of DNA were visualised by gel electrophoresis. The splitting, which resulted in fragments of 37, 67 or 167 base pairs, indicated the normal finding in the position 1691. The splitting resulted in fragments with 67 and 200 base pairs, indicating mutated allele in the position 1691 (Bertina et al. 1994; Kalafatis et al. 2002). The homozygous carriership of the mutation was present, when beside the long fragments with 67 and 200 base pairs the splitting also on fragments with 37 base pairs occurred. Heterozygous carriership was characterised with splitting on fragments with 37, 67, 167 and 200 base pairs of the long segment (Bertina et al. 1994; Kalafatis et al. 2002).

The results of investigation of the FVL in the population with a thromboembolic disease were evaluated with respect to clinical data acquired from the questionnaires.

Results

In the period from 1998 to 2002, FVL was investigated in 350 patients of the age from 2 to 78 years with thromboembolic disease, 216/350 (62 %) were women and 134/350 (38 %) men.

The analysis of clinical data revealed that in the whole set of 350 patients as many as 268 (77 %) suffered from the phlebothrombosis of the lower extremities. Other locations included thrombosis of vena (v.) axillaris in 7/350 (2 %) patients, of v. jugularis in 3/350 (1 %), brain sinuses in 7/350 (2 %), v. retinae in 3/350 (1 %), of v. mesenterica in 3/350 (1 %) and v. lienalis in 6/350 (2 %) patients. The pulmonary embolism without data on thrombosis location as a source of embolism occurred in 53/350 (15 %) of patients.

In the set of 268 patients with a phlebothrombosis of the lower extremities, the thrombus location was defined in 168/268 (63 %) patients, of them ileofemoral

thrombosis (IFT) being in 88/168 (52%) patients, femoropopliteal thrombosis (FPT) in 28/168 (17%) patients, and popliteal thrombosis (PT) in 52/168 (31%) patients. In the rest of patients with lower limb thrombosis – 100/268 (37%), the specific locus of the lower extremities phlebothrombosis was not defined.

By DNA analysis, the mutation of factor V (FVL) was found in 128 (37%) of 350 investigated patients. This group, involving 81/128 (63%) women and 47/128 (37%) men, underwent further analysis. It was realised that 118/128 (92%) patients were heterozygous and 10/128 (8%) were homozygous carriers of the factor V mutation.

A positive family history of thrombosis in close relatives (parents, grandparents, and siblings) of FVL positive patients was found in 60/128 (47%).

The age, when the first thrombotic attack occurred, was indicated in 83/128 (65%) of the carriers of factor V mutation. Of these patients, the first thrombosis occurred in 57/83 (69%) cases before the 40^{th} year of age, in 19/83 (23%) before the 50^{th} year, in 2/83 (2%) before the 60^{th} year and in 5/83 (6%) after the 60^{th} year. 45/128 (35%) patients had no data with respect to the onset of the first thrombotic attack. In all 10 homozygous individuals the first thrombosis was manifested before the 50^{th} year, while in 7 of them the disease was manifested between the 30^{th} and the 40^{th} year of age.

In 108/128 (84%) patients with FVL, no immediate triggering factor for venous thrombus was found. The most frequent potential triggering factor for venous thrombosis among probands with FVL was gravidity, which was present in 14 (11%) patients. Less common were surgical interventions in 4 (3%) patients and oral contraceptives in 2 (2%) patients. No additional triggering factors were present in 9/10 (90%) homozygous carriers of FVL, in 1/10 patient the thrombosis was associated with a surgical intervention.

In carriers of FVL phlebothrombosis of lower limbs prevailed, occurring in 117/128 (91%) patients. A more accurate location of thrombosis was defined in 71/128 (55%) cases. Of these 71 patients, 40 (56%) patients had IFT, 24/71 (34%) PT, and 7/71 (10%) FPT. In 2/128 (2%) patients, thrombosis of mesenteric vein was detected and in 9/128 (7%) patients, the source of pulmonary embolism was not detected. Recurrent thrombosis occured in 39/128 (30%) patients with FVL.

Discussion

The data of authors from countries neighbouring Slovakia indicates a relatively high incidence of mutations of FVL, with the incidence of the heterozygotic form 6.5% in Czech (Paseka et al. 2000), 6.5% in Hungary (Stankovics et al. 1998), 7.5% in Germany (Ehrenforth et al. 1999), and 5.0% in Poland (Herrmann et al. 1997). The incidence of the carriership of FVL is probably similar in the Slovak population.

FVL was detected by PCR analysis in 128 of 350 patients with a thromboembolic disease, while probands originated from various parts of Slovakia. The 37% prevalence of factor V mutation among Slovak patients was in agreement

with findings from other countries, varying between 20 % and 60 % (Bertina et al. 1994; Arruda et al. 1995; Bucciarelli et al. 1999; Dulicek et al. 2000; Nizankowska-Mogilnicka et al. 2003).

The participation of heterozygous (92 %) and homozygous (8 %) carriers of FVL agreed with previous findings of a several-fold higher prevalence of heterozygous than homozygous carriers (Dulicek et al. 2000). Similar to the whole group of patients with thromboses, there were more women than men among patients with FVL. This could be determined by the fact that women may face such conditions as gravidity, puerperium and taking oral contraceptives or hormonal replacement therapy during life. Owing to these external prothrombogenic conditions FVL may have greater potential in women to manifest clinically in the form of thrombosis than in men.

In some patients, PCR analysis of FVL was accomplished after previous APCR investigation by a haemocoagulation method (ProC Global or ProC3 Global with factor V deficient plasma). Calculation of the correlation between the genetic and coagulation method was omitted, since coagulation method was used only in a limited number of patients.

As many as 47 % of FVL positive patients had positive family history of venous thromboses. Supposedly, this high number may be, in part, linked to the autosomal dominant mode of inheritance of FVL. In our set of patients, the first venous thrombotic attack occurred before the age of 40 years in as many as 57 of 83 FVL positive patients, in whom the age of the occurrence of the 1st attack was indicated. This is in conformity with the nature of inherited thrombophilic states, including FVL, which has a typical onset of thrombosis before the age of 45 (Lane et al. 1996; Dulicek et al. 2002; Press et al. 2002). In our study, on the other hand, the relatively late manifestation of thrombosis after the age of 50 years in some heterozygous carriers of FVL and the asymptomatic course till the age of 40–50 years in some homozygous carriers confirmed previous data (Press et al. 2002) that FVL may be a less important risk factor for thrombosis for a particular carrier than other inherited thrombophilias.

In 84 % of patients with FVL (in both homozygous and heterozygous carriers), no additional triggering factor of thrombosis was present. It is assumed that FVL was an independent risk factor for thrombotisation. Other risk factors were present only in a small number of patients, while gravidity was a dominant factor (18 %). This result differs from the finding of Rodeghiero and Tosetto (1999), who have shown that trauma and operative intervention were the most frequent precipitants in patients with FVL. This discrepancy may be associated with different choice of patients, since predominantly patients with thrombosis after surgery were involved in the above-mentioned study (Rodeghiero and Tosetto 1999).

The inherited thrombophilias used to be linked with severity and unusual locations of thrombosis (Press et al. 2002). However, this was not the case in our study, where 91 % of patients had deep venous thrombosis of the lower extremities and the thrombosis of v. mesenterica occurred in only 2 % and the pulmonary embolism of unknown origin in 7 % of FVL carriers.

It is not clear, whether the carriership of FVL increases the risk of recurrence of venous thrombotisation after the first attack (Sheppard 2000; Eckman et al. 2002). In our work, 30 % of patients with FVL had a recurrent thrombosis suggesting that the FVL increases the risk of thrombosis recurrence.

It is known that anticoagulation treatment reduces the risk of thrombosis in FVL positive patients within a particular condition. This strategy must, however, be carefully considered. Even recurrent thromboses should not automatically have anticoagulant treatment. According to Eckman et al. (2002), the only carriers of FVL with recurrent thrombosis for whom testing followed by lifelong anticoagulant, therapy may be a reasonable strategy are those, with no obvious precipitant for venous thromboembolism, and who are at low risk for bleeding complications from oral anticoagulant therapy.

On the basis of our results we suggest that patients with thromboembolic disease occurring before 40 years of age, with venous thrombosis predominantly of the lower limbs, in the absence of triggering factors and with positive family history of venous thromboembolism should be considered for FVL investigation.

Acknowledgements. The support of VEGA grants No. 1/0525/03, 0532/03 and of APVT grant No. 51-013802 is highly appreciated.

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Final version accepted: August 31, 2004