

HEMOPHILIA IN FLORIANÓPOLIS: FREQUENCY AND CARRIER DETECTION

Nadir Ferrari

ABSTRACT

A reference center was set up in Florianópolis to record the different types of hemophilia occurring in the State of Santa Catarina and to detect hemophilia A carriers by pedigree study and laboratory tests. One hundred and two persons were tested: 33 hemophiliacs A from 23 families, 4 hemophiliacs B from 4 families, 3 von Willebrand patients from 3 families, 10 definite carriers, 22 probable carriers and 31 persons with negative results. Twenty seven healthy women were used as controls. The following tests were performed: PT, KCCT, Factor VIII:C, Factor IX:C, Factor VIII:Rag and Factor IX:Ag assays. Discriminant analysis was used to analyze the results obtained for the definite carriers and the probable carriers. Eight of the 18 probable carriers were diagnosed as heterozygotes.

INTRODUCTION

Hemophilia A, hemophilia B and von Willebrand disease are the most important genetic bleeding disorders. Classical hemophilia or hemophilia A is caused by a deficiency of FVIII:C (factor VIII clotting activity) and shows a prevalence of 1/16,000 men (Fischer *et al.*, 1986). The gene coding for FVIII:C is in the region q28 of the X chromosome (Lawn and Vehar, 1985; Gilgenkrantz *et al.*, 1986) and occupies approximately 0.1% of the whole chromosome (Gitschier *et al.*, 1984).

Hemophilia B or Christmas disease is due to the deficiency of FIX:C (factor IX clotting activity) and shows a prevalence of 1/105,000 men (Fischer *et al.*, 1986). The gene coding for this protein is in the q26 region of the X chromosome (Boyd *et al.*,

1984; Giannelli *et al.*, 1984). Both hemophilia A and hemophilia B are recessive traits. Von Willebrand disease is caused by a deficiency of FVIII:RAG (factor VIII related antigen).

Today, with the advances in the management of hemophilia, mainly due to replacement therapy with clotting factor concentrates, many more patients can expect to live to an old age. According to Rizza and Spooner (1983), the average age of death for hemophiliacs in Great Britain is not statistically different from that of the normal population. Nevertheless, the increase in life expectancy does not necessarily mean an increase in reproductive fitness. Kasper and Parton (1974) and Roisenberg *et al.* (1985) observed a lower fertility rate for hemophiliacs when compared to the general population.

Because of their constant need for blood derivatives, hemophiliacs are exposed to the considerable risks involved in the use of these products. Much effort has been directed towards the development of a technology for the production of safe clotting factor concentrates, and the results of DNA cloning have been promising (Brownlee and Rizza, 1984; Toole *et al.*, 1984; Wood *et al.*, 1984; Feldman and Rodell, 1986; Lawn *et al.*, 1986).

It is very important to provide the best possible treatment for hemophilia, so that patients may lead a normal life. Equally important is to control the frequency of the disease by carrier detection and genetic counselling.

A laboratory was set up at the University Hospital of Florianópolis in order to register families with genetic bleeding disorders in the State of Santa Catarina, starting from the city of Florianópolis, with a population of 400,000 inhabitants.

MATERIAL AND METHODS

A letter was sent to every hospital in Santa Catarina with information on the laboratory and on how to refer patients suspected to have hemophilia for diagnosis and treatment. Since only two hospitals answered, the study was limited to the Florianópolis area.

One hundred and two persons were studied: 33 hemophiliacs A, from 23 families; 4 hemophiliacs B from 4 families; 3 von Willebrand patients from 3 families; 10 definite carriers; 22 probable carriers and 31 persons who showed no clotting abnormalities. A sample of 27 healthy women aged 15 to 40 years was also tested.

The following screening tests were used: BT (bleeding time), Tourniquet Test, Platelet count, PT (prothrombin time) and KCCT (kaolin cephalin clotting time).

Factor VIII.C (factor VIII clotting activity) and Factor IX.C (factor IX clotting activity) assays were performed on plasmas with normal PT and abnormal KCCT. The Factor V assay was performed on plasmas with abnormal PT and KCCT. The Factor VIII:RAG (factor VIII related antigen) assay was performed on plasmas

from hemophiliacs, from their families and from patients suspected to have von Willebrand disease. The Factor IX:C_{Ag} (factor IX antigen) assay was performed on plasmas from Christmas disease patients and from their families.

All tests and assays were performed as described by Austen and Rhymes (1975) except for the antigen assays, which were performed by the method of Laurell (1972).

Freeze-dried control plasma and reagents were kindly provided by Dr. Roisenberg, Universidade Federal do Rio Grande do Sul.

Von Willebrand disease patients were classified according to Hoyer (1984).

RESULTS

The data used for statistical analysis are available upon request to the author.

Mean Factor VIII:C and Factor XI:C levels were $99 \pm 25\%$ and $96 \pm 22\%$ respectively, for normal subjects, and $69 \pm 35\%$ and $141 \pm 61\%$, respectively, for definite carriers.

Multivariate analysis of variance was applied to the results obtained for normal women and for definite carriers, using the following formulas as discriminants:

$$X = \text{Ag} (0.02944730) + C (0.10496150) - 6.65290536.$$

$$Y = \text{Ag} (0.10530997) + C (0.01026847) - 7.46982274.$$

where:

Ag = Factor VIII:R_{Ag} level.

C = Factor VIII:C level.

X and Y values were calculated for each probable carrier. If $X < Y$ the laboratory result was considered positive for heterozygosis. If $X > Y$ the result was considered negative.

Eight of the 18 probable carriers, 6 of 7 mothers of sporadic cases and 3 of 5 daughters of definite carriers were identified as carriers using this discriminant.

The data on hemophilia B were not analyzed statistically due to the small number of individuals studied.

The misclassification frequency calculated for the present sample was 5.7% false positive and 2.8% false negative results.

DISCUSSION

Although we were not able to register all genetic bleeding disorders in the

State of Santa Catarina, since only 8 of the patients diagnosed were from the hinterland, we can say that virtually every patient in Florianópolis was studied and his family investigated.

Factor VIII:RAg and Factor IX:Ag levels were normal for hemophiliacs A and B, respectively. Curiously, a considerable number of hemophiliacs showed higher than normal Factor VIII:RAg levels, a fact for which we have no explanation.

Multivariate analysis of variance revealed that the discriminant based on variables Ag and C was superior to that based on the C/Ag ratio. The latter discriminant revealed 86% of the mothers of sporadic cases as heterozygotes, as expected if we consider that the supply of recessive genes for a given generation originates from previous ancestors far more often than from mutation (Simpson and Biggs, 1962).

Due to the wide range of Factor VIII levels in the normal population and to the X inactivation effect (Lyon, 1962), carrier detection is not possible in all cases. The use of quantitative techniques for carrier detection presents the limitation of offering a probability of carriership rather than a yes or no answer. Yet, as qualitative tests such as DNA analysis are just beginning to be available, many laboratories will continue to use clotting and immunoassays for some time.

In genetic counselling, the lack of more precise information about the risks for a given patient or his relatives to have affected offspring adds to the psychological, social and economic problems caused by the disease, as discussed by Alexandrino (1987).

DNA analysis for carrier detection is already being used in more developed countries (Giannelli *et al.*, 1984; Peake *et al.*, 1984; Gitschier *et al.*, 1985; Gilgenkrants *et al.*, 1986; Wion *et al.*, 1986; Ljung *et al.*, 1987). The time has certainly come for the groups working with carrier detection of X-linked recessive diseases to receive appropriate funding to work with these more precise techniques.

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RESUMO

Os objetivos do presente trabalho foram: 1. Criar em Florianópolis um serviço de cadastramento das condições hemofilióides no Estado de Santa Catarina. 2. Levantar genealogias dos casos encontrados. 3. Realizar o diagnóstico diferencial e testes para detecção de portadoras de hemofilia A. Cento e duas pessoas foram testadas: 33 hemofílicos A de 23 genealogias; 4 hemofílicos B, de 4 genealogias; 3 doentes de von Willebrand, de 3 genealogias; 10 portadoras certas; 22

portadoras prováveis e 31 pessoas com resultados negativos. A amostra controle se compôs de 27 mulheres saudáveis, com idades entre 15 e 40 anos. Os testes realizados foram: PT, KCCT e dosagem de FVIII:C, FIX:C, FVIII:Rag e FIX:Ag. Os resultados obtidos com as portadoras certas e prováveis foram utilizados em análise de variância multivariada. Oito das dezoito portadoras prováveis foram diagnosticadas como heterozigotas.

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