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Original Article

Effect of Storage Time and Temperature on Platelet Indices of Ethylenediaminetetraacetic Acid Samples

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ABSTRACT

Platelet function assays are pivotal for proper diagnosing the bleeding tendencies and the pre-analytical variables such as storage time and temperature during the transportation are the most crucial and sensitive part in this process which may cause variations in the laboratory test results **Objective:** To find the effect of storage time and temperature on platelet indices of samples taken in Ethylenediaminetetraacetic acid (EDTA) **Methods:** In this study 100 samples of both genders were included. Complete Blood Count (CBC) samples were taken in two EDTA vials from each patient. All the samples were separated in two groups. One sample group was stored at room temperature (RT) and other at 2-6°C. CBC was run on CBC analyzer Sysmex XN-1000 at 0, 4, 12 and 24 hours **Results:** There was significantly increasing trend for Mean Platelet Volume (MPV), Platelet Distribution Width (PDW) and Platelet to Larger Cell Ratio (P-LCR) at RT and decreasing trend at 4°C when compared with 0 hours. Plateletcrit (PCT) showed significant decreasing trend at 4°C with respect to 0 hours. Significant decrease in platelet count was observed after storage at room temperature as well as at 4°C **Conclusions:** Although 4°C is considered as optimal temperature to transport specimen for CBC but Platelet Indices values varies significantly in comparison to baseline when stored at 4oC and at room temperature. It seems prudent to conclude that if platelet pathology is the major drive for CBC test performance then specimen should be run without delay.

INTRODUCTION

Platelets are the small, anucleoid, disc shaped cells produced by the fragmentation of megakaryocytes in bone marrow. They are the most important component of hemostatic mechanism that maintains the blood flow. They are also a valuable indicator of inflammatory process, tissue remodeling, angiogenesis and wound healing. It has been elucidated that they play role in immunity and communication of other cells independently of their primary role [1]. Complete blood count (CBC) is the most commonly advised test in every clinical setting. Modern hematology analyzers measures platelets and platelet indices as part of CBC. Platelet indices include Plateletcrit (PCT), Mean Platelet Volume (MPV), Platelet to Larger Cell Ratio (P-LCR) and Platelet Distribution Width (PDW) [2, 3]. Various studies have demonstrated the change in platelet indices in different diseases like thrombocytopenia. They are also important markers of platelets activation and have diagnostic value in certain inflammatory diseases such as rheumatoid arthritis, ankylosing spondylitis, ulcerative colitis, inflammatory bowel disease and atherosclerosis [4]. Hematology analyzers measures platelet indices by electrical impedance or by optical scatter method. The choice of method affects their values. The most important variable effecting platelet indices beside analyzer is the

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transport and storage of whole blood [5]. In Pakistan the trend of centralized clinical laboratories is prevailing so specimens have to be transported from distant areas which may include transportation from other cities. The transportation time and even sample storage in lab for re-runs or other investigations affects the platelet indices to various extents. This study was designed to measure the effect of different storage time and temperature conditions on platelet indices in comparison to control.

METHODS

The study was carried out at Department of Pathology, Section of Hematology, King Edward Medical University Lahore after approval from Institutional Review Board (IRB). The study design is case control study that spanned on 2 months. After calculation of sample size 100 samples without the specification of gender were included in this study. Improper, delayed specimen for more than 30 minutes and specimens from patients with myeloproliferative disorder were excluded. After informed consent samples were collected in two EDTA vials from each patient. All the samples were separated in two groups of fifty each i.e. Group A - The samples stored at room temperature (22-26°C) and samples in Group B were stored at 4±2°C (2-6°C). The temperature was monitored through digital thermometer having alarms.CBC was run on CBC analyzer Sysmex XN-1000 at 0 hours, 4 hours, 12 hours and 24 hours for both groups. Results were collected on prescribed proforma for Platelet count, PCT, MPV, PDW and P-LCR. Data was analyzed using Paired sample T test SPSS version 23.0 (Statistical Package for Social Sciences).

RESULTS

The mean age of patients included in this study was 40.36 ± 14.419. Malespopulation were 46% (n=46) and females were 54% (n=54). The Mean of Mean Platelet Volume (MPV) of all the patients at 0 hour was 10.00 ± 1.0461. The mean of MPV at 4 C was as follow; after 4 hours 10.11±1.0688, after 8 hours 9.96±1.0072, after 12 hours 9.73±1.1173 and after 24 hours 9.51±1.1402. The mean of MPV at room temperature (RT) was as follow; after 4 hours 10.20±1.0990, after 8 hours10.27±1.0582, after 12 hours 10.35±1.1010 and after 24 hours 10.73±1.0743. MPV at 0 h was compared with MPV at 4°C and RT at different time intervals. There was significant difference (p value < 0.05) when MPV values were compared at 4°C and room temperature at same time interval except one i.e. after 4 hours (p-value .072)(Figure 1).

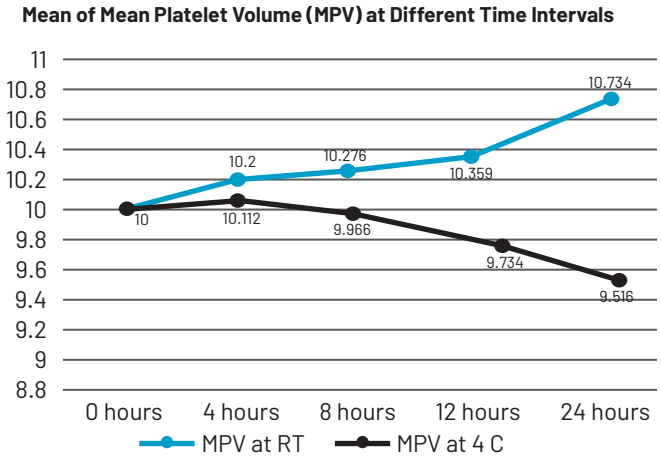

Figure 1: Line graph of mean of mean platelet volume (MPV) showing increasing trend (p-value < 0.05) at RT. There was an initial rise at 4 hours and then decreasing trend

The Mean of Platelet Distribution Width (PDW) of all the patients at 0 hour was 12.41 ± 1.9043. The Mean of PDW at 4C was as follow; after 4 hours 12.78 ± 2.2751, after 8 hours 12.74 ± 2.0633, after 12 hours 12.54 ± 2.4318, after 24 hours 12.21 ± 2.3464. The mean of PDW at room temperature (RT) was as follow; after 4 hours 12.90 ± 2.0779, after 8 hours13.202 ± 2.1912, after 12 hours 13.14 ± 2.4242 and after 24 hours 14.02 ± 2.4216. PDW at 0 h was compared with PDW at 4°C and RT at different time intervals. There was significant difference (p value < 0.05) when PDW values were compared at 12 hours and 24 hours at 4°C and room temperature. But there was no significant difference at 4 hours (0.388) and 8 hours (0.060) at 4°C and room temperature (Figure 2).

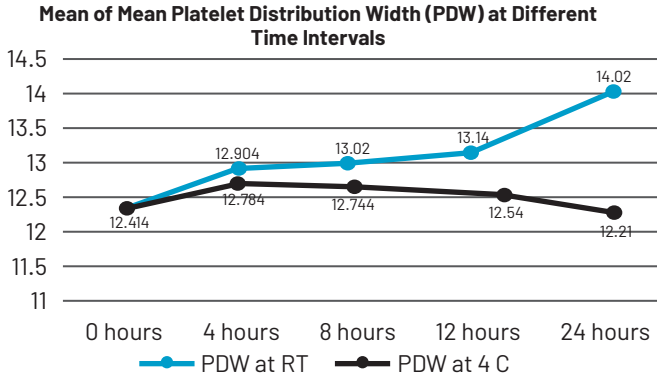

Figure 2: Line graph of mean of platelet distribution width (PDW) showing increasing trend (p-value < 0.005) at RT but at 4°C there was increasing trend at 4 hours and afterwards decreasing

The Mean values of Platelet Large Cell Ratio (P-LCR) of all the patients at 0 hour was 25.60 ± 7.8139. The mean of P-LCR at 4C was as follow; after 4 hours 26.53 ± 8.1135, after 8 hours 25.77 ± 7.4797, after 12 hours 24.21 ± 7.9291 and after 24 hours

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22.68 ± 8.2758. The mean of P-LCR at room temperature (RT) was as follow; after 4 hours 26.97 ± 8.2537, after 8 hours 27.28 ± 8.9750, after 12 hours 28.14 ± 8.4281 and after 24 hours 31.09 ± 8.4908. P-LCR at 0 h was compared with P-LCR at 4°C and RT at different time intervals.There was significant difference (p value < 0.05) when P-LCR values were compared at 4°C and room temperature at same time interval except one i.e. after 4 hours (P value .235)(Figure 3).

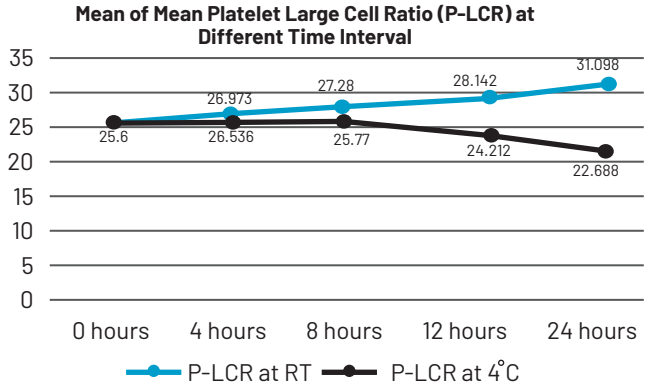

Figure 3: Line graph of mean of platelet large cell ratio (P-LCR) showing increasing trend (p-value < 0.05) at RT and significant decrease (p-value <0.05) after 4 hours at 4°C

The Mean values of Plateletcrit (PCT) of all the patients at 0 hour was 0.27 ± 0.11777. The mean of PCT at 4C was as follow; after 4 hours 0.24 ± 0.11683, after 8 hours 0.23 ± 0.11483, after 12 hours 0.20 ± 0.11496 and after 24 hours 0.16 ± 0.11075 at 4° C. The mean of PCT at room temperature (RT) was as follow; after 4 hours 0.27 ± 0.11742, after 8 hours 0.27±0.11789, after 12 hours 0.27±0.11891 and after 24 hours 0.27 ± 0.12117. PCT at 0 h was compared with PCT at 4°C and RT at different time intervals. There was significant difference (p value < 0.05) when PCT values were compared at 4°C and room temperature at same time interval (Figure 4).

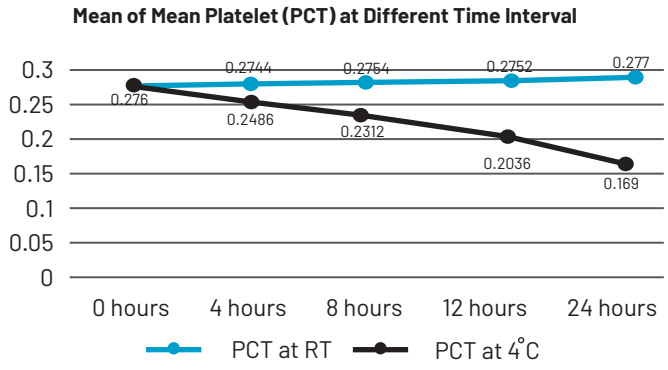

Figure 4: Line graph of mean of Plateletcrit (PCT) showing decreasing trend (p-value <0.05) at 4°C but no significant increase or decrease at RT

The Mean of Platelet count (PLT) of all the patients at 0 hour was 280.86 ± 129.197. The mean of PLT at 4C was as follow; after 4 hours 249.32 ± 122.861, after 8 hours 234.82 ± 118.735, after 12 hours 209.96 ± 113.972 and after 24 hours 179.86 ± 105.653. The mean of PLT at room temperature (RT) was as follow; after 4 hours 274.58 ± 123.037, at 8 hours 272.04±122.314, at 12 hours 269.18±121.975, at 24 hours 266.44 ± 119.717. PLT at 0 h was compared with PLT at 4°C and RT at different time intervals. There was significant difference (p value < 0.05) when PLT count were compared at 4°C and room temperature at same time interval (Figure 5).

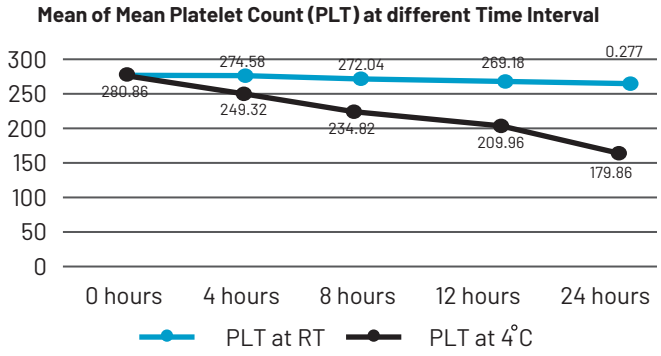

Figure 5: Line graph of mean of platelet count showing decreasing trend (p-value < 0.05) at 4°C and decreasing trend at RT (p-value <0.05) but at 4 hours and at 8 hours with respect to 0 hours p-values were .010 and .014 respectively.

Comparison of p-values of platelet indices at 4°C with platelet indices at RT having same time interval are elaborated in table (Table 1).

Time (Hours)	P value of MPV at 4°C & RT	P value of PDW at 4°C & RT	P value of P-LCR at 4°C & RT	P value of PCT at 4°C & RT	P value of Platelet count at 4°C & RT
After 4 h	.72	.388	.235	.000	.000
After 8 h	.000	.060	.021	.000	.000
After 12 h	.000	.048	.000	.000	.000
After 24 h	.000	.000	.000	.000	.000

Table 1: Comparison of p-values of platelet indices at 4°C with platelet indices at RT at same time interval

DISCUSSION

In clinical laboratories, testing of samples start with the preparation of patients, performance of tests in suitable conditions and finally the report dispatching. Sometimes re-run of test is required to obtain an appropriate result. In many countries, big cities have integrated lab facilities rather than small STAT lab in remote areas. In this situation stabilization of the specimen is necessary during transportation, same is true for Pakistan. There are 4-5 main laboratories located in big cities with many collection centres in small cities and remote areas. Complete blood

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count is one of the commonly advised blood test that gives valuable information to physicians. Platelet indices which include PCT, PDW, MPV and P-LCR are the important markers of complete blood count. These indices are used by clinicians to elaborate causes of thrombocytopenia. Platelets exert pivotal role in many inflammatory diseases, acute pancreatitis, ulcerative colitis, ankylosing spondylitis, rheumatoid arthritis, angina, MI and atherosclerosis [6]. In patients of sepsis, the rise in MPV, P-LCR and PDW with lesser extent indicates a worse prognosis. There are repertoires of mediators actively secreted and expressed by platelets that play their role in inflammation, coagulation, thrombosis and atherosclerosis [7]. So keeping in mind the significance of platelets indices a study was designed to see the effects of temperature and time platelets indices. This study showed that platelets count decreased as storage time increased regardless of storage temperature however, storage at 4oC resulted in marked decrease in count. These results were surprising in respect that most of the studies reported 24 hours stabilization of platelets at RT as well as at 2-6oC [8-11]. However, increase in platelet count is also not uncommon. Increase in storage temperature and time results in morphological changes causing fragmentation of platelets and hence increasing the count [12]. Decrease in platelet count was also observed by different researchers. Jain et al., reported significant trending decrease in platelet count when stored at 33oC for 72 hours [13]. Another study by Wu et al., also reported the same phenomena. It may be due to the shape change from discoid to spherical followed by platelets activation resulting in aggregation that exclude them from platelets thresh hold range for counting [7, 14, 15]. In this study the mean of MPV at 4oC showed an initial rise in MPV and followed by decreasing trend till 24 hours of study while at room temperature a throughout increasing trend was noted. There was significant difference (p value < 0.05) when mean of MPV at 0 h was compared with MPV at 4°C at different time intervals. Our results are in partial agreement to the study of Pinter et al., and Gunawardenet al., who have reported over all increasing trend in MPV at room temperature and 4oC as well. However, room temperature has showed more pronounced effect than 4oC [9, 16]. Sing et al., also reported the increase in MPV at room temperature. This change is MPV is attributed to morphological changes happening during storage [17]. Our findings differ from previous studies in respect to MPV at 4oC as most of the studies reported overall increase in MPV at RT and 4oC but in our study a decreasing trend in MPV was observed at 4oC. Such contrary results may be due to the type of hematological counter used because it also affects the results [14, 16, 19]. International council for standardization in hematology (ICHS) recommends 6 hours storage at 18-22oC and 24h storage at 2-6oC and these guidelines serves best with most of the CBC parameters but not all. It can be clearly concluded from our study that there was significant difference (p value < 0.05) when we compared MPV at 0 h with MPV at 4°C after 4 hours storage. PDW is valuable indicator of platelet anisocytosis. It is reported that there is a direct relationship of MPV and PDW and they usually change in the same direction. In our study PDW and MPV also changed in the same direction. PDW is a MPV dependent parameter so it should be interpreted accordingly [2]. In our study initially there was a rise in MPV and PDW at room temperature and as well at 4oC. However the difference between PDW and MPV at both temperatures was statistically insignificant till 8 hours of incubation. There was significant difference (p value < 0.05) when we compared PDW values at 12 hours and 24 hours at 4°C and room temperature. PDW exhibited gradual decrease at 4oC and gradual increase at room temperature after 4 hours of incubation. PCT denotes volume occupied by platelets in percentage. Under physiological conditions the amount of platelets is maintained in equilibrium by regeneration and elimination process. It is also a derived parameter and it is obtained by multiplying the platelet count ad MPV and then dividing it by 10000. So it is affected by the change in MPV and platelets count [20]. In our study PCT remained stable for entire 24 hours at room temperature however; it showed marked gradual decrease at 4oC. It seems that in calculation of PCT a prior decrease in platelet count and increase in MPV at room temperature adjusted the PCT at near normal range. It also looks prudent to fetch that decrease in platelet count and MPV at 4oC resulted in gradual drop in PCT. P-LCR is a valuable indicator of giant (more than 12fl) circulating platelets. In our study P-LCR exhibited stability till 12 hours of incubation at both temperatures. However statistically significant increase at room temperature while decrease at 4oC was observed at 24 hours of incubation. From this study in can be extracted like other authors that platelets can swell when incubated at room temperature resulting in higher P-LCR [21]. However decrease in P-LCR at 4oC after 24 hours of incubation warrants further work up. From above discussion it could be inferred that whenever platelet indices are required in diagnostic or prognostics scenarios they should be interpreted carefully. Any storage temperature does not suffice stability to all parameters. So in such situations, running the specimen immediately after collection in near vicinity seems right decision rather than transporting it to far flung area.

CONCLUSIONS

The results of our study suggest that care should be taken in interpreting the platelets indices results when sample is

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stored for more than 3 hours. Because storage conditions including 4oC cause significant alterations in the results of platelet indices. Whenever sample transport is required causing delay in analyzing the specimen, platelet indices shall be interpreted with caution.

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