Factor V Leiden gene mutation in young Indian patients with myocardial infarction

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Myocardial infarction (MI) in the young provides a unique model for the investigation of potential interactions between atherosclerotic and prothrombotic coronary risk factors. Factor V Leiden gene mutation, the underlying cause of activated protein C resistance, is the commonest cause for venous thrombosis. It is still not clear whether there is any relationship between this genetic defect and arterial thrombosis. We have studied the prevalence of factor V Leiden mutation in a selective group of young patients with myocardial infarction (MI), aged <45 years, to assess, whether it increases the risk of MI in this group of patients, in whom not only MI is a rare event, but also most of the well known risk factors for MI such as hypercholesterolemia and hypertension are also uncommon. Two cases (5%) were found to be carriers of factor V Leiden mutation, who were also found to be chronic smokers. This preliminary study is an attempt to study a genetic risk factor for thrombosis, i.e. Factor V Leiden mutation to observe whether it is responsible alone or interacting with other risk factors increases the risk for mycordial infarction.

THROMBOSIS of a coronary artery typically occurs in the setting of coronary atherosclerosis and results in the clinical occurrence of acute myocardial infarction[1]. It is quite likely that interactions between atherosclerosis and thrombosis influence the risk of myocardial infarction. In young patients with myocardial infarction, the contribution of thrombosis to athero-thrombotic disease may be particularly important. Furthermore, such types of patients are more common in the Indian population. It is quite logical, therefore, to suspect some genetic factor(s) being responsible for this disease.

A major breakthrough in the understanding of the genetics of thrombosis resulted from the description of activated protein C (APC) resistance as a cause of thrombophilia[2]. Further advance was made with the direct association of this anomaly with a single point mutation in the factor V gene, which produces replacement of Arg^{506} in one of the APC cleavage sites with Gln moiety[3]. Mutated factor Va is less efficiently degraded by APC than normal factor Va and leads to increased thrombin generation and a hypercoagulable state[4,5]. APC resistance is now known to be a major risk factor for venous thrombosis[6,7].

It is not clear whether APC resistance is associated with an increased risk of arterial thrombosis. Conflicting reports are available about the association of factor V Leiden with MI[8–11]. We have tried to investigate whether factor V Leiden mutation increases the risk of MI in a selective group of young Indian patients, among whom atherosclerosis is less prevalent than it is among older subjects.

The study was carried out in 40 adult patients (37 males and 3 females). The criteria for the selection of patients were: (i) age less than 45 years, (ii) the ECG changes suggestive of MI, and (iii) two of the three serum enzymes, i.e. CPK, CPKMB and LDH showing abnormal elevated levels. Forty normal age- and sex-matched subjects served as controls.

PCR analysis. DNA was extracted from peripheral blood leucocytes as described earlier[12]. The PCR amplification was carried out by a standard protocol[13] consisting of 36 cycles (91°C – 40 sec, 55°C, 40 sec, 71°C – 2 min). We used 50 pmol of each of the primers, standard PCR buffer and dNTP concentrations. The amplified product was digested with 4 units of restriction enzyme *Mnl*1 (Boehringer Manheim, Germany) and run in 3% low-melting agarose gel. The age and sex distribution of these patients are shown in Figure 1. There were only three females in this series. Only two patients were heterozygous for factor V Leiden mutation (5%). None of them was homozygous for the mutation (Figure 2).

The other familial risk factors in these patients are tabulated in Table 1. The two cases who showed the presence of factor V Leiden mutation had no history of diabetes or obesity. The relationship between factor V Leiden mutation and arterial thrombosis is still not clear. So far, only one of several case-control studies has shown an association between MI and an increased risk of prevalence of factor V Leiden mutation[8–11]. However, isolated cases of homozygous factor V Leiden mutation

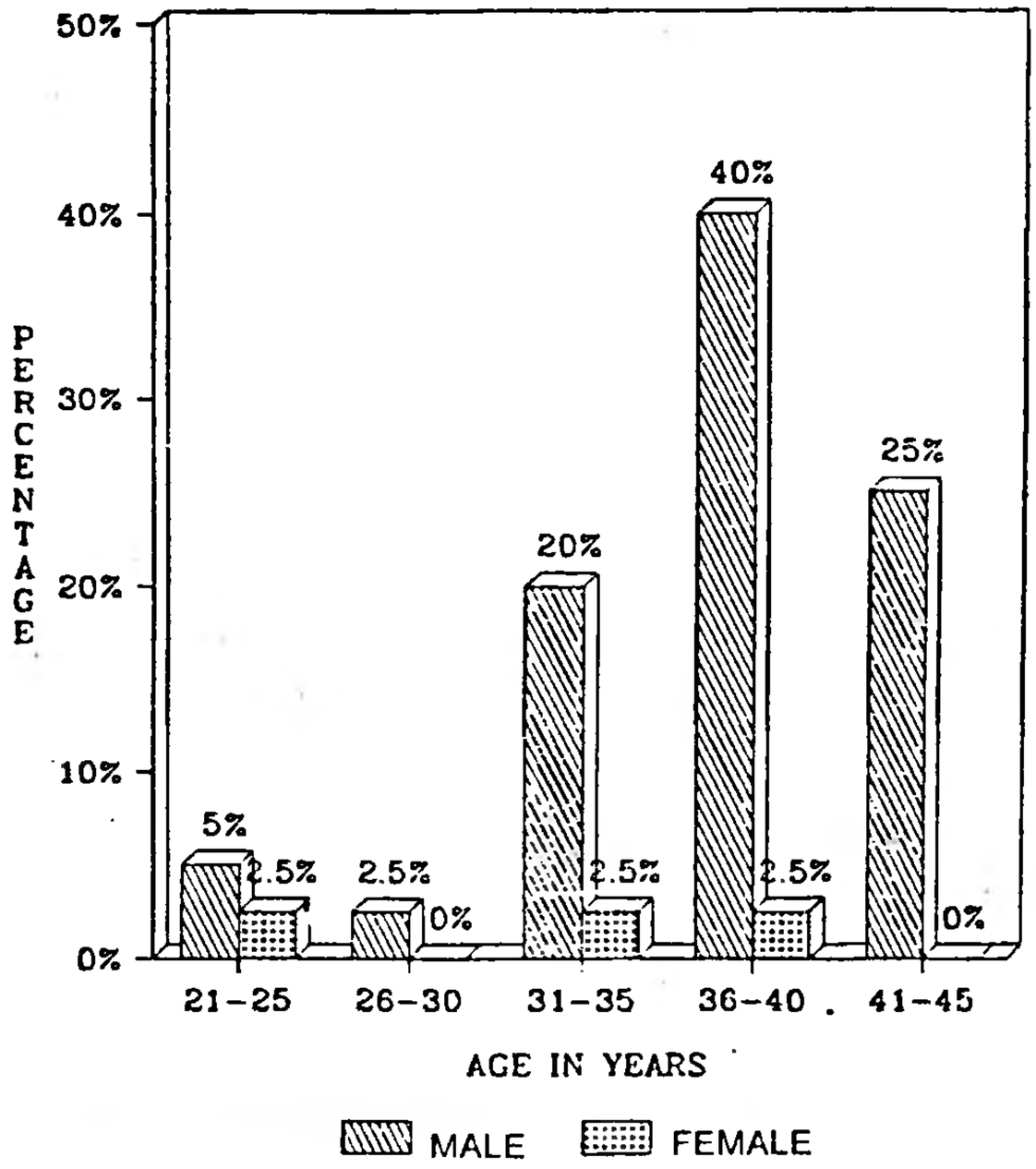

Figure 1. Histogram showing age and sex distribution of young Indian patients with myocardial infarction.

Figure 2. Electrophoretogram of PCR-amplified DNA from patients with myocardial infarction. Factor V gene mutations in patients with myocardial infarction cases. Lanes 1, 2, 4 and 6, Myocardial infarction patients negative for factor V Leiden mutation; lanes 3 and 5, Myocardial infarction patients heterozygous for factor V Leiden mutation; lane 7, PCR-amplified undigested DNA; lane 8, DNA molecular weight marker V (Boehringer, Manheim).

have been reported in a few cases of arterial thrombosis[14,15].

We have screened a selective group of patients less than 45 years of age, in whom most of the risk factors for MI are quite uncommon, to investigate whether this major genetic cause for thrombosis is the underlying explanation in this group of patients. Two of our patients were found to be heterozygous carriers of factor V Leiden (5%). The prevalence is about 4 times higher than its prevalence in our general population which is

Table 1. Prevalence of familial risk factors in Indian patients with myocardial infarction

Disease	No. of patients	Percentage
Diabetes mellitus	1	2.5
Hypertension	5	12.5
Ischaemic heart disease	8	20.0
Peripheral vascular disease	–	–
Cerebrovascular accident	–	–

1.2% (ref. 16). This indicates that this may be a risk factor in this group, but unless a large group is studied, it is difficult to come to a conclusion. Other known familial risk factors were absent in these patients. Screening coagulation tests and blood levels of lipoprotein(a) and fibrinogen were within normal limits.

There are several reports that factor V Leiden mutation has a synergistic effect showing interaction with various genetic and environmental factors[17]. Both the heterozygotes reported here were found to be chronic smokers for the past 10 years. Whether synergistic effect between factor V Leiden and smoking, as reported earlier[18] exists in our population, needs to be studied in a large group of factor V Leiden carriers who smoke and those who do not smoke.

Because of the small number of patients and the low prevalence of factor V Leiden gene carriers, the preliminary finding given here should be considered as a hypothesis generating rather than hypothesis testing. Additional studies are needed to explore potential interactions between behavioural factors (smoking, diet and physical activity), metabolic factors (obesity, hypercholesterolaemia, hypertension and diabetes) and common prothrombotic genetic mutations. In conclusion, factor V Leiden is a risk factor for MI in young patients. Because of its high prevalence relevant to thrombosis, as compared to other genetic factors, the interaction between various risk factors also needs to be analysed.

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