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Platelet Storage Lesion. An institutional study

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ABSTRACT

***Background & objectives**: The biochemical, structural and functional changes that occur during platelet storage under blood bank conditions are collectively known as platelet storage lesions (PSL).As EDTA enhances the storage-induced changes, changes in platelet indices with and without EDTA incubation are promising new tests to monitor the PSL.*

***Materials & methods** :Morphological changes in platelets were monitored by automated hematological cell counter for platelet count and mean platelet volume (MPV).Samples were incubated with K2EDTA FOR 1 h and platelet indices were repeated on the EDTA incubated samples. Difference between pre-and post-EDTA incubation of platelet count (dPLT) and MPV (Dmpv) were calculated.*

***Results**: There was no significant change in the indices without EDTA during storage, however, after EDTA incubation significant changes were noted in dPLT and Dmpv.*

Interpretation & conclusion: The results indicate that storage induced that storage induced lesions take place even in second generation platelet storage containers under recommended conditions of storage.

KEYWORDS : platelet indices, platelets storage lesion, metabolic changes.

Introduction :

Platelet storage lesions (PSL) are detrimental to the post transfusion functional capacity of platelets. As EDTA enhances the storage-induced changes, change in platelet indices with and without EDTA incubation are promising new tests to monitor the PSL.

EDTA is the anticoagulant recommended for full blood cell counts and white blood cell differential analysis principally for its cell preservation properties. However, an attempt to select and standardize on a particular salt of EDTA has been abandoned and replaced by the H1-A standard.[1] The International Council for Standardization in Hematology currently recommends the dipotassium salt of EDTA as the anticoagulant for full blood counts.[1] In Europe and Japan, it is the preferred anticoagulant for this purpose, whereas in the US and the United Kingdom the tripotassium salt of EDTA is more commonly used.[1] Under optimal conditions (appropriate anticoagulant concentration and analysis within 1-4 h after phlebotomy), the choice of dipotassium DETA or tripotassium EDTA makes little difference to the results of full blood cell counts and white blood cell differential analyses.[1] Heparin is not used because it is considered too expensive, it activates platelets and it affects the staining proprieties of cells.[1] Citrate is used as an anticoagulant primarily for coagulation studies.

Two main requirements must be met when monitoring platelet activation ex vivo: (a) a venipuncture procedure must be used that minimize spontaneous platelet activation and (b) blood must be collected into a medium that will not only prevent coagulation, but will also preserve the activation status of platelets until the samples can be analyzed.[2] None of the above-mentioned commonly used anticoagulants is able to prevent platelet activation, and the extent to which preanalytical activation occurs is markedly dependent on the anticoagulant into which blood is collected.[1] If blood is collected into EDTA; platelets quickly change shape from discs with a 2-4 um diameter and a thickness of 0.5um to spheres covered by long thin filopodia.[1] The sphering of platelets in EDTA is initially isovolumtric, but also almost immediately, their apparent size changes over ~1-2 h unit a state a semiequilibrium is reached. [2]

Normal circulating platelets display a log normal size distribution pattern. The platelet size histogram shows evidence of heterogeneity even in healthy subjects. Age, gender, and the number of circulating platelets influence the size distribution. [3]

Circulating platelets may be considered a pool of platelets with different age and size. Platelets size measured as mean platelets volume (MPV) can reflect either the level of platelet stimulation, or rate of platelet production or senescence.[4]

The controversy over whether platelet change volume or density in the circulation is still not clearly resolved. Although the relationship between platelet number and size and megakaryocyte number, size and ploidy has been described[110], the factors that regulate this interaction are till poorly understood. [5]

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 . Survival of platelets, like that of all other living systems, depends on the maintenance of a delicate biochemical balance between different substances including, in particular, glucose and hydrogen ions. 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. These lesions may have an impact on platelet viability and hemostatic function.[6]

Recently platelet indices such as platelet count (PLT), mean platelet volume (MPV), platelet distribution width (PDW), and platelet-large cell ration (P-LCR)have been used as markers for the quality control of PCs, as these reflect storage-induces shape changes in platelets.[7]

Materials and Method

125 random donor platelets were prepared by platelet rich plasma (PRP) method. The whole blood (350 ml) was collected in anticoagulant Citrate Phosphate Dextrose Adenine (CPDA) triple blood bags (HL Hemopack, Hindustan Latex Ltd. Kerala, India). After a resting time of 30 minutes, the whole blood was centrifuged in a Cryofuge 6000i. (Heraeus-Kendro, Hanau, Germany) at 1750 g for eight minute at 22° C to obtain platelet rich plasma (PRP). The obtained PRP was again centrifuged at 3580 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 50 $\pm$ 0.9 ml of plasma.

Sampling was done through sample site coupler with bacterial filter 4C2405 (Baxter, USA), and large bore needle to avoid artificial activation of platelets. The samples were analyzed for platelet count and MPV with and without EDTA incubation using cell counter (KX21 Sys-

tem, Japan). 0.5ml of homogenized platelet concentrate was added to a 4ml vial containing 0.625 mg of K_2 EDTA (Biotech, India) and incubated at 22-24 ° C for 1 h. The dMPV and dPLT were calculated: $dPLT = \text{Platelet count}_{\text{citrate +EDTA}} - \text{Platelet count}_{\text{citrate}}$

$dMPV = MPV_{\text{citrate + EDTA}} - MPV_{\text{citrate}}$

Normal discoid platelets, when exposed to along source and gently rotated or squeezed, refract light and produce a "swirling" phenomenon that can be identified by trained personnel in PCs for transfusion. It was shown already in the 1970s that maintenance of normal discoid morphology during storage may be essential for maintenance of platelet viability. According to a multicenter evaluation swirling seemed a promising method for large-scale quality control of PCs.[8] Swirling can be scored with levels 1-4 according to the study Holme at al.[9]

4	Excellent	6.8-7.3
3	Good	6.7-6.8 or 7.3-7.5
2	Fair	6.4-6.7 or >7.5
1	Poor	< 6.3

The ESC assay measures the proportion of platelets with a discoid morphology in platelets for transfusion. In the test, with the addition of EDTA (to prevent aggregation) and ADP (to activate platelets), discoid platelets will undergo rapid changes to spherical forms. This transformation is associated with a decrease in transmitted light. The extent of decrease in transmitted light has been shown to be directly related to the percentage of discoid platelets in the platelets-rich plasma (PRP).[9]

The HSR measures the ability to platelets to recover their volume after being exposed to a hypotonic environment. In the test, the addition of waters to the cuvette containing PRP causes the platelets to swell because of the influx of water platelets swelling results in a decrease in the refractive index of the platelets and an increase in transmitted light. With maintenance of normal membrance integrity and energy metabolism, platelets are able to extrude the water to regain norm volume.[10]

As ESC and HSR reportedly correlate with the in vivo recovery percentage of radio-labeled stored platelets (R=0.71 and 0.57, respectively), the assays are considered the 'gold standards' for in vitro assay of platelets quality.[11] Although multi-laboratory comparative studies involving ESC have been relatively successful, lack of stable controls has precluded further standardization of these biologic assays even within a single laboratory. Therefore, the use of more quantitative methods would be of value.

The PC sample was first diluted 1 in 20 by adding 50 µl of sample to 950 µl of lysing fluid (1% aqueous ammonium oxalate solution) and kept for 10-15 min at room temperature with intermittent mixing. Improved Neubauer counting chamber was charged with the diluted sample and kept for another 20 min in a moist petridish. PLT were focused under light microscope (40 x objective) (Olympus Corporation, Tokyo, Japan) in five triple lined squares of the central square of the chamber. The PLTs were focused and counted in five fields of equal dimensions and the pictures were magnified and captured using the software "PC TV" (Microsoft, USA). The MS was done by modification of the original method of "Kunicki score" based on the following changes in PLT structure and shape on storage.[12]

PLTs with smooth contours-on two dimensional view under light microscopy discs and spheres could not be differentiated and were categorized in the same group.

dendrites-PLTs that have developed pseudopodia and / or dendritic processes; and long tubular forms-PLTs that have turned into elongated tubes:

balloons- PLTs that have undergone swelling after losing the capacity to maintain an osmotic gradient across their membrane; aggregates-these were clumps of multiple PLTs and could not be counted individually; fragments-these were minute forms pinched off from PLT membrane.

The percentage of discs and spherical forms (i.e., PLTs with smooth contours and normal size) was calculated in one group (a). The percentage of dendrites and long tubular forms was placed in the second group (B) The third group included the balloons, aggregates and the PLT fragments (c). The percentage of each group was multiplied by a series of arbitrary factors as follow: group (a) x2, group "(b) x1 and group (c) x0. The "MS" was obtained by the total of the three numbers thus derived and the maximum score possible was 200, indicating the best quality.

Data was entered into Microsoft excel data sheet and was analyzed using SPSS 22 version software. Continuous data was represented as mean and standard deviation. Independent t test was used as test of significance to identify the mean difference between two groups. p value <0.05 was considered as statistically significant. To assess the correlation between morphology and other biochemical markers of PLT activation Pearson correlation coefficient (r) was applied.

Results:

Table1 shows there was no significant difference in mean platelet values between two groups.

Table2 shows there was significant difference in MPV between two groups.

Table3 shows there was significant difference in PDW between two groups.

Discussion:

Preservation of platelet structure, composition, and function during preparation and storage is one of the primary goals of transfusion medicine practice. Technical advances in the form of introduction of automated cell separators, optimized centrifugation procedures, improved storage containers, leucoreduction, PSL is still a matter of concern.

Various laboratory tests have been recommended to study PSL ranging from most simple test such as pH to more complex test of platelet function. The assessment of mean platelet volume (MPV) is a newly coming up test for studying the PSL a MPV correlates with morphological changes that occur during storage of platelet concentrate (PC) [2]. However, the single determination of MPV at any point of storage is not very much helpful. Incubation of sample with EDTA and calculating the difference in MPV before and after addition of EDTS, known as dMPV, is found to be more reflective of PSL than MPV alone [6]. Similarly, dPLT the difference in platelet count before and after addition of EDTA, represents aggregation capability of functional platelets during storage [6]. Similarly, dPLT the difference in platelet count before and after addition of EDTA, represents aggregation capability of functional platelets during storage [6]. Though a numbers of tests are available, no single test is reflective of PSL in toto.

Platelets get activated following exposures to foreign surface, trauma, low pH agonists (thrombin, ADP) and shear stress. Upon activation, the platelets lose their discoid morphology and become more spherical with multiple pseudopods.[5] Conformational changes in GPII/IIIa complex expose binding sites for adhesive proteins (fibrinogen, vWF) resulting in platelet aggregates test have been adopted to assess PSL.[6]

Various tests have been adopted to access PSL. Corrected count increment after platelet transfusion and bleeding time studies are commonly used for evaluating product efficacy and improved hemostasis, respectively However these are not authentic due to numerous patience variables. Alternatively we can measure measure the in vivo recovery and survival of fresh or stored radiolabelled or biotinylated platelets in healthy volunteers.[7] However, these in vivo studies are expensive, time consuming, and complex to perform In the present study , post incubation with EDTA platelet indices shows significant changes.[8] This could be due to EDTA being a strong calcium chelator, which in-turns causes mitochondrial damage enhancing the storage-induced changes. Hence, effect of EDTA represents the activity of residual platelets in PC which are yet to undergo alternation in shape

changes or platelet aggregation during storage[9]. Therefore, measurement of indices such as dPLT and dMPV after EDTA incubation may help to study PSL. We observe a decrease in dPLT and an increase in dMPV during the storage. A higher dMPV indicates good ability of the platelets to undergone EDTA induced shape changes and thus indicating a better functional activity[10]. A lower dPLT indicates presence of lesser number of aggregates in platelet concentrates. A lower dPLT could also mean decreasing aggregation capacity of platelet. Our results are similar to the studies conducted by the Singh et al and Seghatchian et al.[11]

Conclusion:

Platelets storage remains a major challenge to transfusion services. Technologies and strategies are desired that allow extended storage without causing an unacceptable loss of product quality.

Platelets indices such as the platelet count, mean platelet volume (MPV) and platelets distribution width are used as a marker for maintaining quality control of PC and also considered representative of storage induced shape changes in platelets.[12] The assessment of MPV is an imminent test for studying the PSL as MPV correlates with morphological changes that occur during storage of PC.[13]

According to our results, it may be concluded that platelet storage lesion (PSL) can occur during long-term storage of platelet with a nearly constant slop. Our results also showed that the biochemical and platelet indices as quality control markers can be potential candidates for monitoring the quality of PC and they are, less time consuming easy to perform and obviate observe bias, by using an automated biochemical and hematological analyzers. However, further investigations are required to monitor PSL during PC storage by other platelet quality markers.

Table1: Comparison of platelet count between two groups

Group Statistics					
	Group	N	Mean	SD	P value
PLT	EDTA	124	336.403	270.3259	0.446
	Without EDTA	124	310.535	263.3964	

There was no significant difference in mean platelet values between two groups

Table 2: Comparison of MPV between two groups

	Group	N	Mean	SD	P value
MPV	EDTA	124	3.3266	2.26134	0.018
	Without EDTA	124	2.6323	2.34186	

There was significant difference in MPV between two groups.

Table 3: Comparison of PDW between two groups

	Group	N	Mean	SD	P value
PDW	EDTA	124	14.05	5.05	0.039
	Without EDTA	124	12.79	4.49	

There was significant difference in PDW between two groups.

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