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Evaluation of the correlation between pH and MPV platelet concentrates prepared in Tirana Blood Transfusion Center.

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Abstract

The quality of platelet concentrates is an important option in transfusion therapy. pH and platelet indices have been found to be valuable parameters for monitoring the in vitro quality of platelet concentrates. Platelet activation which leads to loss of its functionality has been demonstrated by changes in those two parameters. The aim of the study was to evaluate the correlation between pH and mean platelet volume (MPV) in platelet concentrates in order to examine the quality of platelet concentrate. 150 units of platelet concentrates were produced by platelet reach plasma (PRP), and stored for 5 days. Then MPV and pH were analyzed by automated hematological cell counter and Ph meter respectively. Regression analysis showed that there was a significant influence of pH changes on the changes in MPV ($P<0.05$). On the other hand, increase in pH lead to decrease in MPV. Storing platelet concentrates up to 5 days may stimulate platelet activity, enhancing its size and resulted in its destruction, so the remaining platelet are those with significantly lower MPV. Also platelet activation was those with an increase in pH. As a result measurements of MPV and pH have a great potential as quality markers of platelet concentrates.

Keywords: MPV, platelet concentrate, transfusion.

Introduction

Since platelets are usually stored as concentrates, the storage of platelet concentrates products in blood banks has been extensively studied.

There is increased demand for stored platelet concentrates (PCs) for therapeutic transfusions such as treatment of patients with disorders resulting in thrombocytopenia and for patients who become thrombocytopenic after chemotherapy or major invasive procedures, such as cardiac surgery. High platelet quality would be expected to result in improved clinical efficacy, determined by count increment, improved hemostasis, and lower risk for adverse reactions in recipients [1]. Increasing the storage time of platelet concentrates have always been a challenge in Transfusion medicine and many studies have been carried out with the aim of improving it. [2, 3, 4]. Platelets are routinely stored in plasma for five days at 22°C. The biochemical, structural and functional changes that occur during platelet storage under blood bank conditions are collectively known as platelet storage lesion. Platelets must be stored under conditions which guarantee that their viability and haemostatic activities are optimally preserved. Storage temperature must be + 20^0C to + 24^0C under constant agitation. The maximum storage time is five days. Storage may be extended to 7 days in conjunction with appropriate detection or reduction of bacterial contamination [7]. Storing platelet concentrates up to 5 days may stimulate platelet activity, enhancing its size and resulted in its destruction, so the remaining platelet are those with significantly lower MPV.

In the present study, we investigated changes of pH and mean platelet volume (MPV) in platelet concentrates in order to examine the quality of platelet concentrates produced in National Blood Transfusion Center, the only institution that provides this human blood product in Albania.

Material and Methods

Platelet counts, agonist-induced aggregability, mean platelet volume (MPV), and pH were monitored in platelet concentrate produced from 150 healthy blood donors, before and after storage of the PC at room temperature. The subjects were voluntary blood donors, age 25–46 years (85 men, 65 women). None had hematologic or bleeding disorders nor had taken any medication for ≥2 weeks. Platelet counts, mean platelet volume (MPV), were analyzed with an electronic counter (ABX Micros-60 cell counter). pH was measured with pH-meter. Those parameters were monitored at the day one and also at the day six of storage of platelet concentrates in room temperature. Bacterial contamination was tested by Bactec for each unit at the day 5 of storage.

2.1. Platelets preparation: Platelet concentrates were prepared by platelet rich plasma (PRP) method. [5] The whole blood (450 ml) was collected in anticoagulant Citrate Phosphate Dextrose Adenine (CPDA) triple

blood bags (Macopharma). After a resting time of 30 minutes, the whole blood was centrifuged in a centrifuge 6000i at 1750 g for eight minutes at 22°C to obtain platelet rich plasma (PRP). The obtained PRP was again centrifuged at 3850 g for eight minutes under same experimental conditions. After the final centrifugation, the supernatant platelet poor plasma (PPP) was separated, and the residual pellet with the platelets was resuspended in a mean volume of 60±0.9 ml of plasma. For storage of platelets in additive solution, 10-15 ml of plasma was left with the platelet concentrates, and eight ml of additive solution was added to every two ml of platelet concentrate to give an expected final concentration of 80% additive solution and 20% plasma. The volume of additive solution and stored platelets had a mean volume of 50±1 ml. Then non-filtered platelet concentrates was placed in a platelet incubator with continuous agitation at 70 cycles/minutes during storage at 22°C.

2.2. Statistical Analysis: Data were reported as means ± standard deviation (SD). The data was compared using paired t-test. The confidence limit was kept at 95%, hence a $p < 0.05$ was considered to be statistically significant.

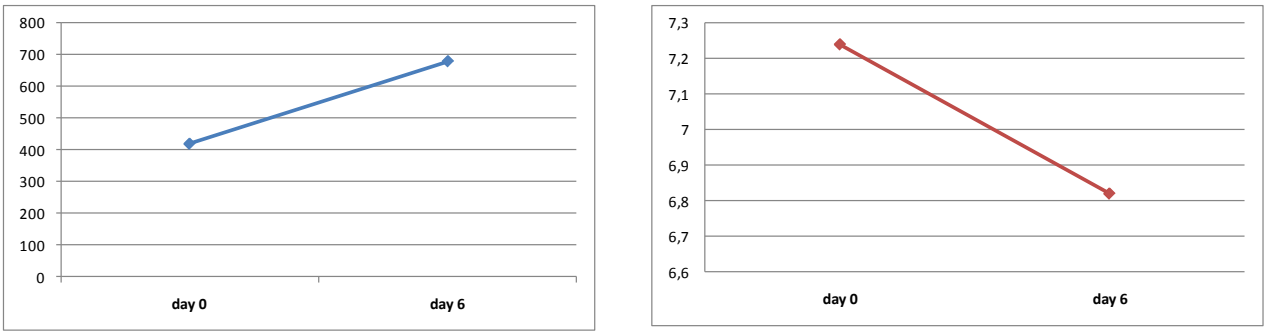
Results and Discussion

In our study, from 150 samples analyzed, two of them were excluded for bacterial contamination. The mean pH of platelet concentrates stored at room temperature was 6.82 at the day six, at which the mean platelet volume was 678.3 x 10^3/ µl. The mean platelet volume on day 0 was 419.25 x 10^3/µl that increased to 678.3 x 10^3/µl on day 6. Significant differences were noted in pH values. (Table 1). Regression analysis showed that there was a significant influence of pH changes on the changes in MPV (P<0.05). On the other hand, increase in pH lead to decrease in mean platelet volume (MPV). (Figure 1.a and 1.b)

Table1. Some metabolic parameters over 5 days of storage of platelet concentrate (PC).

Parameter	*Day 0*	*Day 6*
pH	7.26±	6.82±*
MPV	419.25 x 10^3/µl	678.3 x 10^3/µl

Values are mean ± SD; * P<0.05 compared to day 0.



(a) (b)

Figure 1. Significant influence of pH changes on the changes in MPV. **a**. Changes in MPV after storage of platelet concentrate; **b**. Changes in pH value after storage of platelet concentrate.

The pH measurement is considered a global indicator of the platelet environment, demonstrating the balance between platelet metabolism, bacterial contamination if present and the buffer capacity of the medium, with an acceptable range of 6.4 – 7.4 at 22° C in Europe and > 6.2 in USA in order to retain platelet function [8].

Among metabolic parameters pH showed a significant fall over five days of storage. Fall in pH may affect the quality of final platelet product. The American Association of Blood Banks (AABB) [6] recommended that platelets with pH < 6.2 should not be used for transfusion, and in Europe the same recommendation applies to platelets with pH > 7.4. [7] This limitation in pH decline could be explained by the absence of bacterial contamination as demonstrated by negative culture in all studied PCs and by the fact that the quality of the storage containers allowed proper exchange of oxygen and CO_2 between the outside air and the suspended platelets [9]. The results reported in this study are in accordance to the prior study done by Singh and his colleagues in 2009 [10]. The higher leukocyte contamination in the non-filtered PCs included in our work, resulted in significant glucose consumption over time and consequently its concentration showed significant decrease on day 6 versus days 0.

Metabolic activity of platelets continues during storage. Storage temperature influences pH, glucose consumption, and lactate production. When the pH reaches 6.8, platelet morphology begins to change and it changes dramatically when pH drops below 6.0, and the shape transition becomes irreversible and viability is lost.[11]

In this study, we investigated the changes in pH and MPV in stored platelets concentrates and the demonstration of the swirling phenomenon, based on light scattering by platelets of normal morphology in movement.

Conclusions

Quality control measures such as pH, PLT count per unit and MPV were taken and this provide valuable information to our organization and our experience in producing platelet concentrates.

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