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Death from pulmonary thromboembolism in severe obesity: lack of association with established genetic and clinical risk factors

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Abstract Several clinical and environmental conditions are causally related to sudden death from acute pulmonary thromboembolism (APT). Morbid obesity, despite its frequency and association with adverse health effects, is usually considered at most only an additive risk factor for APT. We reviewed protocols and histories from 7227 consecutive autopsies performed between 1985 and 1996 at the Mayo Clinic, including all deaths from APT where no clinical or environmental risk factor could be identified in the study. Body mass indices (BMI) were calculated and compared with those of age- and sex-matched controls who had died suddenly and naturally without evidence of APT. Resistance to activated protein C is the most common molecular clotting defect predisposing to APT, and it is caused by a point mutation in the factor V gene ($R^{506}Q$). Genomic DNA was extracted from archival tissues of all cases and controls, and the $R^{506}Q$ status was determined by polymerase chain reaction amplification, restriction endonuclease digestion, and direct sequencing. APT was found as the immediate cause of death in 433 patients, with 36 (8%) having no previously established risk factors. Twenty-four of these persons (67%) were morbidly obese (BMI >30 kg/m^2), compared with only five controls (14%, P<0.0001). Four patients in both groups, each with a BMI <30 kg/m^2, had at least one allele positive for $R^{506}Q$. Morbid obesity is an independent risk factor in cases of sudden death from APT after the exclusion of previously established clinical, environmental, and molecular risk factors.

Key words Autopsy · Factor V gene · Obesity · Pulmonary embolism · Sudden death

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Introduction

Venous thromboembolism comprises two interrelated conditions that characteristically accompany serious illness or major surgery: deep vein thrombosis with and without acute pulmonary embolism. The latter accounts for between 5% and 10% of all hospital deaths in the United States [18]. The estimated incidence of clinically significant cases is 600 000 per year [1]. In the absence of risk factors, acute pulmonary embolism is a rare event in healthy nonhospitalized persons, and the identification of additional populations at risk may offer a significant potential health benefit. A number of risk factors for acute pulmonary embolism have been demonstrated, including prior thromboembolism, major surgery, malignant disease, multiple trauma and fractures, pregnancy and puerperium, cardiac and neurological disease, advanced age, and oestrogen therapy, with prolonged immobilization probably representing the predominant common denominator [1, 2].

In some instances, venous thrombosis appears to be hereditary. Several genetic biochemical defects have been identified, including deficiencies of proteins C and S, antithrombin deficiency, hyperhomocysteinuria, dysfibrinogenaemia, increased factor VIII levels, and resistance to activated protein C [17]. Resistance to activated protein C, first described in 1993 [9], causes ineffective down-regulation of the coagulation pathway. This defect, which is found in 20–60% of patients with venous thrombosis, is caused by a single point mutation in the factor V gene of the coagulation cascade, predicting substitution of arginine at position 506 with glutamine ($R^{506}Q$) [8]. Resistance to activated protein C is far more common than the other forms of hereditary thrombophilia [17], with an estimated prevalence of $R^{506}Q$ among whites of 3–15% [8].

In developed countries the prevalence of obesity has increased over the past 30 years [11, 20], and significant overweight is clearly associated with an increased risk of many major diseases [4, 7]. Approximately 8% of health care costs in the United States are spent on obesity-relat-

ed problems [21]. Although obesity was suggested as a risk factor for venous thrombosis some 60 years ago, the evidence from more recent studies is equivocal, and obesity is still considered at most an additive risk factor for venous thromboembolism [1, 2]. The use of the body mass index (kg/m^2) as a measure of obesity is widely accepted, easily determined, and predictive of increased morbidity and mortality in many populations [11]. For clinical utility, morbid obesity should be defined as a condition of excess adipose tissue associated with significant adverse health outcomes [16]. Persons with a body mass index >30 kg/m^2 have a much greater risk of dying early than those with lower levels of fatness [3, 6].

This study tests the hypothesis that morbid obesity is a major risk factor for sudden death from acute pulmonary embolism after correction for other established clinical and molecular risk factors.

Materials and methods

The study was approved by the Mayo Clinic Institutional Review Board. Clinical histories and autopsy protocols from 7227 consecutive autopsies performed over the 12-year period between 1985 and 1996 at the Mayo Clinic were reviewed, yielding 433 patients with acute pulmonary embolism as the immediate cause of death. Of those, all patients with one or more of the established acquired risk factors (prior thromboembolism, malignant disease, recent surgery, significant cardiac or neurological disease, multiple trauma, immobilization for more than 1 day, parturition, oestrogen therapy, and smoking) were excluded. Thirty-six patients were identified who had no clinical or environmental risk factors. For each of these 36 patients, 1 age- and sex-matched control was randomly assigned according to the following criteria: sudden, unexpected death, no identifiable acute or chronic thromboembolic event at autopsy or on review of the clinical history, age matched ±1 year, and year of death matched ±2 years. For each case and control, the post-mortem body mass index (kg/m^2) was calculated. Arithmetic means and standard deviations were calculated, and a paired *t*-test was used to compare the body mass index values in both groups.

Genomic DNA was extracted from archival paraffin-embedded autopsy tissue [19], and a segment of the factor V gene containing R^{506} was amplified in each patient sample with use of a half-nested polymerase chain reaction approach. In brief, an initial 189-base-pair segment encompassing R^{506} was amplified in 35 cycles (1 min 94° C, 1 min 54° C, 1 min 72° C) with standard polymerase chain reaction conditions. A second-round polymerase chain reaction was performed with one original primer and a second, internal, primer, the same polymerase chain reaction conditions, and 1 µl of first-round polymerase chain reaction product as a template, yielding a 147-base pair final polymerase chain reaction product. Primer sequences were as follows:

First-round polymerase chain reaction:

5′-TGCCCAGTGCTTAACAAGACCA-3′ (sense) and
5′-GACCTAACATGTTCTAGCCAGAAG-3′ (antisense)

Second-round polymerase chain reaction

5′-CATGAGAGACATCGCCTCTG-3′ (sense) and antisense primer identical to that in the first-round polymerase chain reaction

Negative controls consisted of tubes with digest buffer left open on the bench during the DNA extraction process, and these were amplified along with the patient samples. Positive controls utilized genomic DNA from individuals with known wild-type, heterozygous, and homozygous $R^{506}Q$ status. The polymerase chain reaction products from cases and controls were subjected to an endonuclease digestion with Mnl1, the fragments were analysed by electrophoresis through 3.5% agarose gels, and visualization was achieved by means of ultraviolet light after ethidium bromide staining. All samples indicative of either a heterozygous or a homozygous $R^{506}Q$ status were reamplified, and the mutation was confirmed by semiautomated dRhodamine terminator cycle sequencing with use of an ABI Prism Automated Sequencer 377XL (ABI, Foster City, Calif.) with the following sequencing primer:

5′-TCGCCTCTGGGCTAATAGGAC-3′

Results

Among of the 7227 autopsies, acute pulmonary embolism was listed as the immediate cause of death for 433 (6%). Most of these patients were found to have had one or more previously recognized clinical or environmental risk factors on review of autopsy protocols and clinical histories (see “Materials and methods”). Thirty-six patients were identified who had died suddenly of a pulmonary thromboembolus and who had none of the established risk factors. This group accounted for 0.5% of all deaths and 8% of deaths from acute pulmonary embolism. Twenty-four of these patients (67%) were morbidly obese, as defined by a body mass index >30 kg/m^2.

The immediate causes of death in the control group were acute myocardial infarction (12), sudden cardiac death with presumed cardiac dysrhythmia (15), ruptured aortic aneurysm (5), acute congestive heart failure (1), and acute gastrointestinal (1) or intracranial (2) haemorrhage. None of those in the control group had evidence of an acute or previous thromboembolic event at autopsy or on history review. Only 5 controls (14%) had a body mass index >30 kg/m^2, and all 5 experienced sudden cardiac death.

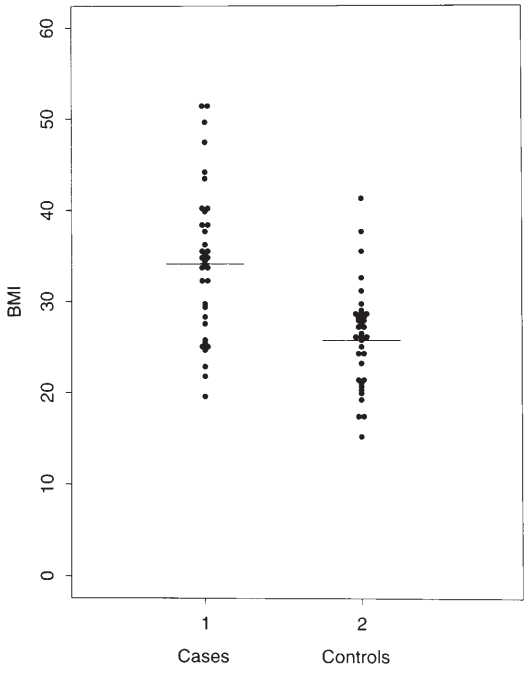

Fig. 1 Distribution of body mass indices (BMI, kg/m^2) in cases and controls

Table 1 Clinical and laboratory characteristics of cases and controls (*BMI* body mass index, *M* (*SD*) mean±standard deviation]

	Age in years M (SD)	Male-female ratio	BMI (kg/m^2) M (SD)	No. of persons with BMI >30 kg/m^2	No. of persons with R^{506}Q and			
					BMI <30 kg/m^2		BMI >30 kg/m^2	
					Hetero-zygous	Homo-zygous	Hetero-zygous	Homo-zygous
Cases								
(*n*=36)	68.0 (15.0)	0.8	34.1 (8.3)	24	3	1	0	0
Controls								
(*n*=36)	68.5 (15.1)	0.8	25.9 (5.6)	5	1	3	0	0

The distribution of body mass index values and their paired differences in cases and controls were symmetrical, with no evidence of outliers or non-normality (Table 1, Fig. 1). Body mass index values were significantly greater in cases than in controls (P<0.0001).

A segment of the factor V gene containing R^{506} was successfully amplified from archival paraffin-embedded autopsy tissue in all 72 patients studied. Subsequent molecular analyses revealed 4 individuals in each group with at least one R^{506}Q mutant allele (Table 1). Each of the 8 patients with R^{506}Q had a body mass index <30 kg/m^2. Further statistical analysis also revealed significantly greater body mass index values in cases than in controls (P<0.0001) when all R^{506}Q-positive patients were excluded or when the 4 case–control pairs in which the cases were positive for R^{506}Q were excluded.

Discussion

The aim of the present study was to investigate the prevalence of morbid obesity in persons who died suddenly of a massive pulmonary thromboembolic event and in whom none of the previously established risk factors was identifiable. A rigorous exclusion of all patients with the slightest hint of any clinical or environmental risk factors identified 8% of all deaths from acute pulmonary embolism as lacking the accepted risk factors. The presence of R^{506}Q as the cause for resistance to activated protein C is only one of several genetic risk factors for thrombosis [5], but it is far more common than other forms of hereditary thrombophilia [17]. The exclusion of patients with positive R^{506}Q status did not change the results of our statistical analysis. It may be noteworthy that none of the individuals carrying R^{506}Q was morbidly obese.

Selection bias is of concern in any study based on autopsy data, since autopsy rates at all major United States medial centres are steadily declining [14]. Hospital autopsy results show a wide discrepancy in the prevalence of pulmonary embolism at death because of differences in the population studied and in how pulmonary emboli are detected and recorded [15]. The present study, however, included only patients with a sudden and unexpected death. This group of patients consists almost exclusively of medicolegal cases with an autopsy rate of virtually 100%. All cases had a well-documented acute pulmonary embolism as the immediate cause of death, whereas none of the controls showed signs of acute or chronic venous thromboembolism of any type at autopsy. All 5 controls with a body mass index >30 kg/m^2 had succumbed to sudden cardiac death, which is consistent with the known association of morbid obesity and sudden cardiac death [7, 10].

Many measures of obesity and fat distribution pattern have been used by various investigators [4, 11, 12]. Body composition for any body mass index varies considerably owing to the effects of age, sex, and ethnicity [4]. This variability has led to the development of methods of increased accuracy for estimating body composition [12]. However, these “direct” methods for measurement of an individual’s body fat mass are cumbersome and expensive and most useful in nutritional and metabolic studies. Body mass index is widely accepted as a measure of obesity for most clinical and epidemiological studies, on the other hand, because it is easily measured and predicts morbidity and mortality in dissimilar populations [4]. Morbid obesity should be defined as a condition of excess adipose tissue associated with adverse health outcomes [16], because the data linking mild overweight (a body mass index of 25–29 kg/m^2) and death are limited, fragmentary, and often ambiguous [13]. In contrast, a body mass index >30 kg/m^2 is clearly correlated with serious health problems and carries a significant risk of mortality [6, 11]. Therefore, this threshold was used to define morbid obesity in the present study.

Despite anecdotal suggestions to the contrary, few hard data link excessive weight to thromboembolic events. Our results suggest that morbid obesity is an independent risk factor for sudden death from acute pulmonary embolism. Since obesity is common in developed countries and since the prevalence of morbid obesity is increasing [11, 20], synergy with other risk factors becomes likely and could account for an even greater number of deaths from pulmonary thromboembolic events. Further studies are needed to confirm our results and to study the interaction of morbid obesity with other thrombophilic risk factors.

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