Study of Normal Values in Coagulation Profile

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Abstract

The objective of the present work was to study normal values of hemostasis parameters in healthy volunteers using Behring Coagulation Timer (BCT). Plasma was divided into 2 parts fresh plasma and lyophilized plasma. It was found that the mean ± SD of prothrombin time (PT) and activated partial thromboplastin time (APTT) in fresh plasma (n=37) were 11.95 ± 0.7 and 40.52 ± 5.30 seconds respectively. The means ± SD of coagulation factors I, II, VII, VIII and IX were 2.55 ± 0.73 g/l (n=36), 82.28 ± 10.28 per cent (n=37), 82.79 ± 19.36 per cent (n=32), 89.13 ± 24.17 per cent (n=37), 94.11 ± 16.29 per cent (n=31) respectively. The normal ranges (p_5-p_{95}) of PT, APTT and coagulation factors I, II, VII, VIII and IX were 10.8-13.3 sec, 31.4-48.0 sec and 1.82-4.65 g/l, 64.83-96.5 per cent, 46.88-113.5 per cent, 52.44-127.61 per cent and 67.87-116.94 per cent respectively. Comparison of PT, APTT between fresh plasma and lyophilized plasma were statistically different (Wilcoxon match-pair signed rank test, $p<0.05$), while F I, II, VIII and F.IX in fresh plasma were increased more significantly than lyophilized plasma.

Key word : Screening Coagulogram, Factor Assay

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Separating the coagulation process into extrinsic and intrinsic systems was determined by laboratory coagulation tests. Prothrombin time was used to evaluate the abnormality of extrinsic coagulation system and monitoring of oral anticoagulant as well as activated partial thromboplastion time measured the intrinsic and common pathways of coagulation including heparin therapy[1,2]. To investigate the cause of bleeding disorders, the screening coagulation profile was performed by platelet count, prothrombin time, activated partial thromboplastin, venous clotting time and thrombin time. However, special laboratory coagulation tests in factor assays were essential for investigation of inherited bleeding disorders[3,4].

The objective of this study was to investigate the intrinsic and extrinisic coagulation tests including factor assays in different types of plasma in normal subjects.

MATERIAL AND METHOD

Blood for coagulation studies was collected by clear venepuncture into polyethylene tubes containing one-tenth (final) volume of 3.8 per cent W/V sodium citrate. Normal blood was obtained from 37 healthy volunteers. Platelet poor plasma (PPP) was performed by centrifuging at 2,500 g for 30 min at 4°C. Aliquots of a single batch of normal PPP were frozen (-70°C) for later use in preparing lyophilized plasma.

Clotting tests

PT, APTT and fibrinogen level were done by the reagents of Thromborel S (Behring Diagnostics), Pathromtin SL (Behring Diagnostics) and Multifibrin U (Behring Diagnostics) respectively.

Factor assays

F II, VII, VIII and IX assays were analysed by the one stage method. Both clotting tests and factor assays were determined in fresh PPP, and lyophilized form by automatic Behring Coagulation Tmer (BCT).

As the values for the 37 normal subjects were expressed with a mean ± SD and the normal

Table 1. Between run precision of commercial standard human plasma (Behring Diagnotics).

No	PT (sec)	APTT (sec)	FI (g/l)	FII (%)	F VII (%)	F VIII (%)	F IX (%)
1	11.5	38.6	2.47	79.54	100.00	84.77	96.48
2	11.24	37.9	2.32	61.60	77.01	80.13	90.6
3	11.5	39.1	2.38	74.42	95.02	73.78	87.29
4	12.10	38.00	2.40	63.51	-	78.94	77.33
5	11.70	38.7	2.31	-	-	96.67	-
6	12.70	37.80	2.39	-	-	-	-
7	12.90	37.40	2.51	-	-	-	-
8	12.60	35.60	-	-	-	-	-
9	12.30	35.9	-	-	-	-	-
10	12.40	35.4	-	-	-	-	-
11	12.6	39.08	-	-	-	-	-
$\bar{X}$	12.24	37.59	2.39	69.77	90.68	82.86	87.92
SD	0.49	1.37	0.07	8.62	12.08	8.65	8.02
CV (%)	3.97	3.64	3.11	12.36	13.32	10.44	9.12

Table 2. Normal values of clotting tests and factor assays in healthy volunteers.

	Clotting tests			Factor assays			
	PT (sec)	APTT (sec)	FI (g/l)	FII (%)	F VII (%)	F VIII (%)	F IX (%)
No of sample	37	37	36	37	32	37	31
$\bar{X}$	11.95	40.52	2.55	82.28	82.79	89.13	94.11
SD	0.7	5.3	0.73	10.28	19.36	24.17	16.29
P_5- P_{95}	10.8-13.3	31.4-48.0	1.82-4.65	64.83-96.5	46.88-113.5	52.44-127.61	67.87-116.94

Table 3. Comparison of PT, APTT and fibrinogen level in fresh plasma (FP) and lyophilized plasma (LP).

	PT (sec)				APTT (sec)				Fibrinogen (g/l)			
	FP (n=37)	LP_1 (n=32)	LP_2 (n=30)	LP_3 (n=36)	FP (n=37)	LP_1 (n=32)	LP_2 (n=29)	LP_3 (n=36)	FP (n=36)	LP_1 (n=32)	LP_2 (n=27)	LP_3 (n=18)
$\bar{X}$	11.95	15.65*	14.09*	14.57*	40.52	59.91*	53.04*	48.87*	2.55	2.04*	2.24*	2.15*
SD	0.70	1.33	1.23	1.51	5.29	9.71	11.42	11.27	0.73	0.53	0.72	0.58
P_5- P_{95}	10.95-13.14	13.43-17.55	12.08-15.76	12.65-16.88	33.08-47.52	46.05-73.95	39.62-70.10	35.58-64.22	1.92-3.72	1.40-2.82	1.52-3.65	1.45-3.02

LP_1 = day 1 of lyophilized plasma stored at -70°C
LP_2 = day 7 of lyophilized plasma stored at -70°C
LP_3 = day 30 of lyophilized plasma stored at -70°C
*$P < 0.05$

range was derived at P_5 to P_{95}. Wilcoxon match pair signed rank test was used in comparison of all the tests between fresh normal plasma and lyophilized plasma.

RESULTS AND DISCUSSION

Routine evaluation of coagulation and clinical manifestation are essential for diagnosis of bleeding disorders. At present, automation analyzer has played a role in the laboratory because it is precise and not time consuming. It is the duty of medical technologists must concern with precision and accuracy of the instrument as well as the normal range of the coagulation profile before tests are done. Collection and types of specimens also play an important role and may affect the value of coagulation tests. Types of specimen could be kept in the form of fresh normal plasma and lyophilized form.

Normal values of clotting tests. PT using the same reagent was similar to the study of Andrew M et al[5] while APTT was higher when the different APTT reagent was used. Commercial APTT reagents are available in different types of activators, the platelet phospholipid substitute and the recommended time for preincubating the test plasma and the activator phospholipid mixture. It is not surprising that there is variation in sensitivity to clotting factor deficiencies, particularly when the deficiency is mild[6].

Mean plasma concentrations of factor I, II, VII, VIII and IX were lower than in the study of Andrew M et al[5].

PT of lyophilized plasma was significantly lower than fresh plasma (Table 3).

Miale and LaFond[7] showed that the PT in freeze-dried reference plasmas (temperature not specified) had changed little after 10 months' storage.

F I, II, VII, VIII and IX assays in fresh plasma and lyophilized plasma were also significantly different because no buffer was added to the preparations of plasma before freeze-drying. The addition of a buffer, such as N-2 hydroxyethylpiperazine N-2-ethasesulphonic acid (HEPES), may improve the stability of the factor. HEPES is known to stabilize the pH of the solution after reconstitu-

Table 4. Comparison of FII, VIII, and IX level in fresh plasma (FP) and lyophilized plasma (LP).

	FII (%) (n=11)		F VIII (%) (n=12)		F IX (%)(n=10)	
	FP	LP_1	FP	LP_1	FP	LP_1
$\bar{X}$	83.30	60.05	80.83	52.73	93.88	58.65
SD	9.19	6.66	24.52	17.18	21.53	13.09

LP_1 = day 1 of lyophilized plasma stored at -70°C
* $p < 0.05$

ting the freeze dried material[8], but little is known about its effect on the longterm stability of the individual factor.

The losses of factor VII activity were estimated to be negligible at -20°C. If stored at 4°C, the activity lost between 2-18 per cent of the original activity[9].

In conclusion, fresh normal plasma should be used to evaluate the normal values in coagulation profile and factor assays.

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การศึกษาหาค่าปัจจัยการแข็งตัวของเลือดของคนปกติ

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ในการศึกษาหาค่าปกติของปัจจัยการแข็งตัวของเลือด โดยใช้เครื่องอัตโนมัติ Behring Coagulation Timer (BCT) พบว่าค่าเฉลี่ย ± SD ของ prothrombin time (PT) และ activated partial thromboplastin time (APTT) จำนวน 37 ราย เท่ากับ 11.95 ± 0.7 และ 40.52 ± 5.30 วินาทีตามลำดับ ค่าเฉลี่ย ± SD ของแฟกเตอร์ I, II, VII, VIII และ IX เท่ากับ 2.55 ± 0.73 กรัมต่อลิตร (จำนวน 36 ราย), 82.28 ± 10.28% (จำนวน 37 ราย), 82.79 ± 19.36% (จำนวน 32 ราย), 89.13 ± 24.17% (จำนวน 37 ราย), 94.11 ± 16.29% (จำนวน 31 ราย) ตามลำดับ ค่าปกติ (P_5 – P_{95}) ของ PT, APTT, แฟกเตอร์ I, II, VII, VIII, และ IX เท่ากับ 10.8 – 13.3 วินาที, 31.4 – 48.0 วินาที, 1.82 – 4.65 กรัมต่อลิตร, 64.83 – 96.5%, 46.88 – 113.5%, 52.44 – 127.61% และ 67.87 – 116.94% ตามลำดับ เมื่อศึกษาเปรียบเทียบค่า PT และ APTT ระหว่างพลาสมาสดกับพลาสมาแห้ง พบว่ามีความแตกต่างกันอย่างมีนัยสำคัญทางสถิติ (Wilcoxon match – pair signed rank test, $p < 0.05$) ค่าแฟคเตอร์ I, II, VIII และ IX ในพลาสมาสดสูงกว่าในพลาสมาแห้งอย่างมีนัยสำคัญทางสถิติ ($p<0.05$)

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