The Limitation of Factor IX Coagulant Activity Determination in the Diagnosis of Hemophilia B Carriers

SARANYA RURGKHUM, B.Sc.*,
SUKANYA CHOTSUPPAKARN, B.A.*,
AMPAIWAN CHUANSUMRIT, M.D.**
WERASAK SASANAKUL, B.Sc.*,
PAKAIMAS PINTADIT, B.Sc.*,

Abstract

A preliminary study of factor IX coagulant activity (FIX:C) for determining hemophilia B carriers was conducted at the Department of Pediatrics, Faculty of Medicine Ramathibodi Hospital in Bangkok. Twenty-eight females (8 obligate, 20 potential carriers) from 17 hemophilia B families were enrolled in the study. Additionally, 25 normal females were included. They were not pregnant and not using oral contraceptives. Then, three cut-off levels of FIX:C including 50 per cent which was the commonly used level; 57 per cent which was the mean-2 SD of normal females and 75 per cent which was the level reported by Knobe and Ljung in 1999 were used for the diagnosis of hemophilia B carriers. The sensitivities of these three cut-off levels were 12.5 per cent (1/8) for 50 per cent, 37.5 per cent (3/8) for 57 per cent and 50 per cent (4/8) for 75 per cent. Also, the specificities were 100 per cent (25/25) for both 50 and 57 per cent, and 96 per cent (24/25) for 75 per cent. Although the low cut-off levels of 50 per cent and 57 per cent had low sensitivities, they yielded a high specificity (100%) compared to the higher level of 75 per cent. In the present study, the sensitivity of the cut-off level at 75 per cent was much lower than that of the study by Knobe and Ljung (93%) since the presented sample size of obligate carriers was rather small. So, enrollment of more subjects should be further carried out. In conclusion, FIX:C determination alone showed a limitation in the diagnosis of hemophilia B carriers. The addition of genetic analysis of linkage analysis or mutation detection is required for a definite diagnosis.

Key word : Hemophilia B, Carrier, Factor IX Coagulant Activity

RURGKHUM S, SASANAKUL W, CHOTSUPPAKARN S, PINTADIT P, CHUANSUMRIT A
J Med Assoc Thai 2002; 85 (Suppl 4): S1071-S1074

* Research Center,
** Department of Pediatrics, Faculty of Medicine, Ramathibodi Hospital, Mahidol University, Bangkok 10400, Thailand.

Hemophilia B is an X-linked hereditary bleeding disorder, commonly affecting males while females are asymptomatic carriers. The accurate diagnosis of carrier state is essential for the prevention of hemophilia, especially in developing countries where health care resources are limited[1]. Since the carrier state is commonly determined by the measurement of factor IX coagulant activity (FIX:C), the present study was designed to determine the cut-off level of FIX:C for the diagnosis of Thai hemophilia B carriers.

MATERIAL AND METHOD

Subjects

Twenty-eight females from 17 hemophilia B families (severe 7, moderate 8 and mild 2) were enrolled in the study, which was conducted at the Department of Pediatrics, Faculty of Medicine, Ramathibodi Hospital, Mahidol University, Bangkok, Thailand. They were classified as obligate carriers (n=8) and potential carriers (n=20) verified by history taking of 100 per cent and 25-50 per cent chance of being carriers, respectively. Five of the 8 obligate carriers were verified by one of the informative restriction fragment length polymorphisms of *Mse* I, *Nru* I, *Dde* I or *Hha* I. The other three were non-informative. In addition, 25 non-carrier females, verified by history taking, were included as the control. Their ages ranged from 18 to 45 years with a mean age of 28 years. None of the subjects were pregnant and were not using oral contraceptives.

Methods

The blood samples were drawn using the two-syringe technique and mixed with an anticoagulant of citrate buffer in the ratio of 9 to 1. The blood samples were immediately put in an ice box and centrifuged at 3,000 rpm for 15 minutes. The plasma was separated, alliquoted in small microtubes and kept in a -70°C freezer. The FIX:C was measured by the one-stage technique using an ACL machine[2]. Plasma from a patient with severe hemophilia B was used as the test base.

Statistical analysis

The unpaired *t*-test was used to assess the difference in FIX:C among the obligate carriers, potential carriers and the normal females. The p-value of less than 0.05 was considered significant.

RESULTS

The levels of FIX:C among obligate carriers, potential carriers and normal females are shown in Table 1. The mean ± SD of FIX:C were 81.4 per cent ± 30.9 (range 48-132) in obligate carriers, 79.2 per cent ± 33.9 (range 24-127) in potential carriers and 110.1 per cent ± 7.1 (range 63-162) in normal females. There was no statistically significant difference among the obligate carriers, potential carriers and normal females. Three cut-off levels of FIX:C were used for distinguishing between hemophilia B carriers and normal females. The first cut-off level was 50 per cent which has been commonly used among hemophilia B carriers. The second cut-off level was 57 per cent which was at the mean -2SD of normal controls in the present study. The third cut-off level was 75 per cent which was the level previously reported by Knobe and Ljung[3]. The sensities and specificities of these three cut-off levels in the diagnosis of obligate carriers are shown in Table 2. These levels were also used for the diagnosis of 20 potential carriers, five (25%) of whom were diag-

Table 1. The levels of factor IX coagulant activity (FIX:C) among obligate carriers, potential carriers and normal females.

Type	Number	FIX:C (%)		
		Individual level	Mean ± SD	Range
Obligate carriers	8	110, 80, 104, 56, 48, 132, 70, 51	81.4 ± 30.9	48-132
Potential carriers	20	89, 108, 42, 113, 104, 130, 40, 90, 127, 75, 48, 52, 25, 24, 68, 72, 72, 69, 126, 110	79.2 ± 33.9	24-127
Normal females	25	83, 94, 105, 162, 98, 88, 79, 107, 85, 110, 138, 125, 63, 111, 75, 109, 139, 111, 130, 143, 99, 128, 83, 134, 153	110.1 ± 7.1	63-162

Table 2. Sensitivities and specificities at three different cut-off levels of factor IX coagulant activity (FIX:C) in the diagnosis of 8 obligate carriers and 25 normal females.

Cut-off FIX:C (%)	Sensitivity (%)	Specificity (%)
50	1/8 = 12.5	25/25 = 100
57	3/8 = 37.5	25/25 = 100
75	4/8 = 50	24/25 = 96

nosed as carriers by using the cut-off level of 50 per cent. When the cut-off level of 57 per cent was used, 6 (30%) females were diagnosed as carriers. If the cut-off level was raised to 75 per cent, 11 (55%) females were diagnosed as carriers.

DISCUSSION

This is a preliminary study in a small number of obligate carriers for determining the sensitivities and specificities of the cut-off levels of FIX:C for the diagnosis of hemophilia B carriers. The sample size was rather small. The determinations of FIX:C is unavoidably influenced by the effect of random X chromosome inactivation. In the present study, when using FIX:C levels of 50 per cent or 57 per cent as the cut-off levels, only one to three out of 8 obligate carriers (12.5-37.5%) and five to six out of 20 potential carriers (25-30%) were diagnosed.

It is suggested that FIX:C levels of less than 50 per cent or the mean-2 SD of normal females should be initially used as a screening cut-off diagnostic criteria. Then, females with their FIX:C's values between 50 and 75 per cent should be further studied by genetic analysis of linkage analysis of restriction fragment length polymorphisms[4]. In this study, 5 out of 8 obligate females (62.5%) were found to be informative for one of the markers of *Mse* I, *Nru* I, *Dde* I and *Hha* I, which was similar to the study in Thai females of Sasanakul, reporting 72 per cent in 2000[5]. These figures were lower than those of Caucasian females in the study by Goodeve, reporting 94 per cent in 1994[6]. Moreover, the application of linkage analysis is limited to 'sporadic' families with no previous history of hemophilia. The female carriers can only be excluded if they do not possess the same polymorphic site as the hemophiliacs. Ultimately, females with non-informative results should receive a more sophisticated mutation analysis.

In conclusion, FIX:C determination alone shows a limitation in the diagnosis of hemophilia B carriers. The addition of genetic analysis of linkage analysis or mutation detection is required for a definite diagnosis.

(Received for publication on September 16, 2002)

REFERENCES

1. Chuansumrit A, Goodeve A, Sasanakul W, et al. DNA polymorphisms for carrier detection of hemophilia in Thailand. Southeast Asian J Trop Med Public Health 1995; 26 (Suppl 1): 201-6.
2. ACL Hundred/Thousand series. Operator's manual P/N 80000-06 March 1996, Instrumentation Laboratory, Italy.
3. Knobe KE, Ljung RC. Haemophilia B carrier detection by factor IX:C analysis; no impact of the type of mutation or severity of disorder. Haemophilia 1999; 5: 238-42.
4. Lillicrap DP, Liddell MB, Matthews RJ, Peake IR, Bloom AL. Comparison of phenotypic assessment and the use of two restriction fragment length polymorphisms in the diagnosis of the carrier state in haemophilia B. Br J Haematol 1986; 62: 557-65.
5. Sasanakul W, Chuansumrit A, Rurgkhum S, Hathirat P. Frequencies of five polymorphisms associated with the factor IX gene in the Thai population. Haemophilia 2000; 6: 715-8.
6. Goodeve AC, Chuansumrit A, Sasanakul W, Isarangkura P, Preston FE, Peake IR. A comparison of the allelic frequencies of ten DNA polymorphisms associated with factor VIII and factor IX genes in Thai and Western European populations. Blood Coagul Fibrinolysis 1994; 5: 29-35.

ข้อจำกัดของการใช้ระดับแฟคเตอร์เก้าในการวินิจฉัยภาวะที่มียีนโรคฮีโมฟีเลีย บี แฝง

ศรัณยา ฤกษ์ข่ำ, วท.บ., วีระศักดิ์ ศาสนกุล, วท.บ.*, สุกัญญา โชตศุภการย์, ศ.บ.*, ผกายมาศ ปิณฑะดิษ, วท.บ.*, อำไพวรรณ จวนสัมฤทธิ์, พ.บ.***

การศึกษาเบื้องต้นของการใช้ระดับแฟคเตอร์เก้าในการวินิจฉัยภาวะที่มียีนโรคฮีโมฟีเลีย บี แฝง ที่ หน่วยโลหิตวิทยา ภาควิชากุมารเวชศาสตร์ คณะแพทยศาสตร์โรงพยาบาลรามาธิบดี โดยทำการศึกษาใน ผู้หญิง 28 ราย (8 obligate, 20 potential carrier) จากครอบครัวผู้ป่วยโรคฮีโมฟีเลีย บี จำนวน 17 ครอบครัว และผู้หญิงปกติ 25 ราย เป็น normal control ผู้หญิงทุกคนที่เข้าร่วมการศึกษานี้ไม่ตั้งครรภ์ หรือรับประทานยาคุมกำเนิด จากการศึกษาพบว่าถ้าใช้ระดับแฟคเตอร์ IX ที่แตกต่างกัน เป็นเกณฑ์ในการวินิจฉัยภาวะที่มียีนโรคฮีโมฟีเลีย บี แฝง จะมีความไวและความจำเพาะแตกต่างกันดังนี้ ระดับแฟคเตอร์ IX เท่ากับ 50% จะมีความไวของการวินิจฉัยเท่ากับ 12.5% (1/8), 57% เท่ากับ 37.5% (3/8) และ 75% เท่ากับ 50% (4/8) และความจำเพาะของระดับแฟคเตอร์ IX เท่ากับ 50% และ 57% คือ 100% (25/25) และ 75% คือ 96% (24/25) ถึงแม้ว่าเกณฑ์ตัดสินของระดับแฟคเตอร์ IX ที่เท่ากับ 50% และ 57% จะมีความไวต่ำ แต่มีความจำเพาะสูงกว่าเมื่อเปรียบเทียบกับระดับที่เท่ากับ 75% ในการศึกษานี้ยังพบว่าความไวของระดับแฟคเตอร์ IX เท่ากับ 75% ต่ำกว่าการรายงานของ Knobe และ Ljung อาจเนื่องจากจำนวนกลุ่มตัวอย่างของการศึกษานี้น้อยกว่า ซึ่งจะต้องมีการเก็บตัวอย่างให้มากกว่านี้ต่อไป

ดังนั้น การใช้ระดับแฟคเตอร์ IX เพียงอย่างเดียวนั้น มีข้อจำกัดในการวินิจฉัยภาวะที่มียีนโรคฮีโมฟีเลีย บี แฝง จึงควรที่จะมีการศึกษาด้านอณูชีวพันธุศาสตร์เพื่อช่วยในการวินิจฉัยที่ถูกต้องต่อไป

คำสำคัญ : โรคฮีโมฟีเลีย บี, ภาวะที่มียีนแฝง, แฟคเตอร์ IX

ศรัณยา ฤกษ์ข่ำ, วีระศักดิ์ ศาสนกุล, สุกัญญา โชตศุภการย์, ผกายมาศ ปิณฑะดิษ, อำไพวรรณ จวนสัมฤทธิ์
จดหมายเหตุทางแพทย์ ฯ 2545; 85 (ฉบับพิเศษ 4): S1071–S1074

* สำนักงานวิจัย,
** ภาควิชากุมารเวชศาสตร์, คณะแพทยศาสตร์ โรงพยาบาลรามาธิบดี, มหาวิทยาลัยมหิดล, กรุงเทพ ฯ 10400