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Original Research Article

Study of refrigerated storage of blood at 4°C on automated hematological parameters & morphological changes in peripheral blood smear: A prospective study

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ABSTRACT

Background: Complete hemogram & peripheral blood smear are primary tests done in day to day practice for correct diagnosis & further treatment of patients. EDTA blood samples are used for this regular screening tests which shows less stability in case of delaying sampling. Hence, the final results of these tests can be affected by different storage conditions & duration. This present study provides data analyzed from EDTA blood samples stored upto 24 hour at both room temperature & 4°C refrigeration.

Materials and Methods: This study includes total 150 blood samples from indoor & outdoor patient without any specific criteria which were collected randomly. These blood samples were analyzed using haematological analyzer for complete blood count & their peripheral smear after storage in both room temperature & 4°C refrigeration for 24 hrs.

Result: There was significant increase in MCV & decrease in MCHC, reduced WBC count & platelet count with storage at room temperature which was prohibited by refrigeration. However, both room temperature & refrigerated storage does not affect RBC count & hemoglobin.

Conclusion: Blood samples stored at room temperature for 24 hrs results in changes in haematological parameters & morphology of cells. Hence, refrigerated storage at 4°C is recommended for accurate results in case evaluation of delayed samples.

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1. Introduction

Hematological reporting is very important aspect in accurate diagnosis & investigations of various organic disease, parasitic manifestations & metabolic disorders as well.[1] It includes complete blood count & peripheral smear which provides a definitive information to physicians or surgeons to diagnose, monitor & further management of patients.[2] Complete hemogram is also necessary for patients who requires blood transfusion to estimate the quantity of blood to be transfused.[3]

The capability of sample to retain the initial value of a measured quantity for defined period within specific limits when stored under defined condition is termed as sample stability.[4] This sample stability is essential to maintain accuracy & reliability of final results in case of delayed evaluation of blood samples.[4]

In routine hematological practice, EDTA is preferrable choice of anticoagulant for automated cell counts due to its general availability, ease of preparation, wide spread use & comparatively low cost.[5,6] However, the EDTA anticoagulated blood shows less stability of parameters & shows changes in morphology of cells in stored blood sample at room temperature in case of delayed analysis & finally result in wrong interpretation of data.[2,6] According

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to previous studies it has been verified that storing blood samples in EDTA for long time before analysis can affects morphology of blood cells, peculiarly red blood cells as they may causes altered osmotic fragility & shows their effects on cellular viability.[6]

Some factors such as lack of manpower & shortage of cell counters, weekends and high environmental temperature which can lead to longer storage of blood samples.[4] In addition, many countries has been developed authorized central laboratories which receives blood samples from peripheral areas or other local laboratories which causes prolonged time of transportation & may lead to delayed evaluation of that particular hematological test.Hence, preanalytical phase needs a careful attention on procedures such as collection of blood samples, patient's identification, preference of collection vials, labelling of specimens, accurate blood suction, clerical errors & storage.[1]

There is simple, modest method to maintain all hematological parameters & morphology of peripheral smear is storage of blood samples at 4°C refrigeration.[7]

Therefore, this study presents a quantitative data and peripheral blood smear changes for blood sample storage at fixed temperature 4°C refrigeration for 24 hrs. It is needful in routine clinical practice to increase accuracy & reliability of haematological investigations in case of delayed blood samples.

2. Aim

To assess the effect of room temperature & refrigerated storage on complete blood counts and on morphological features in peripheral blood smear on automated haematological parameters.

3. Objectives

To compare the effects of stored blood samples both at room temperature & 4°C refrigeration on automated haematology parameters & peripheral blood smear.

4. Materials and Methods

4.1. Subject

Total 150 blood samples from outpatient & inpatient section irrespective of any specific criteria, age & gender were collected randomly.

4.2. Study design

This prospective study was approved & accomplished at Bharati Vidyapeeth medical college & hospital, Sangli in April 2022 to June 2022.

4.3. Sampling collection

Approximately 2 ml of venous blood is collected in EDTA coated vacutainers under standard protocol of aseptic precautions.

4.4. Laboratory testing

The measurement of haematological parameters including WBC, RBC & Platelet count, hemoglobin, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) was done on Sysmex XN 500 5 part differential which is on based on the principle of fluorescence flow cytometry method. Furthermore, their peripheral smears are made by proper mixing of blood samples & using Leishman stain.

In peripheral smear following morphological changes were studied -

4.5. RBC

1. Crenated RBCs
2. Loss of central pallor
3. Spherocytic changes

4.6. WBC

1. Cytoplasmic changes such as vacuolization, degranulation, blebs.
2. Nuclear changes such as lobulations, degeneration, karyolysis, vacuolization, smudge cells.

4.7. Platelets

1. Aggregation of platelets
2. Large platelets

4.8. Room temperature storage

Analysis of 75 blood samples is done at 0 hr for complete blood count as basis of measurement by using automated haematological analyzer 5 part differential & peripheral smear is prepared. Then subsequently these samples were stored at room temperature & re-analyzed after 24 hrs for all haematological parameters & peripheral smear.

4.9. Refrigerated storage

Analysis of another 75 blood samples is done at 0 hr for complete blood count as basis of measurement by using automated haematological analyzer 5 part differential & their peripheral smear is prepared. Subsequently samples were stored at 4°C refrigeration. After 24 hrs, samples were settled at room temperature for 30 mins, then re-analyzed for complete blood count & peripheral blood smear prepared.

4.10. Study area

This study was approved & accomplished in haematology section in department of pathology at Bharati Vidyapeeth (Deemed To Be) Medical College & Hospital, Sangli.

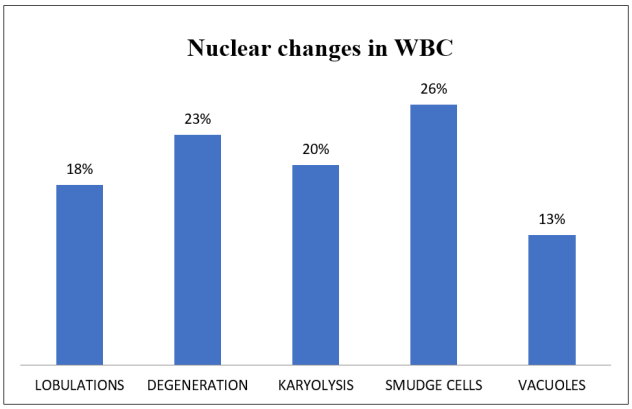
4.11. Statistical analysis

Statistical difference between values of the analyzed parameters at particular time intervals at 0 hr & at 24 hrs. SSPS software system were used to conduct paired T test to compare statistical results. If P value < 0.05, then it was considered as significant result.

5. Results

5.1. Changes in stored blood samples at room temperature for 24 hrs

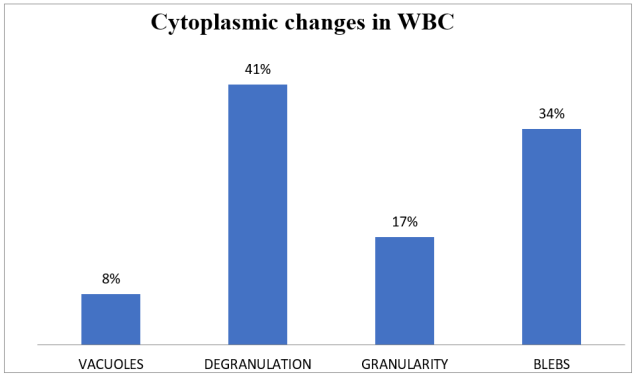
We observed that there is statistical significance (P values < 0.05) in WBC counts in blood samples stored at room temperature. Degranulation, increased granularity in neutrophils, vacuolization & bleb formation in cytoplasm & lobulation, pyknosis, smudging & vacuoles in nucleus are morphological changes seen WBC in stored blood samples at room temperature. Percentage of these artifacts plotted in Graphs 1 and 2 & their images shown in Figures 1 and 2.



Graph 1: Showing percentage of nuclear changes in WBC in stored blood samples at room temperature for 24 hours

The P values are not < 0.05 i.e no statistical significance in variation of haemoglobin values & RBC counts stored at room temperature for 24 hrs. However, some morphological artifacts in RBCs such as crenated RBCs & loss of central pallor revealed with stored sample at room temperature. These changes shown in Figure 3 & their percentage plotted in bar Graph 3.

While, P value < 0.05 i.e statistical significant changes seen in platelet counts in room temperature storage of blood & also shows morphological changes like aggregation of platelets & large platelets. Changes shown in Figure 4 & their percentage in bar Graph 4.



Graph 2: Showing percentage of cytoplasmic changes in WBC in stored blood samples at room temperature for 24 hours

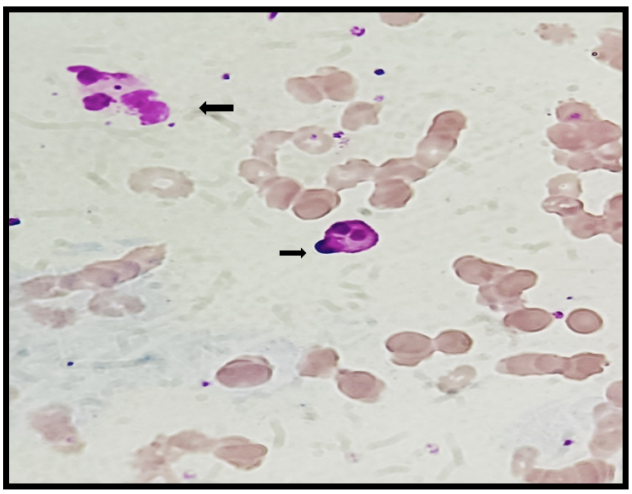


Fig. 1: Leishman stain (100 x showing nuclear degeneration & karyolysis) in stored blood samples for 24 hours at room temp

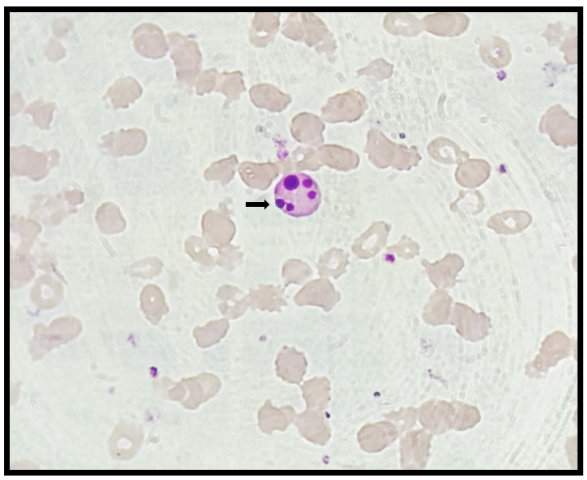


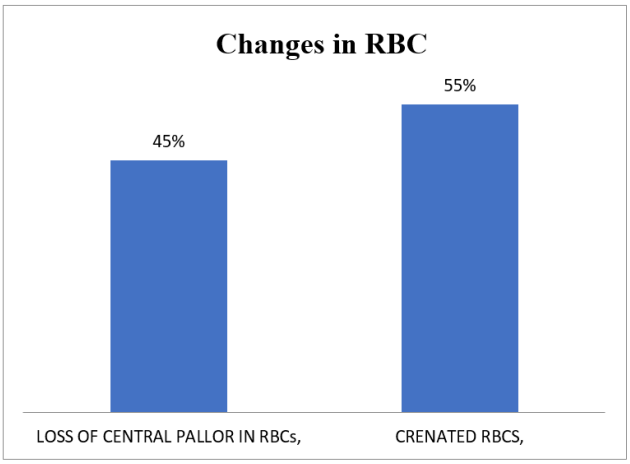
Fig. 2: Leishman stain (100 x showing nuclear karyolysis & cytoplasmic degranulations) in stored blood samples at room temperature for 24 hours

Table 1: Showing results of complete hemogram at room temperature storage of blood

Time & category		No. of samples	Mean	Standard deviation	P value
Pair 1	WBC fresh	75	7369.6	3711.2136	$< 10^{-27}$
	WBC 24 hrs	75	6782.1333	3653.3621	
Pair 2	RBC fresh	75	3.4378667	1.3363	0.7661
	RBC 24 hrs	75	3.4445333	1.3237	
Pair 3	Hb fresh	75	12.2	1.8730	0.5571
	Hb 24 hrs	75	12.18	1.8864	
Pair 4	MCV fresh	75	78.733333	10.3524	$< 10^{-37}$
	MCV 24 hrs	75	90.32	11.3592	
Pair 5	MCHC fresh	75	28.232	4.5379	$< 10^{-31}$
	MCHC 24 hrs	75	24.127273	4.6254	
Pair 6	PC fresh	75	226866.67	75443.7921	$< 10^{-36}$
	PC 24 hrs	75	194266.67	72567.4585	

Table 2: Showing results of complete hemogram at 4°C refrigerated temperature storage of blood

Time & category		No. of samples	Mean	Standard deviation	P value
Pair 1	WBC fresh	75	8139.8667	3025.3463	0.3744
	WBC 24 hrs	75	8134.3867	3022.6047	
Pair 2	RBC fresh	75	3.9220	1.1836	0.4873
	RBC 24 hrs	75	3.9227	1.1840	
Pair 3	Hb fresh	75	13.0987	1.5819	0.3040
	Hb 24 hrs	75	13.0627	1.5815	
Pair 4	MCV fresh	75	84.0000	8.5988	0.1930
	MCV 24 hrs	75	84.2267	8.7789	
Pair 5	MCHC fresh	75	30.9680	3.0829	0.2289
	MCHC 24 hrs	75	30.9253	3.1149	
Pair 6	PC fresh	75	286086.6667	153094.2205	0.1205
	PC 24 hrs	75	287285.3333	153418.8514	



Graph 3: Showing percentage of changes in RBC in stored blood samples at room temperature for 24 hours

In addition, room temperature storage also showed increase in mean value of MCV & decrease in mean value of MCHC with statistical significance (P value < 0.05).(Table 2)

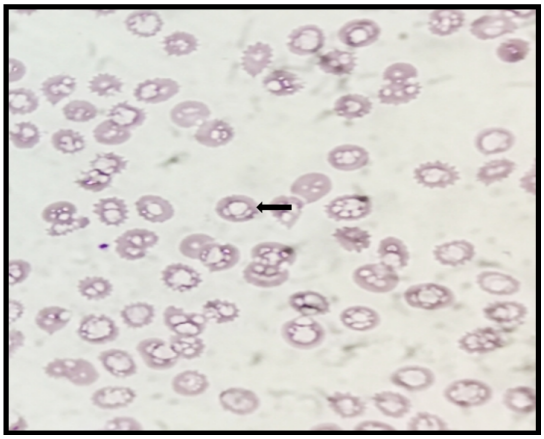
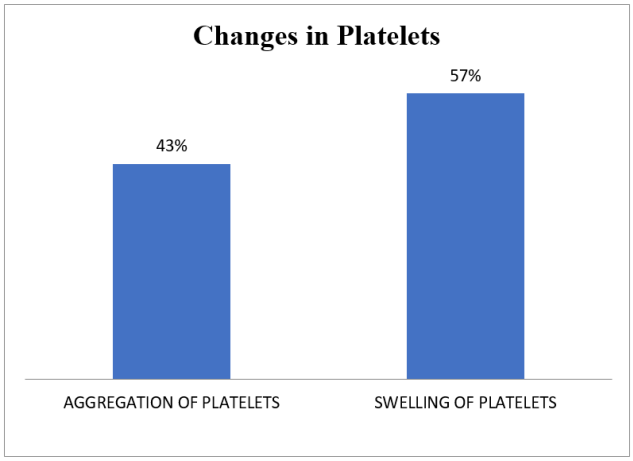


Fig. 3: Leishman stain (100 x showing crenated RBCs)

5.2. Changes in stored blood samples at refrigeration at 4°C for 24 hrs

This study has revealed that there is no statistical significance seen in values of haemoglobin, RBC count, WBC count, platelet count, values of MCV & MCHC.

Changes in counts of haematological parameters & morphology of RBC, WBC & platelets in blood smear



Graph 4: Bar graph showing percentage of changes in Platelets in stored blood samples at room temperature for 24 hours

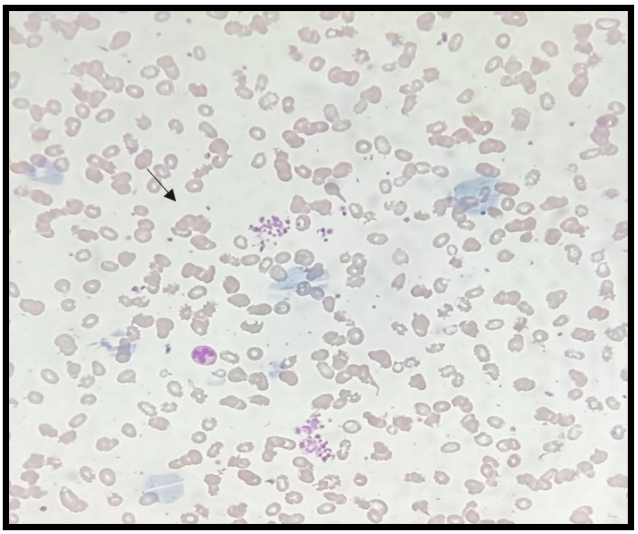


Fig. 4: Leishman stain (40 X showing platelet aggregation)

which was noted in room temperature storage has shown stabilized with refrigeration at 4°C.

6. Discussion

Complete hemogram is basic laboratory investigation which assess the number, size, morphology of hematological parameters like white blood cells, red blood cells, platelets & related indices of the blood such as mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC).[8] According to guidelines of International Society for Laboratory Hematology, morphological artifacts in blood cells begin within 30 minutes of collection of samples.[5,9] while, other previous studies also demonstrated that prolongation of storage of blood samples may lead to significant changes in haematological parameters & their cellular morphology.[9,10]

In regular haematological workup, more than 70% of all errors commonly occurs during preanalytical phase which includes preparation of patient, collection, transportation, storage of collected blood samples & preparation for analysis.[11] It is also necessary to consider the environmental temperature at different places, particularly in hot climate areas e.g. southern countries which affects stability of hematological parameters & final results.[5]

In recent years, a large central authorized laboratories encounters the common problems like proper transfer, storage duration of collected blood samples, long distance dispatchment of specimens which causes the delayed analysis of tests.[12] Therefore, delayed sampling is one of the common problems in routine clinical practice, if quick analysis is not possible or if sample is required for retesting.[11]

According to Imeri et al., RBC counts & haemoglobin values are stable at both room temperature & 4°C refrigeration.[13]

Our study has demonstrated that haemoglobin values & RBC counts are not affected in room temperature storage for 24 hours, but there is significant increase in MCV because of crenated RBC in blood samples stored at room temperature. This change is stabilized by storage of blood samples at 4°C refrigeration for 24 hours**.** These similar findings were observed in prior studies.[7]

Other studies indicated that the degenerative changes in stored blood samples at room temperature allows the influx of water into cellular membrane result into increase in red cell size with time & eventually lead to increased mean corpuscular volume. & other morphological artifacts such as spherocytosis, echinocytosis & sphero-echinocytosis may found after 48 hr of storage at room temperature.[6]

While, MCHC values shows significant reduction in room temperature stored blood samples. MCHC is calculated by ratio of haemoglobin to haematocrit multiplied by 100. Thus, haematocrit increases while haemoglobin remains stable which leads to decrease in MCHC value.[12]

Room temperature storage also shows significant decrease in WBC count which was prohibited by refrigerated storage. Hence, our observance is identical to study Brent L. wood et al.[7] Causes for reduced WBC count can be cytoplasmic changes such as degranulation, vacuolization, degeneration, sometimes blebs formation & karyolitic changes & lobulations in their nucleus.[14]

In relation with prior studies, it is stated that the statistically significant decrease in platelet count at all temperature begins after 6 hrs of sample collection.[2,15] While, in this present study, we have observed that platelet count also has reduced in stored blood samples at room temperature because of aggregation & swelling of

platelets. These changes are corresponds with other previous studies.[4] This change can be misinterpreted as pseudo-thrombocytopenia which can be prevented by refrigeration of blood samples at 4°C.

Overall, these morphological artifacts usually starts within 6 hrs of collection of samples & further increases with time & temperature.[5] In our study we have observed the morphological changes like nuclear lobulations, nuclear karyolysis or pyknosis, nuclear vacuolization & then cytoplasmic changes like degranulation. Platelet aggregates & swelling are also seen. This observance is similar to other prior studies.[14]

Hence, in order to get standard haematological results it is necessary to maintain blood samples at fixed 4°C in conditions of extended period of evaluation, because previous studies proved that blood storage at 2°C may lead to freezing injury to RBCs results in hemolysis. However, storage at > 6°C lead to overgrowth of unspecified bacteria during sampling of blood.[1,14]

7. Conclusion

The result of our study suggests that there are significant changes in haematological parameters from automated cell analyzer & their morphology of cells seen in peripheral blood smear, if blood samples stored at room temperature for extended period of 24 hrs after sample collection. Hence, storage of blood samples at room temperature should be avoided during transportation, within laboratory or in case of delayed evaluation. Finally, we have concluded that refrigerated storage at 4°C is the recommended method of preservation of blood samples for an accurate & standard test results in case evaluation of delayed samples.

8. Abbreviations

RBC – Red blood cells; WBC – White blood cells; PC – Platelet count; HB – Hemoglobin; PCV – Packed cell volume; MCV – Mean corpuscular hmoglobin; MCHC – Mean corpuscular hemoglobin concentration; EDTA – Ethylenediaminetetraacetic acid.

9. Source of Funding

None.

10. Conflict of Interest

None.

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