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TIME AND TEMPERATURE - THE TWIN TOWERS INFLUENCING COMPLETE HEMOGRAM PARAMETERS IN ADULT OUTPATIENTS UNDERGOING CHEMOTHERAPY

Haematology

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ABSTRACT

Background: Hemoanalyser (electrical impedance principle) uses cell size for identification. Degenerated samples due to improper storage alter hematology parameters. Chemotherapy patients monitored for anemia and neutropenia require accurate hematology report. **Aims**: To evaluate effect of storage time and temperature on complete hemogram (CHG) parameters **Setting and Design**: Prospective, analytical study in adult outpatients (OPD) **Methods and Material**: Two ml blood was collected in EDTA vacutainer from 90 chemotherapy patients. Zero-hour reading (ZH) was taken on 3-part-hemoanalyser. Aliquots were stored at 4^0C and room temperature (RT) for 24 hours and reprocessed. Difference of Mean (Mdiff) between values of ZH/ 4^0C (pair A), ZH/RT (pair B) and 4^0C/RT (pair C) was evaluated. **Statistical analysis used**: Quantitative data between qualitative variables was compared by Unpaired t-test. Quantitative data measured twice was compared by Paired t-test. **Results:** Hemoglobin (Hb) and erythrocyte count (RBCC) showed negative Mdiff at RT suggesting erythrocyte lysis. Mean corpuscular volume (MCV) showed positive Mdiff at RT suggesting osmotic ballooning. Erythrocyte distribution width (RDW) showed positive Mdiff at RT suggesting anisocytosis ($p<0.05$). Platelet count and Mean Platelet volume (MPV) had negative Mdiff at RT suggesting fragmentation. ($p<0.05$). Mdiff of leucocyte count (TLC) declined in pairs ABC due to cellular death. Negative Mdiff for absolute neutrophil count (ANC) suggest erroneous recognition of degenerated neutrophils as monocytes ($p<0.05$). **Conclusions:** RT storage affected Hb, RBCC, MCV, RDW-CV, platelet count, MPV and ANC while 24-hour delay at RT influenced TLC, and AMC. Prompt processing of samples achieves best results.

KEYWORDS

complete hemogram, storage time, room temperature, coulter principle

INTRODUCTION:

Specimen collection and storage [1] are major determinants of pre-analytical phase. Degenerated samples affect accuracy and precision of results [2,3]. Semi-automated Hemoanalyser (Coulter's principle) identifies cells by size [4]. Storage at improper temperature & prolonged period alters cell morphology and count, thus affecting parameters on complete hemogram (CHG). [5,6]

Patients on chemotherapy are vulnerable to macrocytic anaemia, neutropenia, and throm bocytopenia. [7] Altered CHG parameters will affect clinical management. [8]

OBJECTIVES:

To study effect of storage time and storage temperature on CHG parameters on 3-part differential hemoanalyser

MATERIALS & METHODS:

An analytical prospective study was conducted over a period of 6 months (3 months of winter and summer each) at a tertiary health care hospital. Adult OPD patients undergoing chemotherapy referred for routine CHG were included.

Two ml blood sample was collected in K2-EDTA vacutainer and immediately processed on 3-part hematology analyser (Zero hour reading ZH). The samples were divided into two aliquots for storage at 4C and room temperature (RT). After 24 hours storage, both aliquots were reprocessed. Thus, each sample had 3 readings (ZH, 4C/24 hr, RT/24hr).

Each reading had following 13 parameters:

RBC parameters:

Haemoglobin (Hb), Red blood cell count (RBCC), Mean corpuscular volume (MCV), red cell distribution width coefficient of variation (RDW-CV), Haematocrit /Packed cell volume (PCV), Mean corpuscular hemoglobin (MCH), Mean corpuscular hemoglobin concentration (MCHC),

Platelet parameters:

Total platelet count, Mean platelet volume (MPV),

WBC parameters: Total Leucocyte count (TLC), Absolute neutrophilic count (ANC), Absolute lymphocytic count (ALC), Absolute monocyte count (AMC).

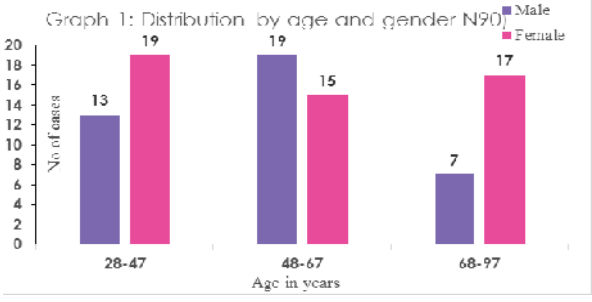
Mean (M) and Standard Deviation (SD) were calculated for every reading. Difference of mean (Mdiff) was calculated between readings of ZH/4C (Pair A), ZH/RT (Pair B), 4C/RT (Pair C) for each parameter. Mdiff (ZH/RT) was compared summer vs winter season. Positive and negative Mdiff respectively indicated increase and decrease in value of parameter.

Statistical analysis:

Data was analysed using PSPP software. Binomial qualitative variables were compared by Unpaired t-test. Quantitative data measured twice was compared by Paired t-test. Study was approved by Institutional Ethics Committee (ECARP/2019/10).

RESULTS:

Total sample size was 90, with 45 cases each in summer and winter. Majority of cases belonged to 38-57 age group. M + SD was 56.22 ± 16.15 years. Female preponderance (51, 56.7%) was noted (**Graph 1**).



All 13 parameters were compared between pairs A, B, C (**Table 1, Graphs 2-5**). Parameters showing parallel changes in 4C/24 hr and RT/24 hr were designated as time dependent. Parameters with greater changes at 4C or RT were designated as temperature dependent (**Table 2).**

Table 1: Difference of Mean (Mdiff) for pairs A, B, C for RBC, platelet, and WBC parameters at ZH, 4C/24 hour and RT/24hr (N = 90)

Parameter (unit of measurement)	ZH	4C/24hr	RT/24hr	Mdiff A	Mdiff B	Mdiff C
Hb (g/dl)	11.48	11.75	11.45	0.27*	-0.03	-0.30*
RBCC (x10 6/ul)	4.01	4.09	3.99	0.08*	-0.02	-0.1*

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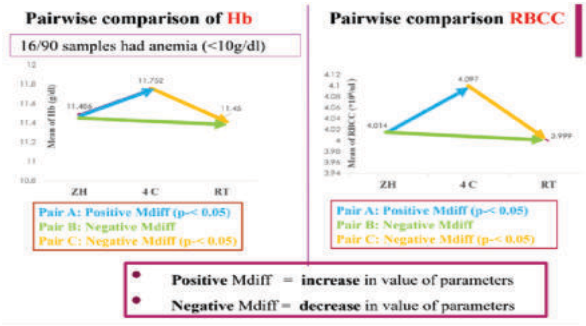
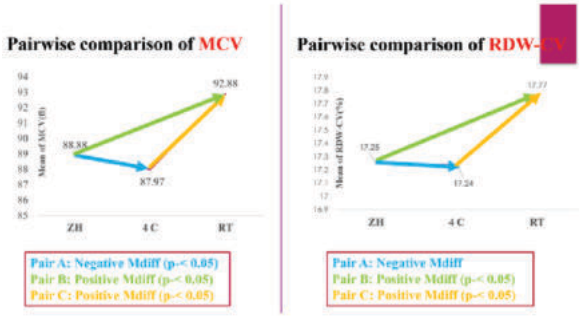
MCV (fl)	88.88	87.97	92.88	-0.91*	4.0*	4.91*
RDW-CV	17.25	17.24	17.77	-0.01	0.52*	0.53*
Hct/PCV	35.42	35.58	36.78	0.16	1.36*	1.2*
MCH (pg)	28.72	28.95	28.8	0.23*	0.08	-0.15
MCHC (g/dl)	32.3	32.9	31.0	0.6*	-1.3*	-1.9*
Platelet count (x10 3/ul)	281.79	293.41	273.88	11.62*	-7.91*	-19.53*
MPV (fl)	7.43	7.72	7.36	0.29*	-0.07*	-0.36*
TLC (x10 3/ul)	7.51	7.45	7.39	-0.06	-0.12*	-0.06*
ANC (x10 3/ul)	4.56	4.6	4.11	0.04*	-0.45*	-0.5*
ALC (x10 3/ul)	2.6	2.51	2.53	-0.09*	-0.07	0.02
AMC (x10 3/ul)	0.44	0.48	0.84	0.04*	0.40*	0.36*

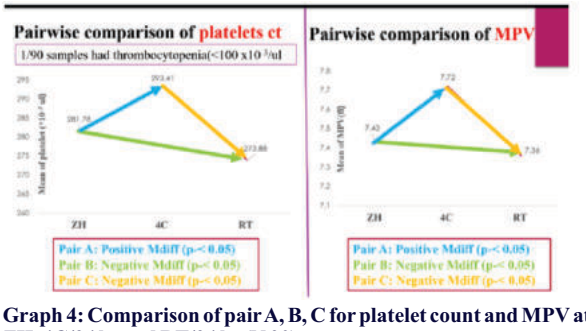
* indicates p <0.05, thus statistically significant



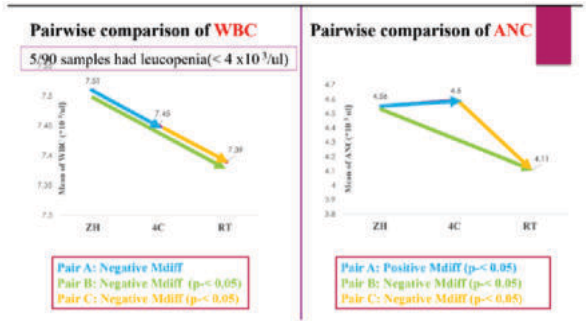
Graph 2: Comparison of pair A, B, C for hemoglobin and RBCC at ZH, 4C/24 hr and RT/24 hr (N 90)



Graph 3: Comparison of pair A, B, C for MCV and RDW-CV at ZH, 4C/24 hr and RT/24 hr (N 90)



Graph 4: Comparison of pair A, B, C for platelet count and MPV at ZH, 4C/24 hr and RT/24 hr (N 90)



Graph 5: Comparison of pair A, B, C for total leucocyte count and absolute neutrophil count at ZH, 4C/24 hr and RT/24 hr (N 90)

Table 2: Time and temperature dependent parameters of RBC, platelet, and WBCs on complete hemogram (N = 90)

	Temperature dependent 4C/24hr	Temperature dependent RT/24hr	Time dependent Both 4C and RT
RBC parameters	↑Hb, RBCC, MCHC	↓ Hb, RBCC, MCHC	---
	↓MCV, RDW-CV	↑MCV, RDW-CV	↑PCV, MCH
Platelet parameters	↑ platelet count, MPV	↓ platelet count, MPV	---
WBC parameters	↑ANC	↓ANC	↓TLC, ALC ↑ AMC

Summer versus winter:
M±SD of RT in summer was 33±2 ^{0}C and 22±2 ^{0}C in winter. Mdiff of pair A was disregarded since refrigeration at 4C would be unaffected by seasonal variation. Changes in Hb, RBCC, RDW-CV, and MPV were significantly greater in summer than winter (p<0.05). MCV showed equal changes in summer and winter. MCH had negative Mdiff in summer but positive in winter. Changes in PCV, MCHC, platelet count, TLC, ANC, and AMC were more in summer than winter (p>0.05). ALC showed more changes in winter than summer (p>0.05)

DISCUSSION:

Present study focused upon chemotherapy patients. Literature studies focused on healthy platelet donors [3], HIV patients [4], and healthy volunteers [9,10].

Room temperature:
RT in western countries [2, 4] have lower ambient temperature. India being a tropical country has higher ambient RT, especially in summer. Most high-end laboratories are equipped with airconditioned (AC) rooms. Our laboratory has non-AC rooms, thus stressing the importance of changes at RT/24hr. Gunawardane et al studied changes at 4, 22 and 37 degrees Celsius [11] RT related studies should specify the temperature range to validate comparison with other studies.

Direct versus derived:
Using Coulter's principle, anucleate particles 2 - 30 fl are recognised as platelets and 25-250 fl as RBCs. Small, medium and large nucleated cells are recognised as lymphocytes, monocytes, and granulocytes. [4,5,6]

Amongst RBCs the direct parameters are Hb, RBCC and MCV, while derived/calculated parameters are RDW-CV, PCV, MCH and MCHC. Platelet count, MPV, TLC, ANC, ALC and AMC are direct parameters.

Factual versus artefactual changes:
Former are due to genuine loss/gain in cell size/count. Latter occur by erroneous recognition of cells due to shrinkage/swelling, clumping and fragmentation. RBC and platelets can be confused since they are evaluated in a common chamber.

RBC parameters:
RT storage correlated with decreased Hb and RBCC due to RBC lysis and elevated MCV due to osmotic swelling. Elevated RDW-CV could be due to swollen RBCs counted as macrocytes and platelet clumps miscounted as microcytes. Raised PCV is a combination of high MCV and low RBCC.

Studies found Hb stable at 4C [3,9] and RT/72 hours [4]. RBCC was stable at 4C and reduced at 27C [9,10]. MCV was stable at 4C [9-11] and raised at 22C beyond 12 hours [9-11]. RDW was stable at 4C, while raised at 22C/24 hours [2,11]. PCV was raised beyond 24 hours at 22C and 37C [4,11]. Derived RBC parameters should not be used as standalone criterion for clinical decisions.

Platelet parameters:
Studies reported reduced platelet lifespan [6] and increased platelet count at 4°C. [3] Gunawardena D et al [11] found fall in platelet count at 4, 22 and 37 Celsius beyond 24 hours. Enlargement at 4C can explain raised MPV. EDTA induced platelet aggregation and fragmentation can manifest as pseudo-thrombocytopenia and pseudo-microcytosis. [6] Platelet clumping manifests as thrombocytopenia.

Leucocyte parameters:
TLC is stable[3] or increased[11] at 4C. Beyond 72 hours there is progressive leucocyte death[4]. Studies report rise in ANC at RT/24

hours [4] and decline at 4°C.[11,12] ALC was reported stable at 4°C and 22C upto 48 hours [11]. Hill V et al [4] found rise in ANC, fall in AMC at RT/24 hours but stable eosinophils and basophil count. EDTA induced degeneration can confuse neutrophils and monocytes.[6]

Unrecorded eosinophils and basophils can account for increased AMC in our study. Concurrent peripheral smear (PS) examination is essential for confirmation.

CONCLUSION:

All temperature dependent parameters (Hb, RBCC, MCV, RDW-CV, MCHC, platelet count, MPV, ANC) were further affected by summer season. Time dependent parameters (PCV, TLC, AMC) had greater changes in summer at RT, suggesting an additive effect of time and temperature. Owing to economic constraints our study was not conducted on 5-part analyser. Many peripheral laboratories are resource poor, being housed in non-AC environment and using 3-part analysers. Patients on chemotherapy are vulnerable to pancytopenia. Vital decisions about dosage and scheduling hinge upon accurate CHG findings.Prompt processing, refrigeration of samples and simultaneous PS examination is recommended.

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