ORIGINAL ARTICLE

The Effect of Storage Temperature of Whole Blood on Platelet Count – A Biophysical Perspective

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ABSTRACT

Introduction: Hematological examinations of blood samples are frequently analysed with significant time delay and this interval is a highly significant pre-analytic variable along with the storage temperature of blood samples. The present study aims to assess the variation in platelet count with time for samples stored at the real room temperature (30°-40°C), at 4ºC and at 25°-27°C.

Materials and Method: Fifty apparently healthy young adults, aged 17-25 years were selected randomly, irrespective of their gender. Blood samples from the subjects were collected and stored in three storage settings: in a refrigerator (FT - 4°C), in a constant temperature bath containing water (PT -25°-27°C) and at room temperature (RT – 30°-40°C). The blood sample were analysed for absolute platelet counting (APC) repeatedly for five days. Room temperature samples were also done with an automatic cell counter. The pattern of fall in APC was observed and analysed.

Results: The mean platelet counts for all the samples stored showed an overall nonlinear decrease in counts with time. The FT, RT and PT cell count decreased rapidly during the initial 6 hours, 15-20 hours and 25 hours respectively. Thereafter the fall slowed down. In contrast, the counts made on automatic cell counter showed the least variation over the duration of study.

Conclusion: The platelet counts and their rate of fall is predominantly a function of platelet metabolism at higher temperatures however their activation and adhesion occurs at lower temperatures.

Keywords: Platelet, complete blood counts, thrombocytopenia.

INTRODUCTION

Hematological examinations of blood samples, though indicated to be made as soon as possible after sampling, are rarely made immediately. In the real scenario, blood samples are frequently delivered to the clinical laboratory after a significant post-collection interval labeled as the "DEAD TIME" of approximately 4 hours[1,2]. On weekends this interval may exceed 72 hours.[2] This interval is a highly significant pre-analytic variable along with the storage condition of blood samples, and thus, remains an intriguing issue for the stability of hematological parameters.

Manufacturers of automated analyzers and published literature often cite that specimens kept at either room temperature or at 4ºC (refrigerated) for up to 24 hours generally yield reliable results for complete blood cell count (CBC) and automated differential leucocyte count[2-4].

However, specific information concerning the suitability or unsuitability of specimens older than one or two days for various tests and the effect of such delay on the reliability of test results is limited, particularly in the recent literature[2].

When the effect of such delay is coupled with the high temperature settings of tropics the suitability of the specimen becomes highly questionable with an increasing probability of unreliability of test results with the storage of samples at increasing temperature, even for short time periods.

The present study aims to assess the variation in platelet count, with time, and the reasons thereof, in healthy young adults, specially at high room temperatures

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of tropical summer for samples stored at the real room temperature, at 4ºC and at 25°-29°C.

MATERIALS AND METHOD

The study was conducted in the Department of Physiology, Government Medical College, Kota Rajasthan. As the study was concerned with the effect of the "real" room temperature, specially the high temperatures, the study was performed in warm, dry days of April to July when room temperature exceeds 30ºC.

After obtaining the approval of the institutional ethical committee, fifty apparently healthy young adults, aged 17-25 years were selected randomly, irrespective of their gender and their written informed consent was taken to participate in the study. Subjects who were smoker, alcoholic, having infections (specially viral, within one month) or any other acute or chronic illness were excluded.

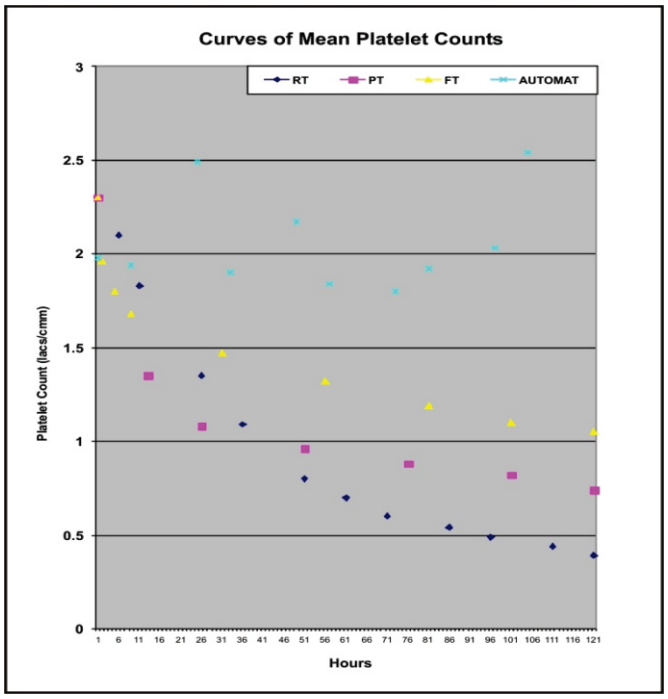
The subjects were divided into ten groups having five persons in each group. Blood samples from each group was studied for five days. Blood samples were collected by drawing 10 ml venous blood from the antecubital vein and immediately anticoagulated in three EDTA vials. After immediate mixing, one vial was placed in a refrigerator (4°C), *(FT sample)* and another in a constant temperature bath containing cold water (25°-29°C), (*PT sample*). The blood from the third vial (*RRT sample*), was immediately subjected to platelet counting using light microscope and hemocytometer (improved Neubauer's chamber). All parts of the sample were subjected to counting everyday for five days. The timing of counting was as follows:

- RRT samples: 0.5, 5, 10, 25, 35, 50, 60, 70, 85, 95, 110 and 120 hours.
- PT samples: 0.5, 12, 25, 50, 75, 100 and 120 hours.
- FT samples: 0.5, 1, 4, 8, 30, 55, 80, 100 and 120 hours.
- RRT samples (with automatic cell counters): 0.5, 8, 24, 32, 48, 56, 72, 80, 96, 104 hours.

The platelet count was done by improved Neubauer's chamber. Platelet counts were performed three times on the same sample and an average of the three counts was used for analysis to minimize the manual error in counting. Refrigerated samples were placed at room temperature for 15 minutes before counting. The time of counting was measured from the time point at which the blood was drawn. All the RRT samples were also done on automatic cell counter repeatedly for comparison with the hemocytometer counts. Room temperature was measured at 6 hour intervals throughout the study. In view of the characteristics of the data set the summary measures method was used for analyzing the data from the study[5-11].

RESULTS

The mean platelet counts for all the samples stored showed an overall nonlinear decrease in counts with time.



RRT samples	0.5 hr	5 hr	10 hr	25 hr	35 hr	50 hr	60 hr	70 hr	85 hr	95 hr	110 hr	120 hr
Mean PC (lacs/cmm)	2.30	2.09	1.84	1.35	1.09	0.80	0.70	0.60	0.54	0.48	0.44	0.39
SD	0.49	0.54	0.48	0.41	0.36	0.27	0.23	0.21	0.19	0.17	0.20	0.21
CV	21.44	25.72	26.16	30.46	33.09	33.82	32.65	34.21	35.11	34.54	45.61	53.94

PT samples	0.5 hr	12 hr	25 hr	50 hr	75 hr	100 hr	120 hr
Mean PC (lacs/cmm)	2.30	1.34	1.08	0.96	0.88	0.81	0.74
SD	0.49	0.32	0.25	0.22	0.20	0.28	0.32
CV	21.44	23.58	22.89	22.68	22.33	34.69	43.67

FT samples	0.5 hr	1 hr	4 hr	8 hr	30 hr	55 hr	80 hr	100 hr	120 hr
Mean PC (lacs/cmm)	2.30	1.96	1.80	1.68	1.47	1.32	1.19	1.10	1.05
SD	0.49	0.50	0.45	0.45	0.39	0.35	0.31	0.26	0.32
CV	21.44	25.29	24.79	26.88	26.90	26.25	25.76	23.92	30.23

Auto RRT	0.5 hr	8 hr	24 hr	32 hr	48 hr	56 hr	72 hr	80 hr	96 hr	104 hr
Mean PC (lacs/cmm)	1.98	1.94	2.49	1.90	2.17	1.84	1.80	1.92	2.03	2.54

Data for Summary Measures Analysis							
Time to reach 75% of initial platelet count (t_{75})				Rate of decrease in platelet counts (Regression coefficients)			
	t_{75}RT	t_{75}PT	t_{75}FT		RT_{RC}	PT_{RC}	FT_{RC}
Mean	12.84	4.72	10.34	Mean	-0.035	-0.049	-0.02
SD	6.12	1.95	11.46	SD	0.009	0.011	0.005
CV	47.68	41.29	110.8	CV	-26.714	-23.264	-24.736

The FT cell count decreased most rapidly during the initial 6 hours followed by the slowest fall and yielded the highest (i.e. most stable) counts after about 20 hours till the end of the study.

The Real Room Temperature (RRT - 30°C to 40°C) counts exhibited the slowest fall in cell counts leading to highest counts among the treatment groups till 15 to 20 hours. After this period the rate of decrease in RRT counts did not change much till about 54 hours and then slowed down further and yielded the lowest count after about 45 hours among the treatment groups.

PT samples exhibited the most rapid drop in counts during the initial 25 hours and slowed down significantly after that. A rate of fall in PT cell counts, intermediate between FT and RT counts was seen during the initial 12 hours. PT counts were lowest between 10 to 40 hours as compared to other treatment groups.

The observation of highest significance was that for all the samples counterchecked repeatedly on automatic cell counter, the platelet counts showed the least variation from the initial count in contrast to the decreasing counts as observed with hemocytometry.

STATISTICALANALYSIS

The present study was a longitudinal, prospective study. The temperature of storage of blood samples was considered as a categorical variable. This type of study yields a longitudinal data set which cannot be subjected to "t-test" because observations were not equally spaced in time, cell lysis near the late stages of study yielded no values, hierarchical nature of the observations i.e. clustering (nesting) within subjects created a problem of serial correlations between observations and successive measurements in a subject were not independent of each other[5-11].

If time-by-time analysis had been opted for analysis it would have resulted in the inflated type I errors[6]

To obviate the problem of multiple comparisons and for the aforementioned problems with the dataset, summary measures method was used for analyzing the data from the study[5-11].

For comparing the rate of change of the platelet count among the three treatments, the summary measure adopted was the regression coefficient. And, for comparing the net absolute change in platelet counts among the groups, the summary measure chosen was the time by which platelet counts decreased to 75 percent of the initial value (t_{75}) because after a drop of 25 percent in counts, it is reasonable to assume that the counts (i.e. the stored sample) were not fair enough representative of the original sample.

To evaluate the statistical significance among the study groups, a one-way repeated measures analysis of variance was performed over the t_{75} and regression coefficients.

The regression coefficients were derived by applying the Ordinary Least Square Regression (OLS regression) to the data points over the following initial hours: 35 hours for RRT, 25 hours for PT and 30 hours for FT.

The mean time by which platelet counts decreased to the 75 percent of the initial counts (t_{75}) were: RRT– 13 hours, PT – 5 hours and FT – 10 hours.

Planned contrast (simple) were obtained to compare mean t_{75} and regression coefficient for each of the three treatment conditions with the mean t_{75} and regression coefficient for the refrigerated samples (controls).

Mean t_{75} and mean regression coefficients during the RT and PT samples were significantly higher than baseline mean t_{75}. Thus, both RT and PT had significantly higher mean than the baseline conditions of 4°C storage.

DISCUSSION

A probable explanation of these variable patterns of decrease in platelet counts can be sought in the altered adhesive and disintegration characteristics of platelet under different storage conditions. These changes in platelet properties appear to be the consequences of platelet activation induced by the various mechanisms of platelet storage lesions, specially by the temperature at which the samples are stored and by adhesion with the glass of the hemocytometer. The development of platelet storage lesion in influenced by physical, chemical and metabolic factors related to platelet (or blood) withdrawal, preparation and storage.

Platelets show a contrasting behavior after storage at 4°C and 22°C. In general, the initial changes are more rapid (within 1 to 10 minute or initial a few hours) and more dramatic for 4°C storage consequent to drastic alterations in the platelet membrane dynamics. The lower metabolic rate at 4°C is the main factor responsible for

slower progression of the changes in comparison to the changes occurring at room temperature (20°C-24°C) storage[12-15].

In contrast, the better preserved membrane integrity at 22°C accounts for slow developing initial changes. Whereas at 22°C, the higher metabolic rate leading to a greater fall in pH results in rapid development of cellular injury. These changes are consequent to the temperature-dependent alterations in platelet membrane dynamics that result in cytoskeletal reorganization and alterations in signal transduction and intracellular processes[16-23].

The works of Zhang[24], Vasin[25], Ruckenstein[26], James[27], Olef[28], and other studies mentioned previously concerned with platelet storage show that Platelets are least activated near 37°C. Platelet activation increases with decreasing temperature. At 4°C nearly all the platelets are activated. Even the room temperature (20° to 24°C) causes marked activation of platelets. The development of platelet storage lesions is much slower at 4°C than at 22°C.

At low temperatures, micro aggregate formation and increased viscosity of blood result in increased shear stress which changes ligand-receptor conformation to increase platelet adhesion[18,27]. At high temperature, increased metabolic rate leads to a fall in pH[20].

When the temperature of storage is lowered, between 37°C to 30°C only subtle changes occur in platelet morphology. However, below 30°C clustering of lipid rafts of plasma membrane occurs due to liquid crystalline to gel phase transition, and is responsible for increasing platelet activation between 30°C and 20°C.[18,24]

This increase in platelet activation subsequent to membrane lipid raft phase transition at 30°C manifests in increased platelet adhesion to glass which reflects in increased rate of fall in platelet counts in PT samples as compared to RT samples.

As the temperature is lowered further, most of the platelets are activated at 20°C. At 15° to 18°C the phospholipids of plasma membrane experience a liquid crystalline to gel phase transition[18,24,29,30]. The cumulative effect of both the phase transitions greatly alters the membrane integrity, fluidity and membrane polarization and may account for the activation of nearly all the platelets at 4°C. Thus, this cumulative effect of temperature-graded differential membrane phase transition greatly increases platelet activation and adhesion to glass to result in a very rapid drop in platelet counts during the first 6 hours in refrigerated (FT) samples.

The altered platelet membrane dynamics causes an increase in cytosolic calcium, cytoskeletal reorganization and altered cellular metabolism and ATP levels, which ultimately causes the lysis of platelets[18,24,29,30,31].

This way, increasing platelet activation with decreasing temperature and subsequent adhesion to glass surface appears to be the prime contributor to the platelet loss during initial hours (specially for refrigerated samples), whereas platelet disintegration plays a major role during later hours when activation is nearly complete[28,32,33].

These two phase of initial platelet adhesion to glass followed by their disintegration reflect as FT curves being mainly adhesive curves and the RT curves being predominantly the disintegration curves.

The most significant observation of the study was the discrepancy between cell counts from hemocytometer and automatic counter. It can be explained as follows:

As activated platelets undergo significant adhesion on glass, decreasing counts are recorded with hemocytometers, whereas for the samples counterchecked with automatic counters, the counts did not decrease because the blood is rapidly flushed through the instrument and consequently the platelets fail to adhere to the tubing of the cell counter. A basis of this adhesive behavior of platelets had been explored biophysically by Ruckenstein et al[26]. The rate of sedimentation and adhesion of platelets onto a horizontal glass surface was studied by Ruckenstein et al.[26] They developed an equation for the rate of cellular deposition through a stagnant solution onto a horizontal surface for the case in which cells are transferred to the surface very rapidly compared with their rate of overcoming the potential barrier between them and the surface, The platelet adhesiveness, can be expressed in terms of the probability, P= 1/ to overcome (i.e. escape over) the potential barrier between them and the surface, and is inversely proportional to the contact period during which appreciable deposition occurs, The time constant, depends exponentially on the potential barrier.

Vasin et al[25] developed a mathematical model of static platelet adhesion on a solid surface, taking the platelet activation in account. As shear stress decreased

the adhesion kinetic curves turned form exponential into sigmoid, as a result of accumulation of free activated cells in the surface vicinity. Platelet adhesion was also seen to be proportional to total activated platelet concentration.

The fall in platelet counts with time recorded with hemocytometers, at higher temperatures is mainly because of metabolic changes in platelets. The contribution of cell disintegration (fragmentation) to the decrease in counts may increase at higher temperatures and in older samples. In contrast, the fall in platelet counts, at lower tempearatures is secondary to the dominance of platelet activation and the biophysico-chemical interactions in the (ionic) nanoenvironment, mediating the adhesion of activated platelets on glass surface[34,35].

These results indicate that even though the pathway for platelet activation is the same, the activation rate can be altered when specific interactions with the surface are involved. The results demonstrate the specificity in the way surfaces of materials activate platelets, depending on the platelet membrane dynamics and the intracellular calcium dynamics[18,24,29,30,31,34,35].

CONCLUSION

Thus it is concluded that: RT curves are predominantly the disintegration curves, whereas the FT curves are mainly adhesive curves.

The fall in platelet counts with time recorded with hemocytometers, at higher temperatures is mainly because of metabolic changes in platelets. The contribution of cell disintegration (fragmentation) to the decrease in counts may increase at higher temperatures and in older samples. In contrast, the fall in platelet counts, at lower temperatures is secondary to the dominance of platelet activation and the biophysico-chemical interactions in the (ionic) nanoenvironment, mediating the adhesion of activated platelets on glass surface.

These results indicate that even though the pathway for platelet activation is the same, the activation rate can be altered by the nanoenvironnment based specific interactions of the surfaces with platelets. These interactions are the key modulators of the platelet membrane dynamics and the intracellular calcium dynamics, and thus they demonstrate the fine regulation of platelet activation secondary to the specificity of the surfaces – platelet interactions.

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