Evaluation of platelet storage lesions in platelet concentrates stored for seven days

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Background & objectives: Platelet storage lesions (PSL) are detrimental to the post transfusion functional capacity of platelets. As EDTA enhances the storage-induced changes, changes in platelet indices with and without EDTA incubation are promising new tests to monitor the PSL. The present study was undertaken to monitor the PSL in 40 units of pooled platelet concentrates harvested by platelet rich plasma (PRP) method and stored for seven days in second generation platelet storage containers using conventional in vitro tests and platelet indices.

Methods: Morphological changes in platelets were monitored by automated haematological 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. Metabolic parameters such as pH, pO₂, pCO₂ and ATP were measured.

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. The mean dPLT on day 0 was 75.15 x 10⁵/µl decreasing to 44.4 x 10⁵/µl on day 7, while dMPV from 0.76 fl on day 0 increased to 1.34 fl on day 7 (P<0.05). Metabolic parameters showed a significant decrease in pH and pCO₂ concurrent with increasing pO₂ during storage (P<0.05). Average ATP level on day 0 was 21.09 µmol/dl falling to 10.59 µmol/dl on day 7.

Interpretation & conclusion: The results indicate that storage induced lesions take place even in second generation platelet storage containers under recommended conditions of storage. Platelet indices especially after EDTA incubation are useful in monitoring PSL. However, how much these changes contribute to poor post transfusion survival and haemostatic function of platelets need to be investigated.

Key words: Platelet concentrate - platelet indices - platelet storage lesions

The biochemical, structural and functional changes that occur during platelet storage under blood bank conditions are collectively known as platelet storage lesions (PSL). These lesions may have an impact on platelet viability and haemostatic function. PSL is associated with morphological changes and platelet activation followed by microvesciculation and loss of function, leading to platelet transfusion failure1-5. Various laboratory tests have been recommended to study PSL ranging from most simple test such as pH to more complex tests of platelet function. The assessment of mean platelet volume (MPV) is a newly coming up test for studying the PSL as 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 EDTA, known as dMPV, is found to be more reflective of PSL than MPV alone. Similarly, dPLT the difference in platelet count before and after addition of EDTA, represents aggregation capability of functional platelets during storage. Though a numbers of tests are available, no single test is reflective of PSL in toto. Therefore, in the present study an attempt was made to analyze platelet storage lesion (PSL) in PCs using platelet indices, such as platelet count, MPV, dPLT, dMPV and metabolic parameters that include pH, ATP, pO2 and pCO2 during in vitro storage for seven days.

Material & Methods

A total of 80 platelet concentrates were prepared by platelet rich plasma (PRP) method in second generation tri-(ethylhexyl)-trimellitate (TEHTM plasticizer) storage containers (Terump-Penpol, Baxter) at Department of Transfusion Medicine, Sanjay Gandhi Post Graduate Institute of Medical Sciences, Lucknow. Two units of ABO group specific platelets were pooled using sterile connecting device (TSCD209A, Terumo, Japan), which prevents contamination, to ensure that a minimum of 50 ml of plasma is left on day 5 after sampling (n=40). Platelet concentrates were stored on horizontal bed platelet shaker with incubator (Helmer, USA) at 20-24°C for seven days. Sampling was done on days 0,1,3,5 and 7 aseptically, through sample site coupler with bacterial filter 4C2405 (Baxter, USA), and large bore needle to avoid artificial activation of platelets. The sealed 5 ml syringe sample was used for measurement of pH, pO2 and pCO2 using blood gas analyzer (Nova ultra C, Nova Biomedical Corporation, USA) at 37°C following the manufacturer’s instructions. The results of ABG analyser were temperature corrected to 22°C. The remaining sample was analyzed for platelet count and MPV with and without EDTA incubation using cell counter (KX21 Sysmex, Japan) as described previously. Briefly, 0.5 ml of homogenized platelet concentrate was added to a 4 ml vial containing 0.625 mg of K, EDTA (Biotech, India) and incubated at 22 – 24°C for 1 h. The dMPV and dPLT were calculated.

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dPLT = \text{Platelet count}_{\text{circ}} - \text{platelet count}_{\text{EDTA}} \\
dMPV = \text{MPV}_{\text{circ}} - \text{MPV}_{\text{EDTA}} \\
\]

ATP was measured in supernatant plasma using commercially available kits from Sigma laboratories (GAPD-G2267, NADH-N4505, PGK-P7634). The optical density was measured on spectrophotometer (Hitachi 150.20, Japan) with 340 nm filter.

Statistical evaluation of data were done by repeated measures ANOVA. (Stata 6, Texas, USA).

Results

Platelet indices with and without EDTA incubation : The mean platelet count on day 0 was 678.3 x 10^3/µl that decreased to 649.25 x 10^3/µl on day 7; the decrease was not significant. Significant differences were noted in PC when sample was incubated with EDTA. The average dPLT on day 0 was 75.15 x 10^3/µl, which significantly decreased to 44.4 x 10^3/µl on day 7 (P<0.05). MPV alone did not change significantly over 7 days of storage. The mean MPV on days 0 and 7 were 9.65 and 9.61 fl respectively. However, dMPV on day 0 was 0.76 fl that increased to 1.34 fl on day 7. This difference was significant (P<0.001).

Metabolic parameters : The pH and pCO2 values of all platelet concentrates decreased during the storage (Table). Values of these parameters obtained from samples analyzed on day 5 and 7 were significantly different (P<0.05) compared to samples analyzed on day 0. Though there was significant fall in pH during storage, average pH was more than 6.8 even on day 7. The pO2 values increased over 7 days of storage. The mean pO2 on day 0 was 19.79 mm Hg that decreased to 13.76 on day 5 but showing a steep rise on day 7 (P<0.004). Statistically significant (P<0.05) fall in ATP was observed over the 7 days of storage of PC.

Discussion

The PSL results from a complex process that is influenced by physical, chemical and metabolic factors related to platelet preparation and storage. Recently, changes in platelet indices during storage of PCs have been found to be useful parameters to study PSL. In the present study non significant change in these indices were observed if measured without EDTA incubation, which is in agreement with other studies. After incubation with EDTA, platelet indices showed significant changes in the present study. This is related to EDTA being a strong calcium chelator, causes mitochondrial damage enhancing the storage-induced changes. Hence, effect of EDTA represents the activity of residual
platelets in PC which have not still undergone shape changes or platelet aggregates during storage. Therefore, measurement of indices such as dPLT and dMPV after EDTA incubation are under evaluation to study PSL.

We observed a decrease in dPLT and an increase in dMPV during the storage. A higher dMPV indicates good ability of the platelets to undergo EDTA induced shape changes and thus indicating a better functional activity. A lower dPLT indicates presence of lesser number of aggregates in platelet concentrates. A lower dPLT could also mean decreasing aggregation capacity of platelets. Since dPLT and dMPV have shown highly significant changes during storage when compared to platelet count and MPV alone, these may be used to study PSL. Other groups have made similar observations.

Among metabolic parameters pH showed a significant fall over seven days of storage. Fall in pH may affect the quality of the final platelet product. The American Association of Blood Banks (AABB) 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. As per the Drugs and Cosmetics Act of India, minimum pH should not be <6 at any given day of storage. Though, a significant drop in pH was observed in the present study, mean pH was 6.8 even on day 7 of storage.

Over these seven days of storage period, an increase in mean pO2 values with concurrent decrease in pCO2 was observed. This is a documented effect of platelet storage lesions with second generation platelet storage containers. During storage, metabolic activity of platelets continues leading to O2 consumption and CO2 production that leads to trend of decreasing O2 and increasing CO2 in container. However, second generation platelet storage containers are more permeable to gases, gaseous exchange take place readily across the container walls which resists accumulation of CO2 and depletion of O2. If the storage container walls were not gas permeable, as in case of first generation plasticizer, oxygen levels would diminish and carbon dioxide levels would increase within the storage containers. Therefore, it can be surmised that second generation plasticizer used in the present study had good gas exchange capability.

ATP levels drop during normal storage of PC. Holme found weak correlation between day 1 ATP levels and post-transfusion platelet recovery. Holme observed that when day 1 PC showed less than 70 per cent ATP levels, there was decrease in percentage of platelet recovery after transfusion. However, accuracy in measuring intracellular ATP levels is difficult, as some of the ATP is in non-metabolic storage pools. The decline in ATP in plasma supernatant was observed to be nearly 30 and 50 per cent on days 5 and 7 respectively in the present study, which is greater than that observed by other investigators. This may be due to difference in methods of measurement of ATP. However, the findings of the present study show a significant decrease in ATP during storage of PCs.

In conclusion, storage-induced lesions take place in PCs, when stored in second generation storage containers under the currently recommended conditions, but how far these change are clinically relevant need to be investigated.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Day 0</th>
<th>Day 1</th>
<th>Day 3</th>
<th>Day 5</th>
<th>Day 7</th>
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<tr>
<td>pH</td>
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<td>7.11±</td>
<td>6.96±*</td>
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<td>32.62±</td>
<td>27.65±</td>
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<td>17.74±</td>
<td>16.63±</td>
<td>13.56±*</td>
<td>10.59±*</td>
</tr>
</tbody>
</table>

N=40 units of ABO specific pooled platelet concentrates. Values are mean±SD; *P<0.05 compared to day 0; **P<0.001 compared to day 0.
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References


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