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ORIGINAL ARTICLE

Estimation of time since death from morphological changes in red blood cells of human cadaver: An autopsy-based study

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Abstract

Estimation of time since death continues to be a significant problem for forensic pathologists. It plays an essential role in medico-legal cases because forensic experts are often asked to assess death time in the investigation. Estimating time since death by studying biochemical changes in blood, CSF, intraocular fluids and other morphological changes in red blood cells is relatively unexplored; very few studies have been done on this topic. A descriptive cross-sectional study was planned to study the morphological changes in Red Blood Cells (RBC) obtained from a refrigerated human cadaver at different postmortem intervals. A total of 210 cases were included in the present study, irrespective of sex. We found that up to 3 hours of post mortem interval morphology of RBCs was normal. In RBC first morphological change observed was irregular and crenated margins at a post mortem interval of 4 hours. We observed complete lysis of RBC at a post mortem interval of 11 hours earliest. Post mortem interval can be estimated by observing morphological changes in a human corpse's blood cells with some degree of accuracy.

Keywords

Blood cells; Cellular changes; Postmortem changes; Time since death.

Introduction

The proper estimation of time since death (TSD) sometimes gives important clues for solving the crime to enforcement agencies. Many changes occur in a dead body after death. These changes can be used to estimate the approximate postmortem interval (PMI). The time of death is documented in-hospital deaths. But in deaths outside the hospital, the autopsy surgeon's help is required by the investigating officer to establish the actual time of death. The traditional methods of ascertaining the time since death is based on naked-eye observation of the dead body's gross changes occurring after death.[1-4] Time since death can also be calculated from the condition of food in the stomach, intestine, and urine in the urinary bladder. Attempts have also been made to estimate time since death by studying biochemical changes in blood, CSF, and intraocular fluids.[5-9]

The study of morphological changes in red blood cells to determine time since death is relatively an untouched topic. Few studies on this topic have been mostly on non-refrigerated dead bodies; however, in common practice, dead bodies are brought to the mortuary and preserved in cold storage before postmortem. Therefore, data related to refrigerated dead bodies will be more useful than non-refrigerated dead bodies. Further, this method for estimating postmortem interval is beneficial as it is simple and less time-consuming. Blood cells show varying degrees of postmortem changes, including morphological changes. These changes occur chronologically during degeneration and vary with regards to the postmortem interval, the study of which may prove useful in determining time passed since death. The present study aimed to study the morphological changes in red blood cells after death concerning the postmortem interval.

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Materials and Methods

The authors conducted a hospital-based, descriptive cross-sectional study in the Department of Forensic medicine in collaboration with the Department of Pathology, University College of Medical Science (UCMS) and Guru Teg Bahadur (GTB) Hospital, Delhi, between November 2015 to April 2017. A sample size of 210 was calculated considering an average rate of 78.75% to interpret the smears with expected results (Rajesh Bardale et al)[11], at Alpha = 5% ($p<0.05\%$), power of study 80% and effect difference of 6%. Cases above the age of 18, where the time of death was known and verified by hospital records were taken for study. Only those cases were included in which the bodies were kept in refrigeration within 30 minutes after documented death. Cases with a known history of blood malignancy, any other blood disorder, and septicemia were excluded from the study.

A total of 210 cases irrespective of sex were brought to the mortuary of UCMS and GTB Hospital for medico-legal

autopsies between 1st of November 2015 to 31st of March 2017, whose exact time of death was mentioned in the hospital death certificate were the subject of the study. All the corpses were kept in a deep freezer at 4^0 C for uniformity. All the Cases were divided into seven groups concerning time since death for convenience of research, and each group contains 30 samples. Written consent was taken from the next of kin of the deceased. The autopsies were done by following the standard procedure of post mortem. The blood sample was collected using a 10ml syringe from the heart chambers taking aseptic precautions. A thin blood smear was prepared immediately and was air-dried. This blood film was stained with Leishman's stain, and microscopic examination of the slides was done under oil immersion lens (100x) and relevant findings such as all Intact RBCs, mixed intact and lysed RBCs, and all lysed RBCs were noted. The presentation of the categorical variables has been done in the form of numbers and percentages (%). On the other hand, the quantitative data with normal distribution were presented as the means ± SD. The data entry was done in the Microsoft EXCEL spreadsheet. The final analysis has been done using Statistical Package for Social Sciences (SPSS) software, IBM manufacturer, Chicago, USA, version 21.0.

Results

In the present study, 210 samples were included out of them, 171 were male, and 39 were female. The mean age of the subjects is 37.33 years ranging from 18 to 76 years. The mean age for a male is 38.14 with age ranging from 18 to 75 years, and in the female, the mean age is 33.23, with age ranging from 18 years to 76 years.

Morphological changes of Red Blood Cells (RBC)

0-6 hours: Up to first 6 hours, all RBCs were found intact (Figure 1), although irregular and crenated margins were seen as early as 4 hours (Figure 2). **6-12 hours:** Complete cell lysis of RBCs was first seen in this group at a post mortem interval of 11 hours (Figure 3). Out of a total of 30 cases, only two smears, i.e., 6.66 % of cases, show mixed, i.e., both intact and lysed RBCs. Rest 28 smears (93.33%) showed all intact RBCs. **12-18 hours:** 11 smears, i.e., 36.66 % of cases showed mixed, i.e., both intact and lysed RBCs. While remaining 19 smears, i.e., 63.33 % of cases consisted of all intact RBCs. **18-24 hours:** 20 smears, i.e., 66.66 % of cases showed mixed, i.e., both intact and lysed RBCs. While remaining ten smears (33.33 %) showed all intact RBCs.**24-36 hours:** 2 smears, i.e., 6.66 % of cases showed complete lysis of all RBCs with no intact RBCs left. However, there were three smears, i.e., 10.00 % cases with all intact RBCs, while the remaining 25 smears (83.33 %) showed mixed RBCs, i.e., both lysed and intact cells were present. **36-48 hours:** In this group, no smear showed all RBCs intact. Eleven slides, i.e., 36.66 % of cases showed lysis of all RBCs, and the remaining 19 smears (63.33 %) showed mixed RBCs, i.e., both lysed and intact cells, were present. **Above 48 hours:** 5 smears (16.66 %) showed mixed RBCs (in all of these samples, time since death was less than 72 hours). The remaining 25 smears, i.e., 83.33 %, showed complete lysis of all RBCs. No intact RBC was seen in any slide with time since death above 72 hours (Figure 4, Table 1).

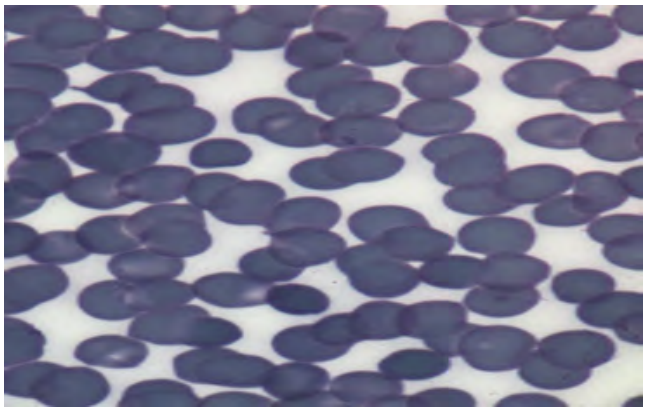

Figure 1: Morphologically normal RBC at PMI of 3.5 hours in blood film stained with Leishman's stain at 100x magnification

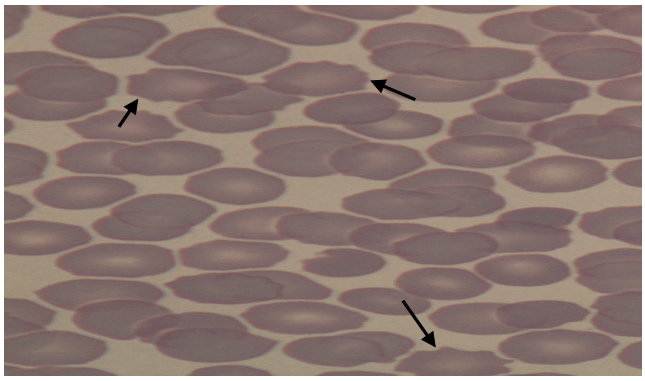

Figure 2: Crenated margins of RBC at PMI of 4 hours in blood film stained with Leishman's stain at 100x magnification

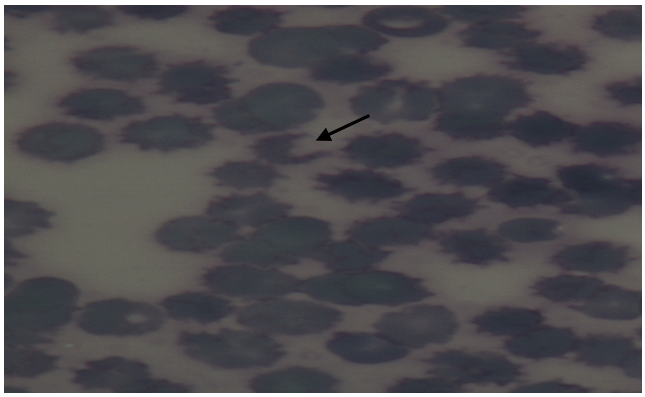

Figure 3: Lysed RBC at PMI of 11 hours in blood film stained with Leishman's stain at 100x magnification

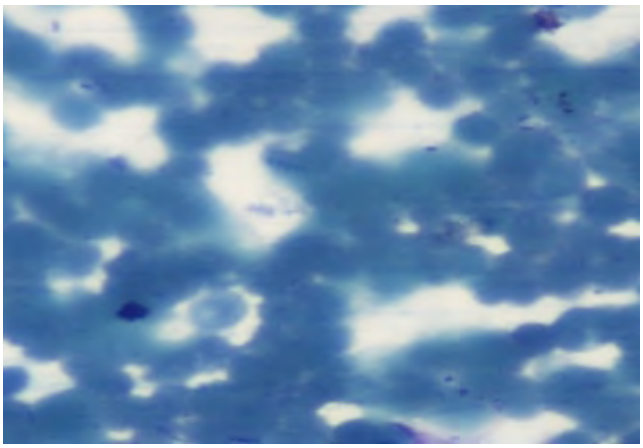

Figure 4: No intact RBC at PMI >72 hours in blood film stained with Leishman's stain at 100x magnification

Table 1: Morphological changes in RBC with respect to post mortem intervals

RBC	Post mortem interval (hours)						
	0-6	6-12	12-18	18-24	24-36	36-48	>48
1) All Intact RBCs.	30	28	19	10	3	0	0
2) Mixed intact and lysed RBCs	0	2	11	20	25	19	5
3) All Lysed RBCs	0	0	0	0	2	11	25
Total	30	30	30	30	30	30	30

Table 2: Comparative findings seen in RBCs of various studies

Findings	Post mortem interval		
	Present study	Bardale et al.[11]	Agarwal et al.[12]
No morphological changes were seen in RBCs.	Up to 2 hours	Up to 2 hours	-
RBCs begin to show crenated margins.	At 4 hours	3-4 hours	-
Lysis of RBC begins.	6-12 hours	6-8 hours	-
No intact RBC was seen.	> 72 hours	> 48 hours	> 19 hours.

Discussion

Numerous blood cells show varying degrees of postmortem changes, and these changes vary with regards to the postmortem interval. In the present study, we found that during the first 6 hours, all RBCs were intact, complete cell lysis of RBCs was first seen at a post mortem interval of 11 hours. However, crenated margins were seen as early as 4 hours. Between 12 – 18 hours, 36.66 % of samples showed mixed and, 63.33 % of the sample still consist of all intact RBCs. Between 18 – 24 hours, 66.66 % of samples showed mixed, while 33.33 % shows all intact RBCs. Between 24 – 36 hours in this group for the first time, two slides, i.e., 6.66 % of samples showed complete lysis of all RBCs with no intact RBCs left. Between 36 – 48 hours, no slides show all RBCs intact. Five slides (16.66 %) showed few intact RBCs above 48 hours since death, but no intact RBC was seen above 72 hours since the death.

Compared with other studies like that of Kumar et al., in all cases examined up to 12 hours after death, the RBCs were intact.[10] In contrast, those in post mortem interval of 12 hours to 18 hours, 94.7% had intact cells, and in 5.3 % cases, the mixture of intact and lysed RBCs was found in the slides. The cases examined at post mortem interval of 18 hours to 24 hours had 58.8% cases with intact cells, whereas in 41.2% cases, the mixture of intact and lysed RBCs was found. Among the cases examined between 24 hours to 36 hours after death, in 55.6% of slides mixture of intact and lysed RBCs were seen, and in 33.3 % of slides, all cells were found lysed & unrecognizable. A post mortem interval of 36 hours to 48 hours, 16.7% of cases had a mixture of intact and lysed RBCs, whereas, in 83.3% of cases, RBCs were lysed entirely& unrecognizable. No RBCs was found intact after 48 hours of death.

In the study of Bardale et al., during the first 2 hours, no changes were seen in the shape or morphology of RBCs.[11] Between 3-4 hours, the RBCs' morphology begins to change from discoid configuration to elliptical shape, to crenated margins, to crumbled discs. 7.5 % of cases showed lysis of RBC as early as 8 hours post mortem interval. No intact RBC was seen after post mortem interval of 20 hours. Agarwal et al. found intact RBC in almost all cases up to post mortem interval of 19 hours.[12] We can see that the present study shows similar findings very much compared to other studies except that in the present study, intact RBCs are seen for a much longer duration of time, i.e., for a post mortem interval of 72 hours. One reason could be that we have conducted our study on refrigerated dead bodies, unlike all other studies.

Conclusion

Post mortem interval can be estimated by observing morphological changes, i.e. percentage (%) of lysed RBCs in a peripheral blood smear of a human corpse with some degree of accuracy; however, more studies are required to narrow down the range of post mortem interval with other parameters because environmental conditions such as humidity and temperature affect the changes in the blood cells after death.

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References

1. Reddy KSN, Murty OP. The Essential of Forensic Medicine and Toxicology. 33rded. New Delhi: Jaypee health science publishers; 2014.p. 142-3.
2. Kannan K, Mathiharan K. editors. Modi's Medical Jurisprudence and Toxicology. 22nded.New Delhi: Lexis Nexis Butterworth's; 2003.p. 246-7.
3. Vij K. Textbook of Forensic Medicine and Toxicology: Principles and Practice. 2nded. New Delhi: Churchill Livingstone; 2000 .p.169-70.
4. Simpson K, editor. Taylor's: Principle and Practice of Medical Jurisprudence.12thed.London: Churchill Livingstone; 1965. p.70-103.
5. Coe JI. Post mortem chemistry update. Emphasis on forensic application. Am J Forensic Pathol.1993 Jun; 14(2):91-117
6. Camps FE. Gradwohl's Legal Medicine.3rd ed. Bristol: John Wright &Sons Ltd; 1976. p.78-98
7. Jaiswal AK. Determination of time of death by estimating the Potassium level in the Human Vitreous, J Indian Acad Forensic Med. 1988; 10:27-9.
8. Platt MS, McClure S, Clarke R, Spitz WU, Cox W. Postmortem cerebrospinal fluid pleocytosis. Am J Forensic Med Pathol.1989; 10:209-12.
9. Wyler D, Marty W, Bar W. Correlation between the postmortem cell content of cerebrospinal fluid and time of death. Int J Legal Med.1994;106(4):194-9.
10. Kumar B, Mahto T, Kumari V. Determination of time elapsed since death from changes in morphology of Red blood cells in Ranchi, Jharkhand J Indian Acad Forensic Med. April-June 2015; 37(2): 971-73.
11. Bardale R, Dixit PG. Evaluation of morphological changes in blood cells of human cadavers for the estimation of postmortem interval. Med. Leg. Update. 2007; 7(2): 4-6.
12. Agarwal SS, Shah K, Kumar L, Chavali KH. Determining the postmortem survival period and blood group antigenicity of red blood cells: a cross-sectional study. Anil Aggrawal's Internet J of Forensic Med and Toxicology [serial online] 2015;16(2):1-8.