Effect of Storage Time and Temperature on Hematological and Biochemical Parameters of Blood

Papiya Majumdar[1], Tirthankar Guha Thakurta[2*].

Departments of Pathology [1] and Physiology [2], KPC Medical College and Hospital,
1F, Raja SC Mullick Road, Jadavpur, Kolkata - 700032
Corresponding Author: Tirthankar Guha Thakurta

Abstract: *Delay in analysis of hematological and biochemical parameters of blood can occur due to referral from remote centres or due to storage, which may cause changes in blood parameters and erroneous laboratory results. We studied the changes associated with storage of blood at different temperatures and for different time-periods. The serial changes in complete hemogram, reticulocyte count and peripheral blood smear morphology, plasma sugar, urea, creatinine and electrolytes were measured. Blood from 60 patients, each collected &stored in K2-EDTA for hematological tests, sodium fluoride for plasma glucose and clotted blood for serum biochemistry. All the samples were analysed by auto-analysers. 20 samples were stored at room temperature (18 - 22°C), 20 samples at 4 - 8°C and 20 samples at 37°C. Analysis of samples was done at 0, 4, 8, 12, 24, 36, 48, 60 and 72 hours.*

When stored at room temperature, the RBC count, hemoglobin concentration remained relatively stable till 48 hours. Platelet, reticulocyte and absolute neutrophil counts changed significantly after 24 hours. Plasma glucose, serum electrolytes and protein varied significantly after 48 hours of storage. Urea and creatinine levels changed less significantly.

Samples stored at 4 – 8 °C were relatively more stable. Constancy ofparameters, like Reticulocyte count and DLC was noted at this temperature. Urea, creatinine and hemoglobin levels can be measured after 48 hours of delay; but protein, glucose and electrolyte estimation may be unreliable.

Keywords: *Preservation injury, Blood cell morphology, Biochemical analysis*

I. Introduction

Delay in analysis of hematological and biochemical parameters of blood can occur due to referral from remote centres or due to storage. This may lead to changes in blood parameters and erroneous laboratory results [1]. The nature and extent of the changes vary with time and temperature of storage [2, 3]. To prevent such storage induced changes, blood is often stored at a low temperature and analysed as early as possible after collection.

It is recommended that traditional CBC (Complete blood count) parameters such as red cell count, white cell count , haemoglobin and platelet count be analysed within 24 hours of sample collection when stored at room temperature [4,5,6].However, parameters useful for diagnosis and monitoring of haematological disorders, such as mean cell volume (MCV), reticulocyte and PBS morphology, are unreliable after 12 hours [7].Osmotic swelling of red cells during storage at room temperature affects volume-dependant variables.Reticulocytes mature into red cells after 24 hours in circulation and also in stored blood. The Clinical and Laboratory Standards Institute (CLSI) recommends that samples stored at room temperature should be analysed for reticulocyte within six hours of collection [8]. It is further recommended that peripheral blood smear examination for morphologic analysis be prepared within four hours, prior to the onset of EDTA-induced changes in red and white cell morphology [8,9,10].

With increased referral from remote centres, the time for transportation of blood samples is increasing. Throughout the transportation process, the collected blood sample may be subjected to ambient temperatures higher than recommended storage temperatures [12].

Moreover, due to increased centralisation of laboratories, there is delay in examination of the collected blood [1]. During this time, blood is often stored at room temperature of the laboratory (around 18-22°C), or occasionally at 4-8°C.

Cell counts are important parameters in evaluating the blood. Cell counts may be determined either manually or by automated hematology analyzers. Whether performed by manual or automated methodologies, the accuracy and precision of the counts depend on proper dilution of the blood sample, uniform distribution of cells and precise sample measurement. Manual counts are done using a microscope after appropriate dilution of

the sample in a hemocytometer, a specially constructed counting chamber that contains a specific volume. Automated methods are superior to manual methods for counting large numbers of cells and minimizing statistical error [13].

II. Aims and Objectives:

In this study, we have studied the changes that may occur in the hematological and biochemical parameters of blood, when stored at different temperatures for different time periods.

III. Materials and Methods

We studied the changes associated with storage of blood at different temperatures and for different time-periods. The serial changes in complete hemogram, reticulocyte count and peripheral blood smear morphology, blood sugar, urea, creatinine, electrolytes, total protein and bilirubin were measured.

***Procedure followed for data collection*:** Blood was collected from 60 patients. Blood from each patient was collected &stored in di-potassium ethylene di-amine tetra acetic acid for hematological tests, sodium fluoride for plasma glucose estimation and clotted blood for serum biochemistry. The samples were randomly distributed in 3 groups of 20 samples each. The first group of blood was stored at room temperature (18 - 22°C), second group at 4 - 8°C and third group at 37°C. Analysis of samples was done at 0, 4, 8, 12, 24, 36, 48, 60 and 72 hours after collection. The laboratory values obtained at 0-hour were taken as controls.

Complete hemogram parameters (Hemoglobin, total counts of RBC, WBC and Platelets, RBC indices, hematocrit, differential count of WBC) were analysed by an automated hematology analyser [13, 14]. Morphologic study of RBC, WBC and Platelets were done manually on a peripheral smear stained by Leishman stain [13]. Differential leukocyte counts obtained by auto-analyser were confirmed manually by peripheral smear examination. Reticulocyte counts were performed manually after staining the blood with supravital stain (Brilliant Cresyl Blue) [13].

Biochemical parameters were measured using an automated serum/ plasma biochemistry analyser. For each sample, bloodsugar, urea, creatinine, sodium and potassium were determined at specified intervals.

The changes in hematological and biochemical parameters were analysed with respect to the control (0- hour reading), in terms of storage time and storage temperature.

The room temperature specimens were placed on the laboratory rack, and room temperature was monitored using a laboratory thermometer. Specimens in the 4-8°C group were stored in a refrigerator at a suitable rack. Specimens for storage at 37°C were placed in an incubator at the specified temperature.
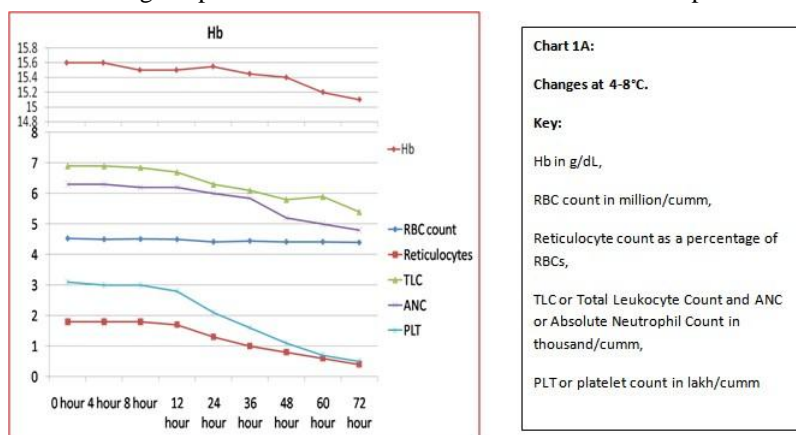
Source of finance: The study was funded by researchers.

Plan of analysis*:* Data were analyzed by using Microsoft Excel 2007 and SPSS-20. After analysis data were presented by charts. The data obtained from current study were compared with similar studies available in literature.

IV. Observations and Results:

The serial changes in blood biochemistry and hematology parameters were noted. The changes are summarised in the charts below.

Chart 1: Changes in hematological parameters of blood at different times and temperature:
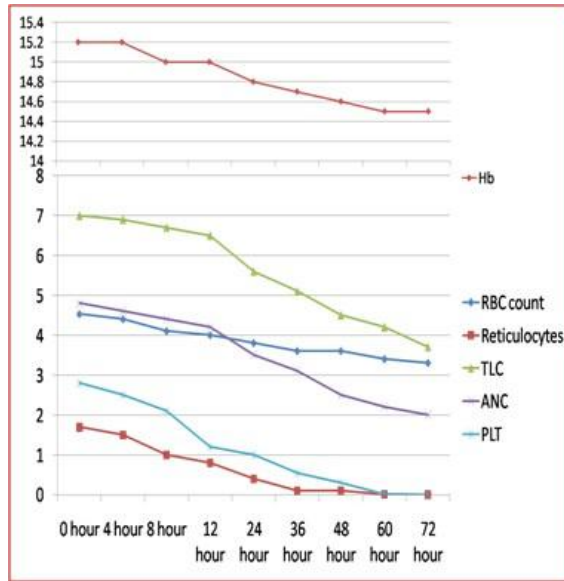

Chart 1A:

Changes at 4-8°C.

Key:

Hb in g/dL,

RBC count in million/cumm,

Reticulocyte count as a percentage of RBCs,

TLC or Total Leukocyte Count and ANC or Absolute Neutrophil Count in thousand/cumm,

PLT or platelet count in lakh/cumm

Chart 1C:

Changes at 37°C.

Key:

Hb in g/dL,

RBC count in million/cumm,

Reticulocyte count as a percentage of RBCs,

TLC or Total Leukocyte Count and ANC or Absolute Neutrophil Count in thousand/cumm,
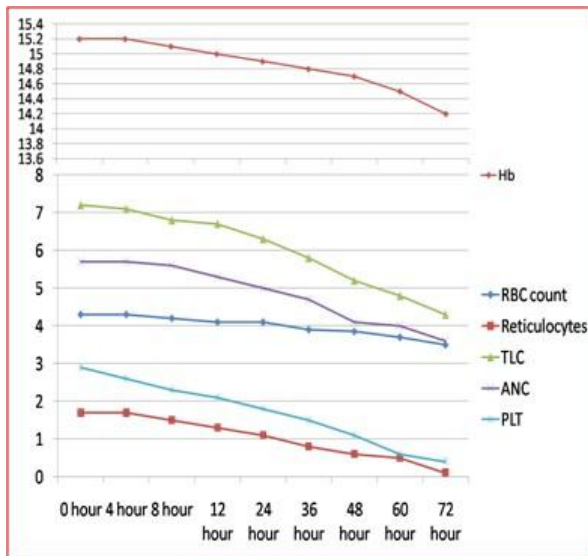
PLT or platelet count in lakh/cumm

Chart 1B:

Changes at 18-22°C.

Key:

Hb in g/dL,

RBC count in million/cumm,

Reticulocyte count as a percentage of RBCs,

TLC or Total Leukocyte Count and ANC or Absolute Neutrophil Count in thousand/cumm,

PLT or platelet count in lakh/cumm

- Most hematological parameters were stable up to 24 hours at 4°C.
- There were insignificant changes in Hemoglobin concentration and RBC count up to 48 hours, in blood stored at 4°C and 22°C (p > 0.05).
- Reticulocyte count, Total WBC Count, Absolute neutrophil count and Platelet count varied significantly after 48 hours in all samples (p < 0.001).
- All parameters, except Hemoglobin concentration were unreliable in blood stored for >24 hours at 37°C.
- The common morphological changes in RBCs were spiculation or crenation and excessive rouleaux formation.
- WBCs showed nuclear degeneration (karyolysis and karyorrhexis) and cellular swelling.
- Platelets were swollen in some samples
- The changes were more at higher temperatures and least when stored at 4-8°C.

Chart 2: Changes in biochemical parameters of blood at different times and temperature:
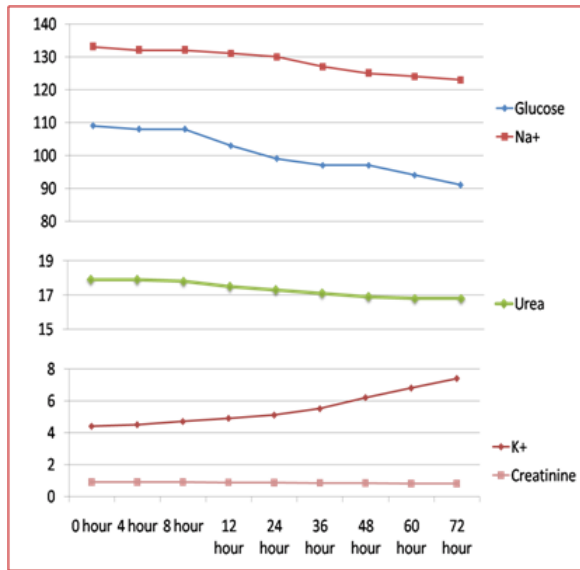

Chart 2A:

Changes at 4-8 degree C.

Key:

Plasma glucose, serum urea and serum creatinine are in mg/dL

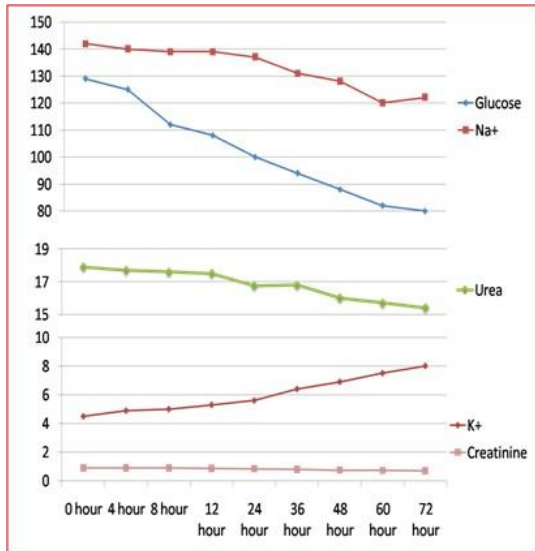Serum Na⁺ and K⁺ are in mEq/L

Chart 2B:

Changes at 18-22°C.

Key:

Plasma glucose, serum urea and serum creatinine are in mg/dL
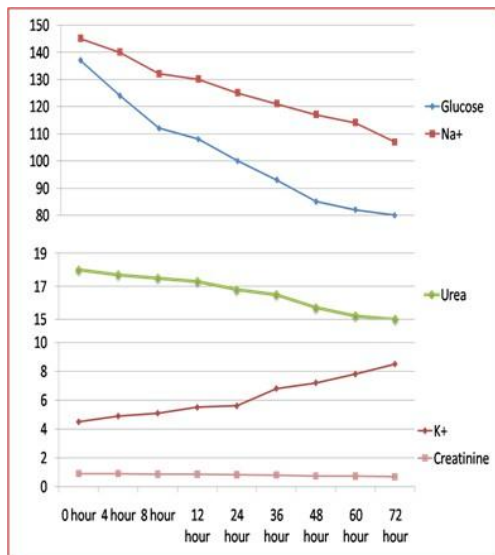
Serum Na⁺ and K⁺ are in mEq/L

Chart 2C:

Changes at 37°C.

Key:

Blood glucose, serum urea and serum creatinine are in mg/dL

Serum Na⁺ and K⁺ are in mEq/L

- Serum Sodium, Urea and Creatinine were stable up to 24 hours at all three temperatures.
- Serum Potassium and plasma Glucose varied significantly after 12 hours of storage ($p < 0.001$). The changes occurred early and were more pronounced at higher temperatures.
- Changes in serum Sodium and plasma Glucose levels were delayed by storage at 4°C.

V. Discussion:

The increasing burden of population and less number of laboratories in many parts of our country necessitates collection of blood from remote centres and transport to referral laboratories. The delay in transport may cause time and temperature dependent alteration of the laboratory findings [1, 2, 3].

It is recommended that traditional CBC parameters be analysed 24 hours after sample collection when stored at room temperature [1]. In our study we found that the platelet count, differential leukocyte count and reticulocyte count change significantly in 24 hours time. The reticulocyte count was most accurate within 6 hours of blood collection, and differential count within 12 hours. The parameters remained more stable at 4-8°C. When the total count of WBC or platelets were too low or too high, an additional manual PBS examination under microscope increased accuracy of the test.

The RBC morphology changes included crenation or spiculation and excessive rouleaux formation. These changes are also seen in different pathological conditions. Spiculated RBCs are often seen in uremia. Excess rouleaux formation may be a feature of chronic inflammatory disorders or multiple myeloma.
Nuclear changes in WBCs included fragmentation, karyolysis and pyknosis. These changes often make differential count difficult and may lead to errors.

Platelets showed great variability in count and morphology. The changes were least, when platelets were examined within 12 hours and when stored at 4°C. Swollen platelets in a background of low platelet count often suggest a disorder of platelet formation, like Immune Thrombocytic Purpura (ITP).
Thus, the morphological changes may confuse the pathologist and clinician to the exact nature of the disease.

Of all biochemical parameters, serum potassium was most susceptible to variation. It showed progressive elevation to near doubling of the value after 72 hours. Therefore potassium levels are best estimated in blood within 4 hours of collection. Serum urea, creatinine and sodium levels were reliable up to 24 hours. Plasma glucose was also reliable upto 24 hours when preserved at 4-8°C. In general storage at 4-8°C improved the accuracy of all tests.

VI. Conclusion:

We can conclude that when blood samples are meant for routine hematological tests, a peripheral smear should be prepared and stained within 4-6 hours. This is to be used for differential count, approximate platelet count and morphological study of blood cells. If reticulocyte count has to be performed, it should be done along with the PBS. Rest of the parameters like Hb, RBC indices, PCV measured by auto analysers should be ideally measured within 24 hours of collection, if stored at room temperature.

Plasma glucose and serum potassium is best measured within 4-8 hours of collection. Serum urea, serum creatinine and serum sodium may be measured within 24 hours, provided the storage temperature is18 - 22°C, i.e. room temperature. And if there is any chance of delay, ideally collected blood for all tests should be preserved at 4-8 °C to obtain the best results..

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