Effects of Hemodilution on Red Blood Cell Fragility Index

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ABSTRACT

Twenty patients with coronary artery disease were selected at random to study the effects of hemodilution on red blood cell fragility index before undergoing extracorporeal circulation. The purpose of this study is to establish accurate base line values based on hematocrit ranges as a prelude to studying red blood cell trauma during extracorporeal circulation. For each patient studied, five separate samples were mixed with various amounts of lactated Ringers solution. The dilutions resulted in a separation of approximately 7 hematocrit between the five samples. The samples were then studied within four hours using the modified Dacie's Method.[1,2] Results of the studies show significant differences in RBC fragility values between the samples from 45 hematocrit to 15. A search of the literature dealing with blood trauma reveal no information relating to the significance of hemodilutions to red blood cell trauma. The authors believe that hemodilution is a major factor to consider in evaluating red blood cell trauma studies and a method of this type should be employed in doing so.

CLINICAL MATERIAL

Twenty patients with coronary artery disease were selected at random for this study. They were chosen because as a group they have 1) normal hematocrit values, 2) their blood has not been subject to any trauma like that of the valve patients, and 3) they are readily available. The twenty patients were, however, screened for their hematological profile[3] before their blood was used in this study.

METHODS

Arterial blood is drawn from the patient before perfusion and put into five separate test tubes labeled A, B, C, D, and E. Each test tube contains 0, ½, 1, 1½, and 2cc. of lactated Ringers respectively. The amount of blood put in each test tube will total 3cc. This will result in various levels of hemodilution.

Each sample is then prepared within four hours using the modified Dacie's Method.[2]

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TABLE I

Samples	Lactated Ringers
A	0 cc
B	½ cc
C	1 cc
D	1½ cc
E	2 cc

TABLE II

Samples	Range	Mean
A	39–44	41.4
B	32–36	34.1
C	26–30	27.5
D	20–26	21.8
E	13–16	14.4

Equal amounts of blood are placed in ten reservoirs of various osmotic strengths. The samples are incubated for 20 minutes at room temperature (25°C.), then centrifuged at 2000 RPM for five minutes and the supernates are poured into each corresponding optical cuvette. The spectrophotometer is set to 540 mm. wavelength. After zero calibration is set, each cuvette is read for absorbance or optical density (O.D.). All values are recorded and calculated for percentages of hemolysis using the following formula.

$$\%\ \text{Hemolysis} = \frac{\text{O.D.}^{x} - \text{O.D.}^{0.85\%}}{\text{O.D.}^{.0\%} - \text{O.D.}^{0.85\%}} \times 100$$

O.D.^{x} = Absorbance of solution of any given osmotic strength of sodium chloride, i.e., 0.65%, 0.60%, 0.55%, 0.50%, 0.45%, 0.40%, 0.35%, 0.30%.

RESULTS

Values as a result of hemodilution are as follows.

Sample A (0 cc.)—39 to 44 hematocrit (Hct.) with a mean of 41.4

Sample B (½ cc.)—32 to 36 Hct. with a mean of 34.1.

Sample C (1 cc.)—26 to 30 Hct. with a mean of 27.5.

Sample D (1½ cc.)—20 to 26 Hct. with a mean of 21.8.

Sample E (2 cc.)—13 to 16 Hct. with a mean of 14.4.

This separates each range of hematocrit by approximately 7.

TABLE III
Expected Normal Values

Reservoirs Labeled	Osmotic Strength of Sodium Chloride Solution	Hemolysis Range
00	0.00%	100
30	0.30%	97—100
35	0.35%	90— 99
40	0.40%	50— 90
45	0.45%	5— 45
50	0.50%	0— 5
55	0.55%	0
60	0.60%	0
65	0.65%	0
85	0.85%	0

TABLE IV
Example

Curvette Labeled	Osmotic Strength Sodium Chloride	O.D.
00	0.00%	0.365
45	0.45%	0.218
85	0.85%	0.005

$$\% \text{ Hemolysis} = \frac{0.218 - 0.005 \times 100}{0.365 - 0.005}$$

% Hemolysis = 59%

Significant measurable hemolysis occurs at .45% and .40% osmotic strenghts.[4] Almost total hemolysis occurs at .35% to .00% osmotic strengths. Therefore, this study will only narrate to the values obtained from .40% and .45% osmotic strengths. Calculated percent hemolysis values obtained from the spectrophotometry readings are as follows:

Sample A (41.4 Hct.)—48%-82% with a mean of 66.5% (.40%) and 12%-71% with a mean of 36% (.45%).

Sample B (34.1 Hct.)—44%-83% with a mean of 69.4% (.40%) and 10%-58% with a mean of 35% (.45%).

Sample C (27.5 Hct.)—77%-85% with a mean of 80% (.40%) and 13%-62% with a mean of 34% (.45%).

TABLE V

Sample A	Hct. 39-44	Mean 41.4	
Osmotic Strength of NaCl (O.S.)	Expected Normal Values	Test Results	Test Mean Results
.40%	50-90	48-82	67%
.45%	5-45	12-71	36.0%
.85%	0	0	0

TABLE VI

Sample B	Hct. 32-36	Mean 34.1	
O.S. of NaCl	Expected Normal Values	Test Results	Test Mean Results
.40%	50-90	44-83	69%
.45%	5-45	10-58	35%
.85%	0	0	0

TABLE VII

Sample C	Hct. 26–30	Mean 27.5	
O.S. of NaCl	Expected Normal Values	Test Results	Test Mean Results
.40%	50–90	77–85	80%
.45%	5–45	13–62	34%
.85%	0	0	

TABLE VIII

Sample D	Hct. 20–26	Mean 21.8	
O.S. of NaCl	Expected Normal Values	Test Results	Test Mean Results
.40%	50–90	54–80	70%
.45%	5–45	8–60	30%
.85%	0	0	0

Sample D (21.8 Hct.)—54%–80% with a mean of 70% (.45%) and 8–60% with a mean of 30% (.40%).

Sample E (14.4 Hct.)—59%–72% with a mean of 65.8% (.45%) and 13%–54% with a mean of 26% (.40%).

On the .40% level, mean values of hemolysis show inconsistencies, especially Sample C, with a mean of 80% and a narrow range of 77%–85% (normal expected values for .40% are 50–90). On the .45% level, however, values show consistent and significant differences through the hematocrit ranges especially between C, D, and E.

DISCUSSION

Although RBC fragility index is a more sensitive study than the measurement of

TABLE IX

Sample E	Hct. 13–16	Mean 14.4	
O.S. of NaCl	Expected Normal Values	Test Results	Test Mean Results
.40%	50–90	59–72	66%
.45%	5–45	13–54	26%
.85%	0	0	0

TABLE X
Summary of Results

	Hct.	Mean Values .40% (O.S.)	.45% (O.S.)
A	41	67	36
B	34	69	35
C	28	80	34
D	22	70	30
E	14	66	26

free hemoglobin, its use and application has not been popular. This, we believe, is due to several reasons: 1) preparation of saline solutions are cumbersome and time consuming, 2) difficulties in the execution of the study, 3) difficulty in the interpretation of the data from the studies. By using a commercially prepared disposable kit,[5] we have eliminated problem number one. After studying approximately sixty dogs, we were able to solve the technical problems of execution. Precision seems to be the key. We find even the position of the cuvette is important. Interpretation of the data was difficult at first, until more data was collected. The keys seem to be volume of data and mathematical analysis.

The .40% O.S. level requires restudy in the human; perhaps using an even more sensitive study, the 24 hour incubation technique.[3] This technique is used successfully in detecting patients with hemolytic anemia.[6]

CONCLUSION

This study achieves a method to produce accurate baselines for various levels of hemodilutions. In studying blood trauma, especially if only free hemoglobin is measured, hemodilution should be considered as a major factor. A search of the literature shows no mention of the effects of hemodilution on hemolysis values.[7,8,9,10] We believe a method of this type should be used as a way of standardizing all the future studies.

SUMMARY

1. Twenty patients with coronary artery disease were selected at random to study the effects of hemodilution on red cell fragility index before undergoing perfusion.

2. Five separate samples were mixed with lactated Ringers resulting in various ranges of hematocrit (41, 34, 28, 22, 14).

3. Each range of hematocrit was studied for their red blood cell fragility index (.40%, .45%, .85%).

4. Results from the .40% level needs further study in relationship to their hematocrit ranges.

5. Results from the .45% (O.S.) level show significant and consistent differences through the ranges especially in the lower ranges.

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