

Evaluation of cryoprecipitate as part of The quality assurance in the Iraqi National Blood Transfusion Centre

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Summary:

Background:- Cryoprecipitate (CRYO) is the cold- precipitated concentration of factor VIII , it is prepared from fresh frozen plasma (FFP) by rapid freezing within six hours of collection and thawed slowly between 1 - 6 C° and removed from the supernatant . The product contain most of F VIII and part of fibrinogen from the original plasma as well as F XIII Von Willebrand (vWF) and fibronectin

Aim of the study:- This study is conducted to provide more information about significant contents of cryoprecipitates in regard to factor VIII, fibrinogen, and von Willebrand factor as part of the quality assurance in blood transfusion centers and to provide competent and efficient therapeutic materials to patients with bleeding disorders.

Materials and Methods:- In this study 98 samples were taken from the Iraqi blood donors at National Blood Transfusion Centre (NBTC) within 9 months from October 2005 to the end of June 2006. The samples were arranged in two groups.

Group I: 56 random samples of CRYO of different blood groups (**before modification**) were used , of them 28 bags were used for FVIII and 22 bags for fibrinogen measurement . Another 25 bags were used for to measure vWF before and after processing .

Group II-A :- 16 random plasma bags were pooled together and divided into 15 bags each containing 200 ml of plasma which were frozen and thawed at (1-6 C°) after 21, 22, and 23 hours.

Group II-B :- Another 25 random samples of cryoprecipitates were taken from NBTC after thawing the plasma for 22 hours and using plasma volume above 150 ml (**after modification**).

The statistical methods used were independent sample T – test and analysis of variance (ANOVA).

Results: - In group I (n=28) only 6 bags (21.4 %) contain more than 70 units of FVIII which did not meet the COE criteria , while 18 bags (81.8 %) contain more than 140 mg of fibrinogen per bag which met the COE criteria.

In group II A (n=16) thawing after 22 hours was the optimal time for separation of FVIII (P=0.027).

In group II B (n=25), FVIII and fibrinogen level separated from 200ml and 170 ml of plasma respectively were significantly more than those separated from less than 200 and 170 ml respectively (P=0.015).

Moreover FVIII and fibrinogen separated from group II B samples were significantly more than those separated from group I. (P=0.001, P=0.027 respectively).

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Conclusions: -

1. Cryoprecipitate prepared by thawing the frozen plasma at fixed temperature between 1-6 C and for 22 hours is recommended for optimal separation of FVIII to meet the COE recommendation. .
2. The volume of the original plasma which is more than 150 ml provides optimal production of FVIII and fibrinogen to meet the COE recommendation.
3. In the Iraqi NBTC the vWF separated from the prepared cryoprecipitate constitute 40-70 % of the total original plasma content of vWF.

Key words : cryoprecipitate , factor VIII and fibrinogen , Quality assurance.

Introduction:

CRYO is defined as the cold-insoluble portion of the plasma. It precipitates when fresh frozen plasma is thawed at 1 - 6 C°. It is essentially a concentrate of high molecular weight glycoproteins; including factor VIII, fibrinogen, von Willebrand factor, factor XIII and fibronectin ⁽¹⁾. Council of Europe (COE) recommends that 75 % of random CRYO

bags should contain more than 70 units of factor VIII and 75% of the bags should contain more than 140 mg of fibrinogen ⁽²⁾.

Many variables affect the clotting factors content of CRYO, especially factor VIII which maybe affected by the blood drawing techniques ^{3,4,5)},

the type of the anticoagulant ^(4, 5, 6), residual cells in plasma ^(4, 5, 7), time and temperature from draw to freezing ^(4, 8, 9, 10), volume of the original plasma ^(11, 12, 13), rate of freezing of plasma ^(14, 15), thawing of the plasma for CRYO preparation ^(11, 14, 16), time for storage of CRYO ^(11, 14), thawing of CRYO ⁽¹⁷⁾, and donor variables ^(18, 19). Only half factor VIII present in plasma is recovered in CRYO bag ⁽³⁾. American Association of Blood Banks (AABB) and Council of Europe (COE) considered the quality control program for CRYO production should include factor VIII, fibrinogen and the volume of CRYO ^(2, 11). Table (1). The total vWF content in each CRYO bag is in the range of 40-70 % of the original vWF present in the initial bag of FFP^(20,21).

Aim of the study

This study is conducted to provide more information about significant contents of cryoprecipitates in regard to factor VIII, fibrinogen, and von Willebrand factor as part of the quality assurance in blood transfusion centers and to provide competent and efficient

therapeutic materials to patients with bleeding disorders.

Materials and Methods

This study was conducted at the National Blood Transfusion Center (NBTC) in Baghdad. CRYO prepared in the center according to COE recommendations⁽¹⁴⁾.

In this study 98 samples were arranged for quality-control assurance of CRYO production in NBTC which were taken from the Iraqi blood donors from October 2005 to the end of June 2006. The samples were arranged in two groups.

Group I: Fifty six random samples of CRYO of different blood groups (**before modification**) were divided into two groups of 28 bags for each, the first 28 bags were used for factor VIII measurement, while fibrinogen was measured in only 22 bags of them because of shortage in the materials at that time.

The other twenty eight bags were taken with samples from their original plasma to obtain the concentration of recovered vWF and its ratio to the original plasma.

Group II A : The plasma from 16 random samples were pooled and divided into 15 bags each contained 200 ml of plasma which were frozen immediately by blast freezing within one hour and were stored at - 30 C .The frozen plasma were thawn after 21 , 22 and 23 hours and FVIII concentration was measured in the collected cryoprecipitate .

Group II B:- Another random 25 samples of cryoprecipitates of different blood groups were prepared in the centre after modification by using the best time-length for thawing which was 22 hours and original plasma volumes of more than 150 ml, where 10 bags contain 150 -170 ml, another 10 bags contain 170 - 200 ml and 5 bags contains > 200 ml and the fibrinogen and factor VIII content were measured in these samples.

Preparing CRYO for factors assay:

Cryoprecipitate was prepared according to the standard procedure applied in the Iraqi National Bank Centre NBIC ⁽²²⁾

Factor VIII assay was done by one stage method using STA-Deficient plasma⁽²³⁾ Fibrinogen assay was done by Clauss method using Fibri-prest Automate 2⁽²⁴⁾, both by a Coagulometer (STA ART 4 Channels)⁽²⁵⁾, in which all are synthesized by Diagnostica Stago. Von Willebrand factor antigen assay was done spectrophotometrically at 540 nm using Liatest vWF by Diagnostica stago⁽²⁶⁾.

The statistical methods used were independent sample T – test and analysis of variance (ANOVA). Data are presented as percentages and means, and the deviations as standard deviation.

Results:-

1. The results before modification in group I:-

Table 2 show that the level of factor FVIII did not meet COE or the AABB recommendations while fibrinogen level did meet the recommendation of COE but not the AABB recommendation

Moreover Von Willebrand factor content per bag was 145.3 ± 69.7 unit and in each CRYO

bag it ranged between 40-70 % of the original vWF present in the initial bag of FFP

2.Group II A :As shown in table 3 , FVIII concentration was significantly more in the plasma thawed after 22 hour than those thawed after 21 and 23 hours ,knowing that the volume of all the fifteen plasma was 200 ml.

3.Group II B :There was a significant difference between factor VIII content of CRYO produced from plasma with volume more than 200 ml and that from plasma with volume 150-200 ml and both groups content of FVIII meet the COE recommendation , as shown in table 4 .

Fibrinogen content of CRYO also affected by volume of the original plasma as there is statistically significant difference between the different original plasma volumes 150-170 vs. more than 170 ml, (P value 0.015) as shown in table 4.

Table (1) AABB and COE standards for cryoprecipitate content of factor VIII and fibrinogen and recommended volumes per bag ^(2,11).

	Factor VIII	Fibrinogen	Volume(CRYO)
COE	≥ 70 U / bag in 75% of bags	≥ 140 mg / bag in 75% of bags	10-20ml
AABB	≥ 80 U / bag in 100% of bags	≥ 150 mg / bag in 100% of bags	10-15ml

Table (2). The results of measurements of factors in CRYO before modification in group I.

Croyppt .	No.	Mean \pm SD	COE recommendations.	
			>140mg / bag In 75% of bags	>70U / bag In 75% of bags
Fibrinogen (mg)	22	253.4 (\pm 133.4)	18 bags (81.8 %)	
Factor VIII (unit)	28	51.74 (\pm 21.48)		6 bags (21.4 %)
von Willebrand Factor (unit)	28	145.3 (\pm 69.7)	—————	

Table (3) Comparison of factor VIII content of CRYO according to the times the plasma reaches the slushy stage in group II-A.

Variable	Time of thawing	No.	Mean \pm SD (Unit)	P value
Factor VIII	21 hours	5	103.5 (\pm 5.5)	0.027
	22 hours	5	127.4 (\pm 19.1)	
	23 hours	5	102.7 (\pm 14.1)	
	Total	15	111.2 (\pm 17.6)	

Table (4). Comparison of factor VIII and fibrinogen content of CRYO according to the volumes of the original plasma in group II-B.

Variable	Volume (ml)	No.	Mean \pm SD	AABB recommendations.	COE recommendations.	P value
				>80 U/ bag In 100%of bag	>70 U/ bag In 75% of bag	
Factor VIII (unit)	>200	5	129.2 (\pm 32.2)	100 %	100 %	0.002
	150-200	20	94.8 (\pm 24.8)	81.8 %	85 %	
Fibrinogen (mg)	>170	15	381.5 (\pm 117)	>150 mg/bag In 100% of bag	>140mg/bag In 75% of bag	0.015
				100 %	100%	
	150-170	10	269.5 (\pm 80)	96%	100%	

The results after modification:-

As shown in table 5, there was significant difference in FVIII before and after modifying CRYO- production, (P value < 0.001), and only those prepared after correction had met the COE recommendations.

Comparing between fibrinogen content before and after modification showed significant difference between these two groups (P value 0.027), although, both of them had met the COE criteria as shown in table 6.

Table (5). Comparison of factor VIII content of CRYO between group I and II-B (before and after modification).

Variable	CRYO	No.	Mean \pm SD (Unit)	P value	COE recommendations >70 U/ bag
Factor VIII	group I	28	51.74 (\pm 21.48)	< 0.001	21.4 %
	group II-B	25	101.4 (\pm 32.6)		88 %

Table (6). Comparison of fibrinogen content of CRYO of group I and II-B (before and after modification).

Variable	CRYO	No.	Mean \pm SD (mg)	P value	COE recommendations >140 mg/ bag
Fibrinogen	group I	22	253.4 (\pm 133.4)	0.027	81.8 %
	group II-B	25	336.7 (\pm 116.8)		96 %

Discussion

This study was conducted to measure factor VIII, fibrinogen and vWF contents of samples of CRYO of different blood groups from Iraqi blood donors at NBTC .The plasma was frozen within 6 hours by blast freezing according to the COE recommendation . In Group I (n=28), in which cryoprecipitate was prepared under the same conditions of the NBCT before modification . Fibrinogen content, as seen in table 2 was > 140 mg / bag in 18 bags(81.8 %) which met the COE but not the AABB recommendations .

Factor VIII content was more than 70 unit / bag in only 6 bags(21.4 %) which did not meet the COE and the AABB recommendations.^(14,11)

Although the measurement of vWF content of CRYO is not recommended by COE or AABB as a part of the quality assurance program for cryoprecipitate^(3, 11), but this factor was measured as a complementary requirements. In this study the total vWF content in each CRYO bag was in the range of 40-70 % of the original vWF present in the

initial unit of FFP, which was in agreement with Kennedy⁽²¹⁾ and Forsberg⁽²²⁾. Also the level of VWF in the cryoprecipitates as seen in table 2 was 145.3 ± 69.7 (M \pm SD) uniwas consistent with the results of Rock G. study which found that the level of vWF in the cryoprecipitate was $151 (\pm 74.2)$ units⁽²⁷⁾.

Two factors were suspected to result in low FVIII level in cryoprecipitate prepared in the NBTC, including the uncontrolled thawing temperature of the old refrigerators and the low volume of the original plasma used for cryoprecipitate production because part of the plasma will be removed for platelet production.

the AABB and COE did not adopt a restriction time for thawing the plasma at 1-6 C°for cryoprecipitate production however this study showed that the best time for plasma thawing using new refrigerators with controlled temperature to obtain FVIII was 22 hours compared to that collected at 21and23 hours knowing that the volume of all the plasma bags were 200 ml ,as seen in table 3 .This result was consistent with the study of Burka EA, et al⁽¹⁶⁾ .Therefore we may conclude that the contolled temperature and

thawing time is important factor in collection FVIII.

In group IIB using the new refrigerators to control the thawing temperature, FVIII in cryoprecipitate prepared from frozen plasma of volume more than 200 ml was significantly more than that obtained from volume less than 200 ml and this was adopted by the AABB⁽¹¹⁾, whereas according to the COE recommendation cryoprecipitate produced from volume more than 150 ml contained acceptable amount of FVIII (Table 4). Therefore we may conclude that the volume of the original plasma play an important role in the level of FVIII in the prepared cryoprecipitate.

Moreover ,table 4 revealed that serum fibrinogen was significantly increased by increasing the original plasma volume and its level had met the COE and the AABB recommendation except those prepared from plasma volume between 150-170 ml where its level did not meet the AABB but it met the COE recommendation. Therefore we may conclude that the volume of the original plasma was an important factor for fibrinogen level in the prepared cryoprecipitate.

By comparing the level of FVIII in group IA with that in group IIB, there was a significant high increase $P < 0.001$ (Table 5). This result was consistent with the Slichter SJ, et al⁽⁹⁾ study who had found that the thawing procedure was an important factor in FVIII production and this was adopted by the AABB¹¹. Also Ness PM et al. study⁽¹³⁾ had found that the original plasma had an impact on the level of both FVIII and fibrinogen .

On the other hand, although fibrinogen level was significantly more in group IIB than in group IA (Table 6), but in group IA in which the thawing temperature was uncontrolled, the prepared cryoprecipitate contain an acceptable amount of fibrinogen which was approved by the COE .Therefore we may propose that the volume of the original plasma and not the thawing procedure had an impact on the level of fibrinogen . This result was approved by Ness PM et al study⁽¹³⁾ .

Conclusions: -

1- Cryoprecipitate prepared by thawing the frozen plasma at fixed temperature between

1-6 C and for 22 hours is recommended

for optimal separation of FVIII which meet

the COE recommendation . .

2- The volume of the original plasma which is more than 150 ml provide optimal production of FVIII and fibrinogen which meet the COE recommendation .

3- In the Iraqi NBTC the vWF separated from the prepared cryoprecipitate constitute

40-70 % of the total original plasma content of vWF.

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