Effect of Storage Time and Temperature on Erythrocytes, Platelets and Leucocytes Pictures of Cattle and Equine Blood

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Abstract: The present study was undertaken to investigate the effect of storage time and temperature on erythrocytes, platelets and leucocytes pictures of cattle and equine blood. Whole blood samples from cows (No = 6) and donkeys (No = 6) were subdivided into two equal groups: refrigerator group (4°C) and room temperature group (30°C). Hematological analysis was performed by automatic blood cell counter at 0, 24, 30, 48, 56 and 72 h. The results revealed that donkey and cow's blood showed insignificant changes in different hematological parameters kept at 4°C when compared with 0 h group. Storage at room temperature affected granulocytes counts only which significantly decreased at 48, 56 and 72 h in donkey's blood and at 72 h in cow's blood. In conclusion, it is possible to analyze blood samples kept refrigerated or at room temperature for erythrocytes and platelets picture within 3 days from blood collection in cattle and equines. On the other hand, granulocytes count must be carried out before 48 h from blood collection in donkeys and before 72 h in cow's blood kept at room temperature.

Key words: Erythrocytes, leucocytes, platelets, storage, temperature, Egypt

INTRODUCTION

It is quite a regular practice in clinical medicine that blood samples should be subjected to hematological examinations as soon as possible after sampling and that the results should be evaluated at the earliest convenience.

Whereas this is also true in occupational and environmental medicine, the collection and analysis of blood may be hindered by various study conditions. For example, it would be expected to spend more than a day for transportation of the samples. The critical question then is how stable the hematological parameters are when kept under different storage conditions.

Despite remarkable progress in blood preservation (Grode et al., 1985; Snyder et al., 1985), the questions regarding stability of hematological findings generally remain unsolved in contrast to serum biochemistry for which effective storage conditions are more or less standardized (Gemba, 1985; Kanno, 1989). The present study was undertaken to investigate the effect of storage time and temperature on different hematological parameters in cattle and equine blood.

MATERIALS AND METHODS

Animals and samples: A total number of 12 blood samples were drawn from the jugular vein of cows and donkeys in vacutainer tubes containing EDTA at the Veterinary Teaching hospital, Assiut University, Egypt. Whole blood samples from cows (No = 6) and donkeys (No = 6) were subdivided into two equal groups: refrigerator group (4°C) and room temperature group (30°C). Hematological analysis was performed using automatic blood cell counter (Medonic CA 620, Sweden).

Hematological analysis: All blood samples were subjected to hematological analysis at the following time intervals at 0, 24, 30, 48, 56 and 72 h. The following hematological parameters were measured.

Erythrocytes picture: Total Red Blood Cells Count (T. RBCs), Haemoglobin Concentration (HGB), Mean Corpuscular Volume (MCV), Red blood cell Distribution Width (RDW), Haematocrit (HCT), Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Haemoglobin Concentration (MCHC).

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Platelets picture: Platelets count (PLT), Mean Platelet Volume (MPV), Platelets Distribution Width (PDW), Plateletcrit (PCT), Large Platelets Concentration Ratio (LPCR).

Leucocytes picture: Total White Blood Cells Count (T. WBCs), lymphocytes count, granulocytes count and monocytes count.

Statistical analysis: Statistical analysis was conducted using SPSS 16.0 for windows (SPSS, Chicago, USA). Statistical analysis was performed by comparing data from 24, 30, 48, 56 and 72 h with those from 0 h using one-way analysis of variance (repeated measures ANOVA). Statistically significant differences were determined at $p \leq 0.05$. Data were expressed as Mean±SD.

RESULTS AND DISCUSSION

Hematological changes in donkey blood
Effect of refrigerator temperature: There were insignificant changes in erythrocytes, platelets and leucocytes pictures in donkey's blood samples stored at 4°C for 72 h (Table 1-3).

Effect of room temperature: There were insignificant changes in erythrocytes and platelets pictures in donkey's blood samples stored at 30°C for 72 h. On the other hand, there were significant decreases ($p<0.01$) in granulocytes counts at 48, 56 and 72 h after storage at room temperature (Table 1-3).

Hematological changes in cow blood
Effect of refrigerator temperature: There were insignificant changes in erythrocytes, leucocytes and platelets pictures in cow's blood samples stored at 4°C for 72 h (Table 4-6).

Effect of room temperature: There were insignificant changes in RBCs and platelets pictures in cow's blood samples stored at 30°C for 72 h. On the other hand, there were significant decreases ($p<0.05$) in granulocytes counts at 72 h after storage (Table 4-6).

Samples of blood obtained from animals for hematological analysis are often collected at sites away from the analytical laboratory and delays may occur between collection and analysis. Storage conditions of specimens during this period may affect their quality and the results of subsequent testing. In the present study, donkey and cow's blood showed insignificant changes in

Table 1: Erythrocytes picture in donkey before and after incubation

Incubation	0 h (No = 3)	24 (No = 3)	30 h (No = 3)	48 h (No = 3)	54 h (No = 3)	72 h (No = 3)
Refrigerator 4°C						
T. RBCs count (10^6)	6.24±0.99	6.01±0.82	6.02±0.89	5.92±0.890	5.73±0.63	5.55±0.66
MCV (fl)	48.00±4.51	48.26±4.84	47.90±4.68	39.23±11.93	48.20±4.81	49.03±5.10
RDW (%)	24.73±2.07	24.83±2.86	24.90±2.42	25.30±2.400	25.26±2.93	25.46±3.02
HCT (%)	29.80±1.83	28.73±0.97	28.60±1.32	28.26±1.680	27.43±0.25	27.00±0.40
HGB (g dL^{-1})	10.46±0.66	10.36±0.40	10.20±0.36	10.16±0.550	10.13±0.15	9.66±0.25
MCH (pg)	16.93±1.65	17.43±1.71	17.06±1.82	17.33±1.650	17.83±1.94	17.60±1.83
MCHC	35.23±0.05	36.16±0.15	35.60±0.43	35.96±0.560	37.03±0.61	35.90±0.60
At room temperature 30°C						
T. RBCs count (10^6)	5.95±0.62	5.55±1.06	5.57±1.07	5.54±0.970	5.33±0.90	5.21±0.97
MCV (fl)	48.06±4.98	51.16±5.00	51.86±5.26	51.73±3.700	52.66±3.82	51.56±3.77
RDW (%)	24.80±2.91	23.73±2.55	23.63±2.70	21.73±3.060	21.23±2.66	20.33±1.90
HCT (%)	28.36±0.72	28.10±3.36	28.56±3.30	28.43±3.230	27.83±2.82	26.66±3.30
HGB (g dL^{-1})	10.10±0.11	9.66±1.11	9.36±1.20	9.26±1.040	9.23±1.00	8.80±0.95
MCH (pg)	17.10±1.90	17.60±1.70	17.00±1.60	16.90±1.600	17.50±1.50	17.10±1.60
MCHC	35.56±0.45	34.36±0.15	32.83±0.37	32.60±0.960	33.33±1.20	33.16±1.70

Data expressed as Mean±SD

Table 2: Platelets picture in donkey before and after incubation

Incubation	0 h (No = 3)	24 h (No = 3)	30 h (No = 3)	48 h (No = 3)	54 h (No = 3)	72 h (No = 3)
Refrigerator 4°C						
PLT (10^3)	204.67±73.18	168.33±103.19	153.67±93.4700	141.0±67.500	153.0±79.890	182.0±78.000
MPV (fl)	5.90±0.500	6.70±0.1000	7.10±0.26000	7.30±0.200	7.36±0.200	7.00±0.100
PDW (%)	9.60±1.250	11.23±0.4900	12.20±0.52000	12.83±0.550	12.83±0.350	11.56±0.370
PCT (%)	0.12±0.050	0.11±0.0700	0.11±0.06000	0.10±0.050	0.11±0.050	0.12±0.050
LPCR	12.10±3.600	16.83±1.9600	19.46±2.54000	22.30±2.150	22.26±1.280	17.43±1.190
At room temperature 30°C						
PLT (10^3)	164.33±95.00	169.00±94.620	168.330±68.090	161.67±68.25	177.33±50.10	182.67±87.31
MPV (fl)	6.23±0.490	5.80±0.1000	5.760±0.0500	5.66±0.410	5.630±0.35	5.83±0.600
PDW (%)	10.46±1.150	9.43±0.3700	8.930±0.2800	8.83±0.750	8.660±0.64	9.00±1.150
PCT (%)	0.093±0.05	0.09±0.0500	0.093±0.0400	0.09±0.040	0.096±0.03	0.1±0.0500
LPCR	15.40±3.200	9.26±0.3700	8.200±0.8700	7.53±3.100	6.660±3.23	8.23±4.610

Data expressed as Mean±SD

Table 3: Leucocytes picture in donkey before and after incubation

Incubation	0 h (No = 3)	24 h (No = 3)	30 h (No = 3)	48 h (No = 3)	54 h (No = 3)	72 h (No = 3)
Refrigerator 4°C						
T. WBCs count (10^3)	20.93±4.56	21.30±4.43	20.75±5.10	20.20±5.02	19.23±4.71	19.13±4.210
Lymphocytes count (10^3)	6.73±0.11	7.20±0.60	6.36±0.11	5.90±0.36	5.70±0.45	6.20±0.520
Granulocytes count (10^3)	12.83±4.30	11.96±2.36	13.10±4.70	12.83±4.74	12.13±4.44	11.33±3.880
Monocytes count (10^3)	1.36±0.20	2.13±1.45	1.46±0.28	1.46±0.20	1.40±0.10	1.60±0.000
At room temperature 30°C						
T. WBCs count (10^3)	21.13±4.61	21.00±6.64	20.23±6.88	17.73±6.61	18.60±7.56	16.93±7.520
Lymphocytes count (10^3)	7.13±1.00	8.20±3.05	8.06±3.40	9.83±3.98	11.26±4.99	11.36±5.540
Granulocytes count (10^3)	11.76±2.54	10.10±1.65	9.83±1.62	5.00±1.66**	4.26±1.53**	2.66±1.070**
Monocytes count (10^3)	2.23±1.36	2.73±1.96	2.33±1.88	2.90±1.03	3.06±1.06	2.90±1.210

Data expressed as Mean±SD; **:Significant at $p<0.01$

Table 4: Erythrocytes picture in cattle before and after incubation

Incubation	0 h (No = 3)	24 h (No = 3)	30 h (No = 3)	48 h (No = 3)	54 h (No = 3)	72 h (No = 3)
Refrigerator 4°C						
T. RBCs count (10^6)	5.60±0.90	5.60±0.80	5.60±0.90	5.50±0.90	5.50±0.900	5.50±0.90
MCV (fl)	46.80±4.40	46.70±4.30	47.10±4.70	46.70±4.70	47.00±4.400	48.10±4.40
RDW (%)	23.40±0.30	23.70±0.60	23.50±0.50	23.50±0.40	23.30±0.400	23.50±0.30
HCT (%)	25.80±2.30	25.80±1.90	26.00±1.80	25.20±1.80	25.70±2.100	26.30±2.20
HGB (g dL^{-1})	9.20±0.80	9.10±0.80	9.10±0.80	9.10±0.80	9.30±0.800	9.00±0.80
MCH (pg)	16.50±1.30	16.50±1.30	16.40±1.30	16.70±1.40	17.00±1.500	16.50±1.40
MCHC	35.40±0.60	35.50±0.50	35.00±0.70	35.90±0.68	36.30±0.300	34.30±0.40
At room temperature 30°C						
T. RBCs count (10^6)	5.30±0.67	5.30±0.69	5.48±0.41	5.39±0.44	4.90±1.180	5.12±0.56
MCV (fl)	50.56±1.30	50.66±1.15	51.10±0.62	50.96±0.70	52.20±0.650	52.40±0.83
RDW (%)	23.40±0.20	22.96±0.15	22.36±0.45	22.63±0.15	22.10±0.200	22.30±0.26
HCT (%)	26.73±2.80	27.00±2.97	28.03±1.89	27.43±1.94	25.56±5.930	26.70±2.76
HGB (g dL^{-1})	9.46±1.10	9.33±0.98	9.76±0.49	9.63±0.55	9.00±1.900	9.30±0.68
MCH (pg)	17.90±0.35	17.56±0.47	17.86±0.47	17.90±0.45	18.50±0.700	18.36±0.73
MCHC	35.50±0.51	34.70±0.28	34.90±0.60	35.10±0.73	35.46±1.050	35.03±1.20

Data expressed as Mean±SD

Table 5: Platelets picture in cattle before and after incubation

Incubation	0 h (No = 3)	24 h (No = 3)	30 h (No = 3)	48 h (No = 3)	54 h (No = 3)	72 h (No = 3)
Refrigerator 4°C						
PLT (10^3)	189.30±53.00	184.30±54.1	152.00±68.50	160.30±72.40	133.00±55.0	134.70±32.10
MPV (fl)	5.60±0.200	6.20±0.10	6.30±0.100	6.20±0.400	6.30±0.30	6.10±0.100
PDW (%)	8.60±0.200	9.60±0.20	9.80±0.200	9.50±0.500	9.60±0.40	9.40±0.100
PCT (%)	0.10±0.030	0.10±0.03	0.09±0.040	0.09±0.040	0.08±0.03	0.07±0.020
LPCR	4.20±0.100	6.10±2.10	6.70±0.700	5.40±0.500	5.80±0.30	6.10±0.500
At room temperature 30°C						
PLT (10^3)	146.67±38.78	117.67±6.65	111.67±31.65	118.67±15.94	110.00±9.53	116.00±19.92
MPV (fl)	6.00±0.430	6.13±0.30	6.40±0.720	6.50±0.250	6.36±0.45	6.20±0.360
PDW (%)	9.30±0.550	9.60±0.51	9.50±0.360	9.70±0.600	9.90±0.62	9.00±0.300
PCT (%)	0.08±.0280	0.06±.005	0.07±0.026	0.07±0.020	0.06±0.01	0.07±0.017
LPCR	6.46±1.200	6.40±0.95	9.56±4.000	9.30±1.800	8.36±1.26	7.63±2.180

Data expressed as Mean±SD

Table 6: Leucocytes picture in cattle before and after incubation

Incubation	0 h (No = 3)	24 h (No = 3)	30 h (No = 3)	48 h (No = 3)	54 h (No = 3)	72 h (No = 3)
Refrigerator 4°C						
T. WBCs count (10^3)	9.16±0.23	8.90±0.51	8.93±0.51	7.86±1.26	7.60±0.43	7.50±1.100
Lymphocytes count (10^3)	4.46±0.89	3.70±1.99	3.93±1.70	3.10±0.80	3.20±0.90	3.53±1.100
Granulocytes count (10^3)	3.96±1.01	4.36±2.60	4.06±2.30	3.96±2.10	3.70±1.60	3.10±0.660
Monocytes count (10^3)	0.73±0.11	0.83±0.15	0.93±0.25	0.80±0.17	0.70±0.26	0.83±0.150
At room temperature 30°C						
T. WBCs count (10^3)	8.60±0.34	9.00±0.65	8.60±2.38	7.56±2.65	8.10±1.40	7.73±0.060
Lymphocytes count (10^3)	4.60±1.30	6.03±1.50	5.30±4.50	4.90±4.10	5.30±1.60	6.20±0.450
Granulocytes count (10^3)	3.13±1.10	2.23±1.50	2.70±2.30	2.10±1.85	2.10±1.92	0.90±0.300*
Monocytes count (10^3)	0.86±0.21	0.76±0.15	0.66±0.25	0.53±0.40	0.66±0.10	0.63±0.100

Data expressed as Mean±SD; *: Significant at $p<0.05$

different hematological parameters at 4°C (Table 1-6). Storage at room temperature affected some blood parameters as granulocytes counts which significantly decreased at 48, 56 and 72 h in donkey's blood and at 72 h in cow's blood. Studies on the storage of human blood have shown that refrigeration results in better stability of hematological values (Cohle *et al.*, 1981; Bellamy and Hinchliffe, 1990; Wood *et al.*, 1999). Results

of the present study agreed with previous studies, Hirase *et al.* (1992) reported that T. RBCs, HGB, HCT, T. WBCs and PLT from human blood were stable for 1 week when kept refrigerated or at room temperature Hamilton and Davidson (1973) stated that T.RBCs, HGB and T.WBC were stable for 3 days.

In addition, Cohle *et al.* (1981) found that PLT, T. RBCs, HGB and T.WBCs were stable over 5 days period. None of the previous studies investigated the effect of storage time and temperature on PCT, MPV, PDW and LPCR. The use of automatic blood cell counter helped the analyses of these parameters.

CONCLUSION

This study taking in consideration the condition of blood collection and storage in the present study, it is possible to analyze blood samples kept refrigerated or at room temperature for RBCs and platelets picture within 3 days from blood collection in cattle and equines. On the other hand, granulocytes count must be carried out before 48 h from blood collection in donkey's blood and before 72 h in cow's blood kept at room temperature.

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