Evaluation of haemogram parameters in refrigerated therapeutic whole blood at the blood transfusion center of the Douala General Hospital Cameroon

ABSTRACT

Blood transfusion is a substitute therapy which consists to administered blood or one of its components to one or more sick person. hematological components retain their stability if the cold chain is maintained between 2 and 6°C. The quality of storage of therapeutic blood bags is essential for good transfusion performance. The aim of this work was to evaluate the stability of haemogram parameters under the influence of cold chain on whole blood bags seronegative from infections transmissible by blood transfusion (ITT)

Methodology: Methodology: A cohort of 200 blood collected into the citrate phosphate dextrose adenine (CPDA) blood bag collected consecutively at the blood transfusion center of the Douala General Hospital constituted the population of the study conducted from March 1st to September 30th, 2018. 5ml of this blood from the donor's blood bag was collected into a dry tube (for the blood count using URIT 3000 Plus machine) and the rest was stored in a BIOBASE brand refrigerator at a temperature between 2 and 6. On the day of delivery, a second sample was taken by section of the tubing of the bag for the analysis of a second haemogram of the same blood bags. Temperature, refrigerator opening frequency and blood were collected. Data analysis was done using by SPSS 20.0 for Windows software. The results were considered significant at $p<0.05$.

Results: The storage temperature significantly ($p\leq0.05$) decrease the rate of leukocytes, erythrocytes and hemoglobin. When the shelf life and the frequency of opening the refrigerator increased, hemoglobin, hematocrit, MCV and lymphocyte decreased significantly while leukocyte, MCHC, thrombocytes and granulocytes increase significantly.

Conclusion: This study showed that, the decrease in leukocytes, red blood cells and hemoglobin levels was significantly related to shelf life and frequency of opening the refrigerator. Knowledge on this variation could be very useful in the selection of refrigerated blood or pint and the efficiency of transfusion.

Keywords: Cold Chain, Storage, Blood Transfusion, Variation.

INTRODUCTION

Blood transfusion consists of administering blood or one of its components (red blood cells, platelets, granulocytes, plasma, proteins) from one or more subjects called (donors), to one or more sick subjects called (recipients) [1]. It is a substitute therapy which occupies a place of choice in the therapeutic arsenal of many countries in sub-Saharan Africa hence the existence

of a cold chain for the conservation of blood. The latter is defined as a systematic process intended to ensure the safe storage and transport of blood from its collection from the donor until its administration to the patient who needs transfusion. According to WHO, hematological components are thermolabile and therefore retain their stability if the cold chain is maintained between 2 and 6°C for a period between 21 and 42 days depending on the type of anticoagulant [2]. However, during this storage process, several events may occur, such as voltage fluctuations, repeated opening of the refrigerator. These events associated with the long shelf life of more than 21 days, can be the cause of a number of variations in hematological parameters in the blood bag [3]. It is in view of these factors of variation that we proposed to evaluate the profile of haemogram parameters of blood bags after collection and at the time of delivery to the Douala General Hospital-Cameroon.

MATERIALS AND METHODS

An evaluative study of 200 bags of whole blood was conducted in the blood transfusion service of the Douala General Hospital between the months of March 1st to September 30th, 2018. After collecting samples of blood bags (containing 63ml of CPDA anticoagulant to collect 450ml of blood) by frank venipuncture at the bend of the donor's elbow with asepsis containing 70° alcohols, a quantity of approximately 5ml was dispensed into a dry tube for the first blood count and the tubing of each blood bag was cut after welding with an electric sealer, without breaking the safety of the blood product and stored in a BIOBASE brand blood bank refrigerator at a temperature between 2 and 6°C. On the day of delivery, part of the tubing was cut and then its contents introduced into a dry tube for a second blood count, using the same URIT 3000 plus hematological counter. Donor data (age, sex) were collected from the donor candidate selection form and the donor register. From the day of collection to the day of delivery of the blood bags, the temperatures and the frequency of opening the refrigerators were noted. The data collected during this study was entered using Microsoft Office Excel 2013 software and analyzed by SPSS 20.0 for Windows software. The data was presented in the form of frequency distribution tables. The comparison of frequencies and the tests of association were carried out using the Chi 2 test. The results were considered significant at $p<0.05$.

RESULTS

Table 1 presents the comparison of the means of the blood count parameters at collection and at delivery for the entire study population. According to this table, we noted a general variation Is it the blood bag of all the parameters of the haemogram between the day of

collection of the blood bags and its delivery. A significant decrease in leukocytes, granulocytes and thrombocytes count was observed. However, a significant increase in lymphocytes was observed on the delivery blood count.

Table 1: Comparison of means of blood count parameters at collection and at delivery

	COLLECTION		DELIVERANCE		VARIATIONS	
Parameters	**Mean**	**Standard deviation**	**Mean**	**Standard deviation**	**Mean**	**Standard deviation**
Leucocytes	4.16	1.257	3.043	1.38	1.1261	1.099
Erythrocytes	4.43	0.51	4.42	0.54335	0.0966	0.11729
Hemoglobine	11.911	1.51	11.9	1.5349	0.0879	0.15294
Hematocrite	36.15	4.57	36.453	5.0348	1.0432	1.41494
MCV	81.58	8.04	82.235	8.3653	1.0243	2.11685
MCH	27.171	5.2598	27.223	5.1676	0.4804	0.45623
MCHC	32.873	0.9171	32.556	1.1479	0.7965	1.00797
Thrombocytes	204.347	45.9037	117.265	54.4165	87.1558	46.38542
Granulocytes	42.076	9.8033	22.302	14.0259	20.9272	13.48464
Lymphocytes	46.077	10.1625	64.617	15.9613	20.0923	14.93952

Keys: MCHC: Mean Corpuscular Hemoglobin Concentration; MCH: Mean Corpuscular Hemoglobin Content; MCV: Mean Cell Volume
Note: The average storage time was 7.32 ± 6.79 days with a minimum of 2 days and a maximum of 18 days.

Table 2: summarizes the correlations between the variation of blood count parameters and the storage temperature. A negative correlation coefficient was observed in leucocyte ($r = -0.232$, $p = 0.001$), erythrocytes ($r = -0.172$, $p = 0.015$), and haemoglobin ($r = -0.219$, $p = 0.002$)

Table 2: correlation between the variation of blood count parameters and storage temperature

PARAMETERS	r	p-VALUE
variation of leucocytes	- 0.232	0.001
variation of erythrocytes	- 0.172	0.015
variation of hemoglobin	- 0.219	0.002
variation of hematocrite	- 0.028	0.693
variation of MCV	- 0.020	0.776
variation of MCH	- 0.050	0.479
variation of MCHC	- 0.027	0.710
variation of thrombocytes	- 0.057	0.426
variation of granulocytes	- 0.136	0.056
variation of lymphocytes	- 0.038	0.600

Keys: MCHC : Mean Corpuscular Hemoglobin Concentration; MCH: Mean Corpuscular Hemoglobin Content; MCV: Mean Cell Volume; r: correlation factor

Note: The average storage temperature was 3.44 ± 0.85°c with a maximum of 4.88°c and a minimum of 0.50°C

Table 3: presents the correlations between the variation of blood count parameters and storage time. A negative correlation coefficient was observed hemoglobin (r = -0.326, p =0.0001), hematocrit (r = - 0.568, p = 0.0001), MCV (r = - 0.803, p = 0.0001), lymphocytes (r = - 0.282, p = 0.0001) and a positive correlation coefficient in leucocyte (r = -0.276, p = 0.0001), thrombocytes (r = -0.374, p = 0.0001), MCHC (r = - 0,547, p = 0.0001), and granulocytes (r = -0.327, p = 0.0001)

Table 3: Correlation between the variation of blood count parameters and storage time

PARAMETERS	r	r-VALUE
variation of leucocytes	0.276	0.0001
variation of erythrocytes	- 0.121	0.089
variation of hemoglobin	- 0.326	0.0001
variation of hematocrit	- 0.568	0.0001
variation of MCV	- 0.803	0.0001
variation of MCH	0.041	0.565
variation du MCHC	0,547	0.0001
variation of thrombocytes	0.374	0.0001
variation of granulocytes	0.327	0.0001
variation of lymphocytes	- 0.282	0.0001

KEYS: MCHC: Mean Corpuscular Hemoglobin Concentration; MCH: Mean Corpuscular Hemoglobin Content; MCV: Mean Cell Volume; R: correlation factor
Note: The average storage time was 7.32 ± 6.79 days with a minimum of 2 days and a maximum of 18 days

Table 4 presents the correlations between the variation of blood count parameters and the frequency of opening of the refrigerator. From this table, we observed a negative correlation coefficient in erythrocytes (r = -0.254, p = 0.0001), haemoglobin (r = -0.334, p =0.0001), hematocrit (r = -0.595, p = 0.0001), MCV (r =- 0.803, p = 0.0001), lymphocytes (r = -0.280, p = 0.0001) and a positive correlation coefficient in leucocyte (r = -0.254, p = 0.0001), thrombocytes (r = -0.334, p = 0.0001), MCHC (r = - 0.598, p = 0.0001), and granulocytes (r = -0.298, p = 0.0001).

Table 4: correlation between the variation of haemogram parameters and the frequency of opening of the refrigerator.

PARAMETERS	r	p-VALUE
variation of leucocytes	0.254	0.0001
variation of erythrocytes	-0.147	0.046
variation of hemoglobin	-0.334	0.0001
variation of hematocrit	-0.595	0.0001
variation of MCV	-0.821	0.0001
variation of MCH	0.073	0.325
variation of MCHC	0.598	0.0001
variation of thrombocytes	0.334	0.0001
variation of granulocytes	0.298	0.0001
variation of lymphocytes	-0.280	0.0001

Keys: MCHC: Mean Corpuscular Hemoglobin Concentration; MCH: Mean Corpuscular Hemoglobin Content; MCV: Mean Cell Volume; R: correlation factor
Note: The daily refrigerator opening frequency obtained was 12 ± 4 times.

DISCUSSION

This study revealed a decrease in erythrocytes which was significantly correlated with the increase in the frequency of opening the refrigerator, the increasing number of days of storage and the drop in the storage temperature of the blood bag in the cold chain. Indeed, a red blood

cell has a lifespan of about 120 days and can be eliminated by two mechanisms, namely extravascular hemolysis which is predominant and intravascular hemolysis [4, 5]. During storage, erythrocytes are subject to lesions resulting from the alteration of energy metabolism (decrease in ATP, acidosis, increase in calcium concentration) as well as oxidative stress (oxidative attacks on structural proteins) thus causing modifications to metabolic, functional, morphological and rheological erythrocytes. These lesions appear gradually from the first days of storage and accumulate rapidly from the second week, hence their decrease observed over time [6,7,8,9]. In addition, greater hemolysis is reflected with the long stay of erythrocytes in the cold chain by a decrease in the level of hemoglobin. These results are comparable to those of [10] who observed a variation in hemoglobin in dogs.

The number of thrombocytes decreases upon delivery. According to some studies, when standard platelet concentrates are prepared and well preserved, they can only last a maximum of five days (in vitro), a period which guarantees their viability for a satisfactory platelet transfusion yield [3]. However, increased thrombocytes was observed when they are varied with respect to increased in the frequency of opening the refrigerator and the shelf life. This increase can be attributed to pseudothrombocytosis due to fragments of hemolyzed red blood cells, proteins, etc. which can be read as platelets by automaton [11]. Storage is accompanied by a decrease in antioxidant defenses and an increase in oxidative attacks with the formation of Reactive Oxygen Species (ROS) [6, 7-8]. Causing fragmentation of structural proteins, reduced deformability, osmotic fragility and increased mechanical fragility that can be read as platelets [12,13]

Leukocytes decreased on the day of delivery compared to the day of donation. This result could also be due to oxidative stress during storage. This result is in agreement with those of several authors [14,15]. Granulocytes are neutrophils, eosinophils and basophils. The URIT 300Plus machine counts them and associates them with the monocytes. With the influence of storage, in the long run between 2-6 degrees Celsius, the probable morphological changes are read as lymphocytes by the automaton; hence the increase in the rate of lymphocytes at the detriment of granulocytes with the increase in the number of days of storage and the frequency of opening the refrigerator.

CONCLUSION

The results of this study show a decrease in leukocytes, granulocytes, thrombocytes and an increase in lymphocytes on the day of delivery was correlated with the increase in frequency

of opening the refrigerator, number of days of storage and a significant variation of storage temperature of the blood bags in the cold chain.

ETHICAL CONSIDERATIONS

For this study, we received a research certificate from the Dean of the Faculty of Medicine and Pharmaceutical Sciences of the University of Douala: the administrative authorization of the Director of the General Hospital of Douala and the ethical clearance issued by the institutional ethics committee for human health research of the University of Douala.

DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded

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