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Factors affecting the quality of cryoprecipitate

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Abstract:

BACKGROUND: Many variables affect the quality of cryoprecipitate (CRYO). We investigated the effect of freezing techniques and ABO blood groups on the quality of CRYO with respect to factor VIII: C and fibrinogen levels.

MATERIALS AND METHODS: Ninety-six whole blood units each collected from in-house (Group I) and blood donation camps outside the hospital premises (Group II) were processed for CRYO preparation. Within each group, half the number of plasma units was frozen using blast freezer and another half using the conventional freezer. The CRYOs from blood groups A, B, and O were equally distributed, i.e. 32 within each of the Groups I and II. The fibrinogen and factor VIII: C levels in CRYO were analyzed using single-stage clotting assay.

RESULTS: In Group I, the mean ± standard deviation percentage recovery of factor VIII levels in CRYO prepared using the conventional freezer and blast freezer were 58.5% ±16.2% and 66.7% ±16.4%, respectively, and in Group II, it was 55.3% ±17.6% and 70.4% ±13.4%, respectively. Recovery of factor VIII was higher in CRYO prepared using blast freezer than that of CRYO prepared using conventional freezer ($P < 0.000$). In Group II, CRYOs prepared using blast freezer had higher percent recovery of fibrinogen than that of Group I. In both the groups, the mean factor VIII levels in blood group A were higher than that of factor VIII levels in the blood group O CRYO.

CONCLUSION: The factor VIII recovery in CRYO improves significantly with higher baseline factor VIII: C levels, blood group A donor, and rapid freezing using blast freezer. Rapid freezing also increases the fibrinogen yield.

Key words:

ABO blood groups, blast freezer, cryoprecipitate, factor VIII: C, fibrinogen

Cryoprecipitate (CRYO) is the cold-insoluble fraction of plasma protein recovered by centrifugation when fresh frozen plasma (FFP) is thawed at 4°C. Although recombinant factor concentrates are readily accessible, in a developing country such as India, CRYO is still an important source of concentrated form of factor VIII: C. CRYO has approximately 40–70% of the factor VIII and 30–50% of fibrinogen of the starting plasma.

As per the Drugs and Cosmetics Act, 1940 and the Rules therein,[1] the antihemophilic factor VIII activity in the final CRYO product should not be <80 IU/bag. There are no specifications for fibrinogen content in the CRYO product. The quality control criteria set forth by the Directorate General of Health Services (DGHS), Ministry of Health and Family Welfare, Government of India, for fibrinogen levels is 150–250 mg/bag and factor VIII activity of 80–120 IU/bag.[2] Factor VIII is a labile coagulation factor, and all steps of CRYO production should be optimized to prevent a reduction in factor VIII activity.[3,4] Factors such as donor variation in factor VIII levels, time and temperature between donation and start of freezing process, the rate of freezing, and blood group of the donor may affect the factor VIII levels in plasma.[5]

The study was conducted to assess the quality of CRYO by measuring fibrinogen and factor VIII: C levels with respect to (i) freezing techniques: Conventional freezer versus blast freezer and (ii) ABO blood groups.

Materials and Methods

This study was conducted in the Departments of Transfusion Medicine and Hematology at a Tertiary Care Hospital in North India, which is also a Regional Blood Transfusion Centre. It was conducted over a period of 3 months from February 2013 to April 2013. The study was performed on 96 units of whole blood (WB) collected in-house at the Blood Donation Centre located in the hospital premises (Group I) and 96 WB units collected from blood donation camps

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conducted outside the premises of the hospital (Group II). Thus, a total of 192 units were included in the study (Group I: n = 96, Group II: n = 96) [Figure 1]. The study was approved by the Institute Ethics Committee.

Blood collection and component preparation

The donors were selected in accordance with the Government of India regulations (Drugs and Cosmetics Act and Rules, 1940).[1] Using aseptic technique, phlebotomy was done after getting informed consent from the donor. The WB units were collected in triple blood bags (Terumo Penpol Ltd., Trivandrum, Kerala, India) of 450 ml collection with 63 ml of CPDA1 (Citrate phosphate dextrose adenine 1) as an anticoagulant-preservative solution. The WB units collected from blood donation camps were only from those camps where the transport time was <30 min from the hospital premises and cold chain maintenance was ensured during the transport of blood. The temperature of the transport box containing the blood bags was noted using a calibrated digital thermometer with sensor probe (CIE, S. no. 1103390), once the units were received in the component preparation laboratory. The temperature probe was kept between the blood bags at the bottom of the transport box just above the layer of gel packs. The temperature reading was noted when the display became constant. Subsequently, the blood grouping was done from the donor samples in the pilot tubes to select an equal number of blood units of A, B, and O blood groups. Within each of the Groups I and II in the study, there was an equal number of units of blood groups A, B, and O (32 each) [Figure 1].

The component preparation was done by following steps: (i) The WB units were weighed and balanced prior to centrifugation and tapped properly to avoid red cell contamination, (ii) using refrigerated centrifuge (Cryofuge, Heraeus, Kendro laboratory, 6000 iC, Germany), these WB units were separated into packed red blood cells and plasma using the hard spin (3950 rpm for 5 min) at a temperature of 4°C with acceleration and deceleration values of 9 and 4, respectively, and (iii) the plasma was expressed into satellite bag with the help of manual plasma expressor.

To eliminate variability in fibrinogen and factor VIII: C levels in CRYO, constancy of volume of liquid plasma and CRYO

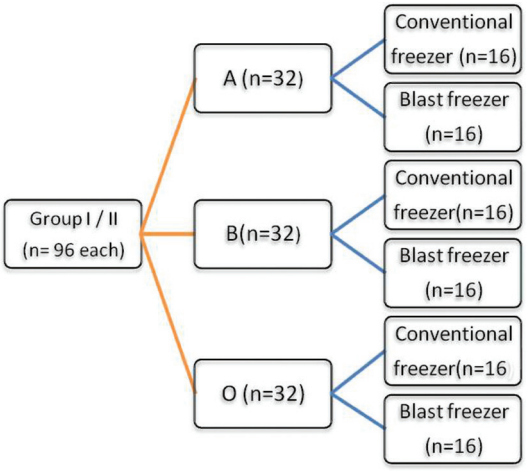

Figure 1: *Study design*

was ensured. The plasma was weighed after separation, and the residual plasma was transferred to the primary bag before sealing the satellite bag from the primary bag so that the final volume was 200 ml. The transfusion transmissible infections screening using samples in pilot tubes was also done for human immunodeficiency virus (HIV) (anti HIV-1 and anti HIV-2), hepatitis B surface antigen, anti-hepatitis C virus, syphilis (venereal disease research laboratory) and malaria. Only those blood units negative/nonreactive for these tests were included in the study.

Sampling of plasma before freezing

The transfer tube of the bag containing the plasma was adequately stripped to ensure that there is a continuous column of plasma in the tubing after inversion. Subsequently, a segment of the transfer tube of 8 cm length was prepared aseptically using a handheld tube sealer (XS1000T, Terumo Penpol Ltd., Trivandrum, Kerala, India). This segment containing the plasma was detached from the bag tubing, and the plasma bag was then frozen. After cutting one end of this tube segment with a sterile surgical blade, the plasma (1 ml) was transferred into a plastic cryovial and used for the determination of factor VIII: C and fibrinogen levels using semi-automated coagulometer. Thus, the testing in plasma was performed on the same day of collection and separation and these levels of factor VIII: C and fibrinogen were taken as the baseline values.

Freezing of plasma units

Within each of the Groups I and II, half of the plasma units (n = 48) were frozen using blast freezer (Dometic, MBF21, Luxembourg, Europe) in 30–60 min and the remaining half (n = 48) were frozen using conventional deep freezer at −80°C (Terumo Penpol Ltd., Trivandrum, Kerala, India) in 4–6 h. The plasma units frozen using blast freezer were then immediately transferred to a deep freezer and stored at −80°C. All these units were labeled as fresh frozen plasma (FFP). The sample size of each of the blood groups A, B, and O was 16 each for both the freezing techniques [Figure 1].

Thawing of fresh frozen plasma units

Within 1 week of storage in a deep freezer (−80°C), the FFP units were subjected to thawing for the preparation of CRYO. Thawing was done using 4°C circulating water bath (Remi Elektrotechnik Ltd., Maharashtra, India; and Terumo Penpol Ltd., Trivandrum, Kerala, India) by packing FFP units in individual polythene wrappers and placing them vertically inside it for about 3 h. The end point of thawing was considered when there was a slush formation in these units.

Separation of cryoprecipitate

The thawed units were weighed and balanced before centrifugation, and they were subjected to hard spin centrifugation of 3950 rpm for 5 min at a temperature of 4°C with acceleration and deceleration values of 9 and 4, respectively, and separated into CRYO and cryo-poor plasma using manual plasma expressor. After separation, some amount of plasma was transferred to a bag containing CRYO so that the final volume was 20 ml.[2] Following thawing, for determination of factor VIII: C (IU/bag) and fibrinogen levels (mg/bag), samples were taken from the tube segments of the CRYO units aseptically using the handheld tube sealer as it was done for a sampling of plasma before freezing. To achieve this, the tubing

of the CRYO bag was first stripped to get a representative sample of contents of the bag in the tubing and then the tube segment (about 8 cm) was prepared. This segment containing the sample was detached from the bag tubing, and its one end was cut with a sterile surgical blade to get 1 ml of the sample into a plastic cryovial for testing. The testing was done on the same day of separation of CRYO.

Measurement of fibrinogen and factor VIII: C levels in plasma and cryoprecipitate

FVIII: C and fibrinogen were measured using ST art® 4, a semi-automated coagulometer (Diagnostica Stago, France). Factor VIII: C was analyzed using the single-stage activated partial thromboplastin time (aPTT), and fibrinogen was measured by the Clauss method. The percentage recovery of factor VIII: C and fibrinogen levels in CRYO were also calculated.

Statistical analysis

All quantitative variables were expressed as mean ± standard deviation (SD). Comparisons between continuous variables were done using unpaired Student's *t*-test. Within each group, factor VIII: C and fibrinogen contents per bag were analyzed using ANOVA test. Correlation of fibrinogen and factor VIII levels of CRYO and percentage recovery in CRYO with baseline levels in plasma were done using Pearson's correlation (*R*). All statistical tests were two-sided and performed at a significance level of $\alpha = 0.05$.

Results

Baseline levels in plasma

In Groups I and II, the mean (±SD) fibrinogen levels in plasma were 618.5 ± 103.6 and 602.7 ± 106.6 mg/bag, respectively ($P = 0.299$). The mean (±SD) factor VIII: C levels in plasma in Groups I and II were 311.7 ± 149.7 and 373.1 ± 153.66 IU/bag, respectively, with Group II having a significantly higher level ($P = 0.006$) [Table 1].

Effect of freezing techniques on factor levels in cryoprecipitate

In Group I, the mean percentage recovery of fibrinogen levels in CRYO were 39.4 ± 11.2 and 42.3 ± 9.2 using conventional and blast freeze techniques, respectively ($P = 0.13$), while in Group II they were 42.8 ± 10.9 and 52.7 ± 15.6, respectively, which was statistically significant ($P = 0.000$) [Table 2]. For percentage recovery of factor VIII: C, it was observed that in both the Groups I and II, it was significantly higher in CRYO prepared using blast freezer ($P = 0.016$ and 0.000, respectively) [Table 2].

Table 1: Baseline levels of fibrinogen and factor VIII: C in plasma before freezing

Group	Mean±SD (range)	
	Fibrinogen level in plasma (mg/bag)	Factor VIII: C in plasma (IU/bag)
Group I (*n*=96)	618. 5±103.6 (430.4-891.0)	311.7±149.7 (63-1166)
Group II (*n*=96)	602.7±106.6 (399.2-832)	373.1±153.66 (86-1181)
*P**	0.299	0.006

**P* value compares the factor levels between Group I and Group II.
SD = Standard deviation

Effect of ABO blood groups and freezing techniques on factor VIII: C and fibrinogen levels in cryoprecipitate

In both Groups I and II, the mean factor VIII: C levels in blood groups A and B were significantly higher than that in the blood group O CRYO, with blood group A having the highest levels. In Group I, there was no significant difference between factor VIII: C level in plasma and CRYO of blood groups A and B ($P = 0.155$ and 0.459, respectively). However, there was no significant difference between different ABO blood groups ($P = 0.67$) with respect to percentage recovery of factor VIII: C. In Group I, blood group A CRYO had higher percentage recovery of factor VIII: C in CRYO prepared using blast freezer than that of conventional freezer (70.7% vs. 51.9%, $P = 0.006$), while in Group II blood group O CRYO, it was higher in CRYO prepared using the blast freezer (67.9% vs. 39.1%; $P = 0.000$) [Table 3 and Figure 2].

Regarding fibrinogen level, there was no significant difference between ABO blood groups in both the Groups I ($P = 0.92$) and II ($P = 0.9$) CRYO. In Group I CRYO, none of the ABO blood groups had statistically significant percentage recovery of fibrinogen using blast freezer as compared to the conventional freezer ($P = 0.9$ for blood group A, $P = 0.801$ for blood group B, and $P = 0.2$ for blood group O) [Table 3 and Figure 3]. However, in Group II CRYO, the blood group O CRYO had significantly higher fibrinogen level and percentage recovery using blast freezer ($P = 0.004$, 0.000).

Correlation between factor VIII: C and fibrinogen levels in plasma and cryoprecipitate

In Groups I and II, there was a significant correlation between factor VIII: C levels in plasma and that of CRYO (correlation coefficient, $R = 0.81$ and 0.83; $P = 0.000$ and 0.000). Moreover, there was no significant correlation between factor VIII: C levels in plasma and its percentage recovery in CRYO ($R = -0.19$ and 0.72; $P = 0.06$ and 0.48). Similarly for fibrinogen level, in both the Groups I and II, there was a significant correlation between its content in plasma and that of CRYO ($R = 0.224$ and 0.39; $P = 0.03$ and 0.000). However, there was a negative significant correlation between fibrinogen levels in plasma and its percentage recovery in CRYO in Group I ($R = -0.43$; $P = 0.000$), but not in Group II ($R = -0.17$; $P = 0.096$). The correlation between factor VIII: C and fibrinogen levels in plasma and CRYO with respect to two freezing techniques is described in Table 4.

Discussion

CRYO has approximately 40–70% of the factor VIII and 30–50% of fibrinogen of the starting plasma. Because of its low volume of isoagglutinins and absence of red blood cells, CRYO can be transfused without regard for the ABO group or Rh type of the original unit, though ABO compatibility is preferred in neonates.[6] CRYO is an invaluable source of factor VIII: C in patients with hemophilia by providing a low-volume concentrated source of factor VIII, eliminating problems of volume overload, in situations where factor concentrates are unavailable and/or unaffordable.

In our country, CRYO is prepared only in a few institutes due to lack of infrastructure and facilities to measure factor VIII levels. As our center is a regional blood transfusion center and also

Table 2: Effect of freezing techniques on factor levels in cryoprecipitate

Groups	Type of freezing	Fibrinogen (mg/bag)	Percentage recovery of fibrinogen	Factor VIII: C (IU/bag)	Percentage recovery of factor VIII
Group I	Conventional freezing (*n*=48)	240.1±62.2 (156.16-509.70)	39.4±11.2 (18.92-76.41)	175.2±60.7 (81.6-424.3)	58.5±16.2 (27.9-92.9)
	Blast freezing (*n*=48)	255.8±56.9 (160.00-414.80)	42.3±9.2 (26.92-63.44)	205.5±124.4 (39.4-641.5)	66.7±16.4 (35.3-98.3)
	*P**	0.2	0.13	0.67	0.016
Group II	Conventional freezing (*n*=48)	250.5±71.4 (136.60-455.8)	42.8±10.9 (20.96-65.18)	205.3±125.4 (34.4-640.1)	55.3±17.6 (14.8-91.8)
	Blast freezing (*n*=48)	320.2±102.6 (153.60-608.20)	52.7±15.6 (22.9-81.7)	266.6±106.8 (90.6-597.6)	70.4±13.4 (41.21-93.37)
	*P**	0.011	0.000	0.011	0.000

Data presented as mean±SD (range). *The *P* value compares the percentage recovery of factor levels between the two freezing techniques in both the groups. SD = Standard deviation

Table 3: Effect of ABO blood groups and freezing techniques on percentage recovery of factor VIII levels in cryoprecipitates

Blood group	Freezing technique	Group I or II (*n*=16 each)	Mean±SD (range)			
			Factor VIII: C (IU/bag)		Percentage (%) recovery in CRYO	
			In Plasma	In CRYO	Factor VIII	Fibrinogen
A	Conventional freezer (*n*=32)	I	355.8$^{\bullet}$±105.6 (189.6-612.8)	177.8±77.3 (106.8-424.3)	51.9*±19.3 (27.9-87.4)	46.0$^{£}$±11.2 (32.2-76.4)
		II	452.3$^{\bullet}$±252.4 (148.0-1181.2)	310.3±141.3 (102.6-640.0)	70.9*±12.8 (51.3-91.8)	44.8$^{£}$±9.1 (31.0-65.2)
	Blast freezer (*n*=32)	I	407.2$^{\blacksquare\blacksquare}$±262.0 (161.6-1165.6)	277.4±164.6 (112.3-641.5)	70.7**±17.6 (40.5-98.3)	43.4$^{££}$±8.2 (30.8-63.4)
		II	472.4$^{\blacksquare\blacksquare}$±80.6 (296.2-640.0)	366.5±96.4 (190.4-597.6)	77.6**±10.9 (61.7-93.4)	49.5$^{££}$±18.1 (22.9-81.7)
B	Conventional freezer (*n*=32)	I	295.7$^{\blacksquare\blacksquare\blacksquare}$±92.6 (172.9-511.2)	186.3±49.2 (106.2-290.6)	64.9***±13.6 (47.3-92.9)	37.9§±11.3 (18.9-67.4)
		II	348.2$^{\blacksquare\blacksquare\blacksquare}$±96.7 (192.0-493.6)	194.5±68.6 (103.5-371.8)	55.9***±10.0 (35.4-75.3)	44.0§±11.4 (30.2-63.7)
	Blast freezer (*n*=32)	I	338.1$^{\#}$±115.7 (135.2-505.6)	213.3±70.1 (93.4-314.5)	64.6$^{\bullet}$±12.8 (45.5-86.9)	41.7§§±8.7 (26.9-60.1)
		II	395.9$^{\#}$±135.5 (184.2-651.5)	249.6±70.6 (117.1-381.7)	65.7$^{\bullet}$±13.6 (41.2-88.3)	52.8§§±15.7 (26.4-79.3)
O	Conventional freezer (*n*=32)	I	279.3$^{\#\#}$±82.3 (132.1-392.0)	161.3±53.1 (81.6-256.1)	58.6$^{\bullet\bullet}$±13.3 (41.7-88.4)	34.1$^{¥}$±7.7 (25.2-53.0)
		II	292.3$^{\#\#}$±85.2 (85.6-428.8)	111.3±41.7 (34.4-175.3)	39.1$^{\bullet\bullet}$±12.6 (14.8-61.9)	39.3$^{¥}$±11.8 (20.9-61.1)
	Blast freezer (*n*=32)	I	193.8$^{\#\#\#}$±68.0 (62.8-333.6)	125.7±63.8 (39.4-301.0)	64.7$^{\bullet\bullet\bullet}$±18.5 (35.3-90.2)	41.7$^{¥¥}$±10.8 (27.1-61.7)
		II	277.7$^{\#\#\#}$±95.2 (150.4-511.2)	183.8±56.4 (90.5-322.2)	67.9$^{\bullet\bullet\bullet}$±13.0 (44.1-86.8)	55.9$^{¥¥}$±12.8 (37.6- 80.2)

*$\mathbf{P=0.006}$, **P=0.59, ***P=0.2, $^{\bullet}P$=0.994, $^{\bullet\bullet}\mathbf{P=0.002}$, $^{\bullet\bullet\bullet}P$=0.9, $^{\blacksquare}P$=0.4, $^{\blacksquare\blacksquare}P$=0.777, $^{\blacksquare\blacksquare\blacksquare}P$=0.54, $^{\#}P$=0.46, $^{\#\#}P$=0.9, $^{\#\#\#}\mathbf{P=0.03}$, $^{£}P$=0.99, $^{££}P$=0.5, $^{§}P$=0.48, $^{§§}P$=0.06, $^{¥}P$=0.5, $^{¥¥}\mathbf{P=0.003}$, Statistically significant *P* values are shown in bold. SD = Standard deviation, CRYO = Cryoprecipitate

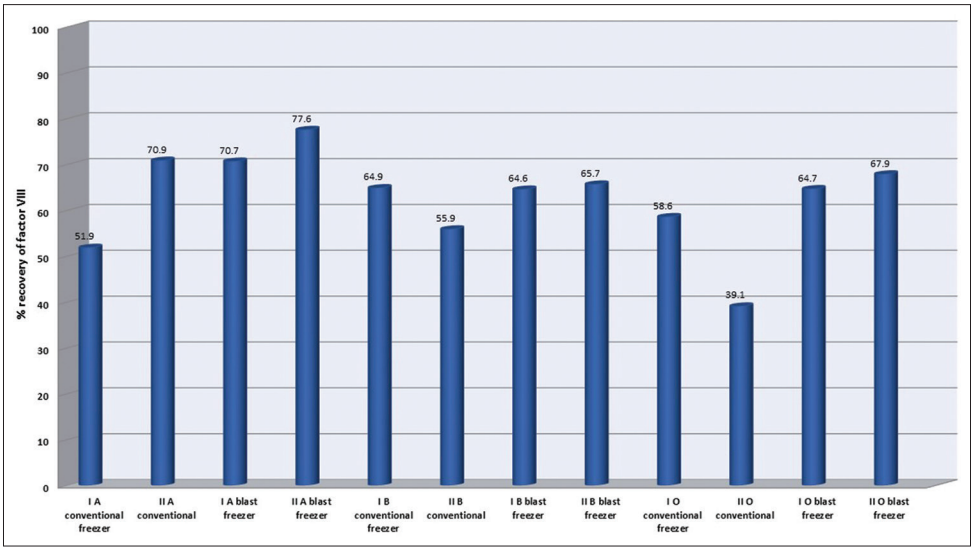

Figure 2: *Intergroup comparison of percentage recovery (%) of factor VIII in cryoprecipitate with respect to ABO blood group and freezing techniques*

Table 4: Correlation between factor VIII: C and fibrinogen levels in plasma and cryoprecipitate prepared using two different techniques

Freezing technique	Factor VIII: C (IU/bag)		Fibrinogen (mg/bag)		Percentage recovery in CRYO	
	In plasma	In CRYO	In plasma	In CRYO	Factor VIII: C	Fibrinogen
Conventional freezing (*n*=96)	337.3*±142.7** (85.6-1181.2)	190.2*±97.9 (34.4-640)	608.3$^{\#}$±112.2$^{\#\#}$ (400.2-891.0)	245.3$^{\#}$±66.8 (136.6-509.7)	56.9±16.9** (14.8-92.9)	41.1±11.1$^{\#\#}$ (18.9-76.4)
Blast freezing (*n*=96)	347.5•±165.7•• (62.8-1165.6)	236.1•±119.3 (39.3-641.5)	612.9$^{\$}$±98.0$^{\$\$}$ (399.2-868.6)	287.8$^{\$}$±88.6 (153.6-608.2)	68.5±15.0•• (35.3-98.3)	47.5$^{\$\$}$±13.8 (22.9-81.7)

Data presented as mean±SD (range). *P=0.000, R=0.81, **P=0.48, R=–0.07, •P=0.000, R=0.85, ••P=0.51, R=–0.68, $^{\#}P$=0.001, R=0.32, $^{\#\#}P$=0.000, R=–0.37, $^{\$}P$=0.003, R=0.29; $^{\$\$}P$=0.018, R=–0.24. SD = Standard deviation, CRYO = Cryoprecipitate

a part of tertiary care institute, CRYO is prepared regularly to meet the patient needs. Blood group distribution in the Indian population reveals that O and B are more common than A and AB when compared to the Western population, where A is more prevalent.[7,8] Voluntary blood collection occurs mostly from blood donation camps held outside the premises of hospital. Hence, we studied three critical variables, namely the collection site, freezing technique, and ABO blood group of the donor on the yield of factor VIII: C and fibrinogen in CRYOs.

The baseline fibrinogen level in Group I and Group II CRYO did not differ significantly [Table 1], but the CRYO prepared using blast freezer in Group II had higher percentage recovery of fibrinogen than that of Group I. The finding that rapid freezing could have an effect on fibrinogen recovery in CRYO has not been reported previously. Group II plasma (baseline) factor VIII: C level was higher than that of Group I which could be explained by higher factor VIII: C levels in blood group A plasma in Group II (P = 0.006).

Comparison of fibrinogen and factor VIII yields in other published studies [Table 5] revealed that mean fibrinogen level per unit in our CRYO was comparable, and the mean factor VIII content per CRYO unit was higher than that of factor VIII content in CRYO observed in other studies. The possible reasons for these differences could be that the previous studies had smaller sample size (n = 10 on an average), preselection of donors for the study, lower volume of the original plasma as it was used for platelet preparation, lack of standardization of techniques for the preparation of CRYO, analysis of stored samples in a batch after thawing, factor VIII assay used (Chromogenic assay versus single-stage clotting assay for aPTT), and volume of the final product of CRYO.

The quality control criteria set forth by DGHS, Technical Manual[3] for fibrinogen levels are 150–250 mg/bag and factor VIII activity of 80–120 IU/bag. Only 8 out of 192 units of CRYO (4.17%) did not meet the requirements (1 CRYO unit did not meet the specification for both factor VIII and fibrinogen levels), whereas all others (95.83%) qualified.

In both of our study groups, the mean factor VIII:C levels in blood group A CRYO were higher than the mean factor VIII:C levels in blood groups O and B CRYO (although it was not statistically significant in Group I). With respect to blood group A, Group II CRYO units had higher factor VIII: C levels than that of Group I (P = 0.001) CRYO units. Our study findings on blood group as a major determinant of factor VIII levels in CRYO are supported by a study reported by Mohanty *et al*.[16] where the authors revealed that factor VIII: C levels were highest in blood group A individuals compared to O and B blood groups. A study by Alakech *et al*.[15] showed that factor VIII: C levels in blood group A CRYO was 169 ± 44 IU/bag and that of blood group O CRYO was 128 ± 55 IU/bag. A study conducted by Freedman and Rock[17] showed that the mean factor VIII: C levels were lower in blood group O CRYO than that of blood group A and blood group B CRYOs. Table 6 shows a comparison of factor VIII levels with respect to ABO blood groups in various studies.

In our study Groups I and II, percentage recovery of factor VIII was higher in CRYO prepared using blast freezer (66.7%

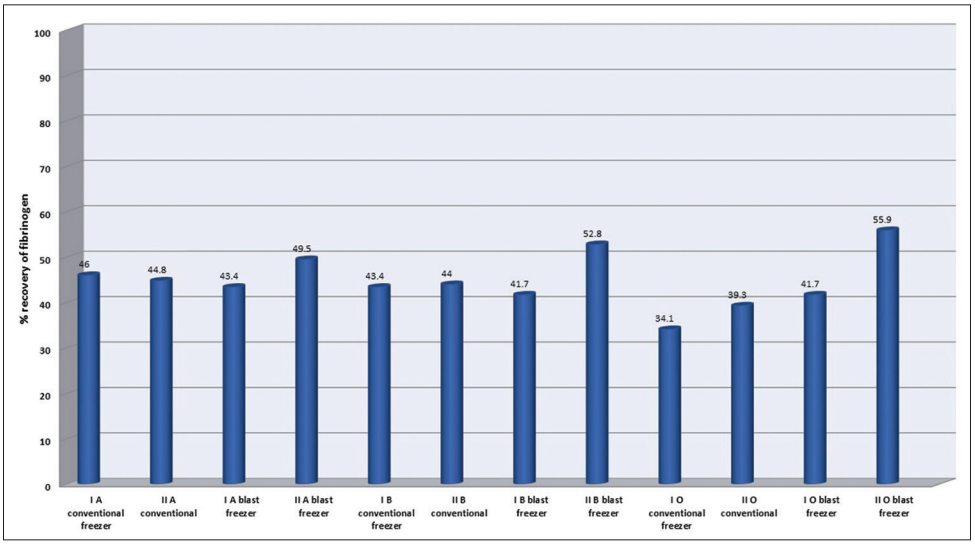

Figure 3: *Intergroup comparison of percentage recovery (%) of fibrinogen in cryoprecipitate with respect to ABO blood group and freezing techniques*

Table 5: Comparison of our study results of factor VIII: C and fibrinogen levels in cryoprecipitate with other studies

Authors	Sample size	Freezing technique (units)		Blood groups	Mean±SD	
		Conventional freezer	Blast freezer		FVIII: C (IU/bag)	Fibrinogen (mg/bag)
Our study						
Group I	96	48	48	A, B, and O	190.3±98.6	247.9±51.8
Group II	96	48	48	A, B, and O	236.0±118.9	285.3±94.6
Yazer *et al*.[9]	20 (controls)	Not mentioned		O and AB	252.4±70.1	455.8±172.6
Yousef *et al*.[10]	10 (controls)	-	Yes	Not mentioned	82.8	286
Hornsey *et al*.[11]	-	-	Yes	A and O	149*±29; 106**±14	271*±31; 288**±82
Ness and Perkins[12]	88	Yes	-	Not mentioned	145±78	266±83.4
Caudill *et al*.[13]	10	Yes	-	O only	133±37	183±44
Omidkhoda *et al*.[14]	96	-	Yes	A, B, and O	86.09#±12.83; 70.76§±12.8	NT
Alakech *et al*.[15]	10	Yes	-	A and O	148±51	221±64

*Results from center A control CRYO (*n*=16), **Results from center B control CRYO (*n*=8), #Chromogenic method for factor VIII assay, §Factor VIII assay – single-stage clotting test. NT = Not tested, SD = Standard deviation, CRYO = Cryoprecipitate

Table 6: Comparison of our study results of factor VIII: C levels and ABO blood groups of cryoprecipitate with other studies#

Blood group	Our study		Other studies				
	Group I (*n*=32 each)	Group II (*n*=32 each)	Mohanty *et al*.[16]*	Alakech *et al*.[15] (*n*=5 each)	Freedman and Rock[17] (*n*=5 each)	Wensley and Snape[18] (*n*=16 each)	Gunson *et al*.[19]** (*n*=6 each)
A	227.6±136.2	338.4±122.4 (*P*=0.001)$	109.28±20.8 (*n*=20)	169±44	59.6±10.8	90±26	89.7
B	199.8±61.1	222.1±74.0 (*P*=0.19)	90.5±18.8 (*n*=26)	NT	51.3±22.4	NT	NT
O	143.5±60.6	147.5±61.1 (*P*=0.7)	96.76±21.4 (*n*=39)	128±55	52.0±11.0	62±60	70.2

#Data presented as mean±SD, IU/bag, $Significant (Group I vs. Group II), *Samples tested were volunteer plasma and measured as IU/dl, **Report of a working party of the Regional Transfusion Directors Committee, England. NT = Not tested, SD = Standard deviation

in Group I; 70.4% in Group II) than that of CRYO prepared using conventional freezer (58.5% in Group I; 55.3% in Group II). In a study by Slichter *et al*.,[20] CRYO units were prepared using different techniques of freezing of plasma, namely controlled rate liquid nitrogen (−80°C) with freezing time of 30 min, ethanol and dry ice (−70°C), and −85°C freezer. The mean factor VIII: C levels in CRYO per bag using these freezing techniques were 122, 131, and 128 IU, respectively. The percentage recovery of factor VIII: C levels in CRYO were 53%, 55%, and 57%, respectively. There was no significant difference in the yield of factor VIII using these different techniques of freezing. The percentage recovery of factor VIII: C in CRYO in this study is lower using controlled rate liquid nitrogen (−80°C) when compared to our study results using blast freezer. The recovery mainly depends on the time for phase change at the freezing point which is achieved earlier with blast freezers or snap freezing with ethanol boxes, preserving the factor VIII: C activity in plasma.

Conclusion

From our study, we conclude that the best quality CRYO in terms of factor VIII yield can be obtained from blood group A donors by utilizing the rapid freezing technique. Higher the baseline factor VIII: C levels, better is the quality of CRYO obtained. Blood collection site does not affect the factor yield provided the cold chain is maintained during transport. Rapid freezing also increases the fibrinogen yield.

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Conflicts of interest
There are no conflicts of interest.

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