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Original Article

Postmortem Evaluation of Autolytic Changes in Morphology of Red Blood Cells and Haemogram Pattern for Estimation of Time since Death

Ashish Tyagi[1]*, Shilpa Garg[2] and Hitesh Chawla[3]

[1]Assistant Professor, [3]Associate Professor, Department of Forensic Medicine, Shaheed Hasan Khan Mewati Government Medical College, Nalhar, Nuh, Haryana, India
[2]Associate Professor, Department of Pathology, Shaheed Hasan Khan Mewati Government Medical College, Nalhar, Nuh, Haryana, India
*Corresponding author email id: drashishfm@gmail.com

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ABSTRACT

The accurate estimation of time since death sometimes is very important for forensic experts in administration of justice. Various cells and tissues of the body showed significant changes with respect to post-mortem interval and one such sample is blood. The aim of this study is to assess the post-mortem autolytic changes in morphology of red blood cells and haemogram pattern for estimation of time since death. The present study was a cross sectional one and non random purposive sampling was done. The blood samples from 60 dead bodies which were brought for postmortem examination in the mortuary and preserved in <4°C temperature was taken after taking into consideration inclusion and exclusion criteria. The time since death was recorded and studied with respect to autolytic changes in RBC morphology and haemogram pattern. On meticulous examination of all the blood samples there were certain changes observed in the RBCs shape, intactness, central pallor and peripheral redness with respect to post-mortem interval. However, no statistical significance was correlated in the haemogram (RBC count, Hemoglobin, haematocrit, MCV, MCH and MCHC) with respect to time since death.

Keywords: Autolytic changes, Haemogram, Peripheral blood smear, Red blood cell morphology, Time since death, Post-mortem interval

INTRODUCTION

Postmortem interval or time since death is the time between death and post-mortem examination. It has importance not only in criminal matters but also in civil cases. The gross and microscopic changes in the body after death start to appear soon after death. As the time progresses various changes are variable and some remain constant. The study of these changes in the body helps us to determine time since death which has significant medico-legal importance. Although time since death is not an accurate estimation, variability in the assessment with regard to different factors affecting it can help us to estimate post-mortem intervals with ease. The basic criteria which are necessary for methods to determine time of death are their being easy applicability and non invasiveness. It is easy to obtain postmortem blood

samples even at the scene and it takes not more than a few minutes. The main problem is to provide a reliable method that could be based on blood analysis [1].

In the red blood cell the changes after death are not only restricted to integrity, morphology or shape of RBC but also central pallor and peripheral redness. In the present study autolytic changes in the morphology of Red Blood Cell and hemogram pattern are being studied to estimate time since death or post-mortem interval.

MATERIALS AND METHODS

The present study was conducted in the Department of Forensic Medicine in collaboration with Department of Pathology of a tertiary care hospital and Medical College of rural Southern Haryana. The cross-sectional study was conducted for a period of one year and sixty blood samples were taken from the dead bodies that were brought to the mortuary for medico-legal autopsies. The sample design was non-random purposive sampling. Blood samples from the dead body were retrieved after taking informed consent from next of kin of the deceased and approval from Institutional Ethical Committee. The blood samples were taken irrespective of age and sex from all such cases of hospital or institutional deaths where complete treatment record, cause of death and time since death has been documented. All the dead bodies were preserved at minimum 4°C in the deep freezer before the corpse was brought to the mortuary for post-mortem examination. The samples which were excluded from the study were those having prior clinical history of haematological disorders (malignancy, disease or infection), decomposition or putrefaction has started, brought dead cases or non institutionalised deaths where certified time of death is not available.

After initial dissection of the body, a postmortem blood sample was collected from chambers of Heart with the help of 5ml disposable syringe and was divided into two portions and kept in EDTA vials for study. First blood sample was immediately sent to the haematopathology lab for estimation of haematological parameters such as total count of RBC, Haemoglobin percent, Haematocrit, MCV, MCH and MCHC by automated cell counter. Any samples with clotted blood in the EDTA vial were discarded. Automated Cell Counter generates a paper strip which contains the identification no, total count of RBC, WBC, Platelet, percentage of Haemoglobin, value of Haematocrit along with MCV, MCH, MCHC, R/W etc. Another blood sample was used to prepare thin blood smear which were then 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 were noted. All the morphological changes observed in Red blood cells were observed in terms of change in their appearance, shape, central pallor, integrity and lytic activity in the cells and their internal structures. These observations were categorized and tabulated.

All the data was statistically analysed using SPSS version 20.0. Student t test was used to test the significance. P value less than 0.05 was considered significant. Pearson correlation was also used to establish and quantify the strength and direction of the relationship between two variables.

RESULTS

The blood samples were collected from 60 corpses (34 males and 26 females) which arrived at the mortuary of the Department of Forensic Medicine for post-mortem examination. Blood smears were examined to note the morphology of red blood cells for correlation with time since death. The study was majorly based upon autolytic morphological variations in Red Blood Cells which were noted in following manner [2]:

a. Integrity: Intact, Mixture of intact and lysed, and Lysed & not recognizable

b. Shape: Intact, Slightly dysmorphic and grossly Dysmorphic

c. Central Pallor: Intact, Reduced and Lost

d. Periphery: Red and Pale

Table 1: Distribution of cases according to time since death

Time since death (hours)	Male (%)	Female (%)	Total (%)
0-6	4 (11.76)	3 (11.54)	7 (11.67)
6-12	11 (32.35)	8 (30.77)	19 (31.67)
12-18	8 (23.53)	4 (15.38)	12 (20)
18-24	3 (8.82)	4 (15.38)	7 (11.67)
24-36	2 (5.88)	2 (7.69)	4 (6.67)
36-48	4 (11.76)	3 (11.54)	7 (11.67)
>48	2 (5.88)	2 (7.69)	4 (6.67)
Total	**34 (56.67)**	**26 (43.33)**	**60(102.02)**

For the purpose of classifying the observation systematically, the dead body samples were grouped in the following manner based on the known time elapsed since death i.e. in hours duration group.

In our study (Table 2) among the cases examined during the first 6 hours and 6-12 hours after death, in all cases (100%) morphology of RBCs was found to be normal. Whereas in 12 to 18 hours duration, they started to lyse initially and in 18 to 24 hours nearly half of them were lysed but morphologically remained intact. A mixture of intact, lysed and non-recognisable cells were seen after 24 to 36 hours of death. RBCs were not recognizable at all >48 hours after death in present study.

Among the cases examined (Table 3) during the first 6 hours after death one fourth of RBCs started to become dysmorphic. In 6 to 12 hours after death a mix picture of intact, slightly and grossly dysmorphic RBCs appeared with more preponderance towards slight dysmorphism. Whereas, in 12-18 and 18-24 hours after death, a mix picture of slightly and grossly dysmorphic RBCs seen in all of the cases. After 36 hours of death, all RBCs were found to be grossly dysmorphic (100%).

Table 2: Integrity of RBC morphology with respect to time intervals

Time since death (hours)	RBC intact	RBC intact and lysed	RBC lysed and Not recognisable	Total
0-6	7 (100%)	-	-	7
6-12	19 (100%)	-	-	19
12-18	9 (75%)	3 (25%)	-	12
18-24	4 (57.14%)	3 (42.86%)	-	7
24-36	1 (25%)	2 (50%)	1 (25%)	4
36-48	-	4 (57.14%)	3 (42.86)	7
>48	-	-	4 (100%)	4

Table 3: Shape of RBC with respect to time intervals

Time since death (hours)	RBC intact	RBC slightly dysmorphic	RBC grossly dysmorphic	Total
0-6	5 (71.43%)	2 (28.57%)	-	7
6-12	5 (26.32%)	11 (57.89%)	3 (15.79%)	19
12-18	-	5 (41.67%)	7 (58.33%)	12
18-24	-	2 (28.57%)	5 (71.43%)	7
24-36	-	1 (25%)	3 (75%)	4
36-48	-	-	7 (100%)	7
>48	-	-	4 (100%)	4

Table 4: Central pallor of RBC with respect to time intervals

Time since death (hours)	Central pallor intact	Central pallor reduced	Central pallor lost	Total
0-6	6 (85.71%)	1 (14.29%)	-	7
6-12	7 (36.84%)	12 (63.16%)	-	19
12-18	-	7 (58.33%)	5 (41.67%)	12
18-24	-	3 (42.85%)	4 (57.14%)	7
24-36	-	1 (25%)	3 (75%)	4
36-48	-	-	7 (100%)	7
>48	-	-	4 (100%)	4

Table 5: Periphery of RBC with respect to time intervals

Time since death (hours)	Periphery RBC red	Periphery of RBC Pale	Total
0-6	6(85.71%)	1(14.29%)	7
6-12	11 (57.89%)	8 (42.11%)	19
12-18	3 (25%)	9 (75%)	12
18-24	-	7 (100%)	7
24-36	-	4 (100%)	4
36-48	-	7 (100%)	7
>48	-	4 (100%)	4

In Table 4, RBCs central pallor was intact upto 6 hours after death but the pallor started to reduce in 6-12 hours interval in half of the cases. The reduction of pallor in RBCs progressed to loss of pallor in the next six hours duration i.e. 12-18 hours and remained constant till 18-24 hours. The loss of pallor becomes more prominent after 36 hours of death, when all the cases showed similar findings.

In Table 5, periphery of RBCs remains red in the majority of cases in the first 6 hours after death which thereafter changes to paleness in nearly half of the cases in 6-12 hours. The percentage of pale RBCs increased to 75% in next 12-18 hours. After 18 hours, all cases showed paleness of RBCs in 100% of cases.

DISCUSSION

The majority of cases in the present study comprised of those where time since death at the time of autopsy was 6-12 hours and 12-18 hours and maximum upto 48 hours similar to Kumar *et al.* [2–5] have observed corpses with post-mortem interval ranging from 1.7 to 270.4 hours in their study. Babapulle and Jayasundera [6] observed corpses from 0 to 84 hour period in their study. Bardale and Dixit[7] have also observed the changes in corpses only up to 24 hours after death. However, Shah *et al.* observed the blood cells in corpses with time since death varying from 2.5 to 19 hours [8].

The results of present study demonstrated that the RBCs morphology was intact till 18-24 hours after death in all cases and a mixture of lysed, unrecognisable and intact cells were observed after 24- 36 hours of death in most of the cases. Beyond the 36 hours' time period following death, the RBCs completely lysed and lose their integrity. Study conducted by Jat *et al.,* Bardale and Dixit, Shah *et al.* and Kumar *et al.* also concur with the RBC morphology findings observed in this study. An earlier

Table 6: Correlation study of different parameters of Haemogram with Time Passed since Death

	RBC	Haemoglobin	Hematocrit	MCV	MCH	MCHC
Pearson's Correlation	-0.259	-0.220	-0.243	0.270	0.112	0.056

*Correlation is significant at the 0.01 level (2-tailed).

No statistically significant correlation was found in all the variables with respect to time since death.

study conducted by Bardale and Dixit [7] concluded that a rise in temperature can lead to hastening of decomposition in living substances. The results of the present study are also quite in coherence to those of Shah *et al.* who found that intact RBC's could be observed in all the cases up to 19 hours post-mortem and earliest post-mortem interval at which RBC's were found to be broken was 7 hours [3,7,8,2].

Time since death also showed significant relationship with the shape of RBCs. The shape of RBC also changed from slightly dysmorphic to grossly dysmorphic with the passage of time which is also shown in Table 3. The process of dysmorphism started earlier in our study i.e. within 6 hours of death. In next 6 hours this change in shape of RBCs accelerates with a mix picture of intact, slightly and grossly dysmorphic RBCs appeared. RBCs appearance becomes grossly dysmorphic to total lyses in more than 36 hours after death. Our findings in this regard also concur with the studies conducted by Jat and Kumar Penttila and Lahio states that when corpses were kept at >4°C the red cells were quite rapidly transformed from discoid configuration to crumbled discs, echinocytes and spherocytes, but no debris or burst cell configurations were seen. Up to 12 hours most red cells were quite normal or slightly crumbled, between 12 and 48 hours most of them were encountered discs, and from 48 to 168 hours the spiculated or crenated cells were dominant. The number of the more smooth surfaced cells later increased. Bardale. also observed that morphology of most of the RBCs was unidentifiable beyond a period of 30 hours [4,5].

With regard to the central pallor and peripheral colour of the RBC which also changes and becomes paler with the passage of time. For the initial 12 hours the central pallor and peripheral red colour of RBC remains intact in some samples, however with the passage of time these two findings become obsolete and colour started to become faint in all the samples beyond the period of 24 hours after death. Our findings are similar to the observations seen by Jat and Kumar [2,3] .

All the parameters of haemogram in relation with RBC were found to be statistically insignificant with respect to time since death in this study which was not the case in the studies earlier conducted by Penttila *et al.* [4,5,9]. The red cell count and haematocrit were found to be positive correlated with regard to time since death as both showed slight increase in number upto 12-36 hours after death which was shown by Penttila and Lahio. The increase in haematocrit is mainly due to the loss of the liquid phase of the blood into the surrounding tissues rather than to the increase in the volume of red cells. Kundu established a negative statistical correlation of RBC count in relation with post-mortem intervals. The different result may be due to small sample size of current study and because of different techniques or machines used by different authors for haemogram analysis.

CONCLUSION

The present study proves that changes in the morphology of red blood cells can be useful as a supplementary procedure for estimating time since death. However, there is continuous need for the development of an accurate method, by which the post-mortem interval can be determined in the blood components**.**

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REFERENCES

[1] Maeda H, Ishikawa T, Michiue T. Forensic biochemistry for functional investigation of death: concept and practical application. Legal Medicine. 2011;13(2):55–67.

[2] Kumar B, Mahto T, Kumari V. Determination of time since death from changes in morphology of Red Blood Cells. Journal of Indian Academic Forensic Med. 2015;37(2):148–51.

[3] Jat SS, Punia RK, Khichi MK, Sharma S. Effect of time since death on morphological changes of red and white blood cells-an autopsy based study at S.M.S. medical college & attached group of hospitals, Jaipur during the year 2016-2017. Medico-legal Update. 2019;19(2): 145–50.

[4] Penttila A, Laiho K. Autolytic changes in blood cells of human cadavers- Morphological studies. Forensic Science International. 1981;17(2):121–32.

[5] Lahio K, Penttila A. Autolytic changes in blood cells and other tissue cells of human cadavers I. Viability and ion studies. Forensic Science International. 1981; 17:109–20.

[6] Babapulle CJ, Jayasundera NPK. Cellular changes and time since death. Medical Science Law 1993;33(2):213–22.

[7] Bardale R, Dixit PG. Evaluation of morphological changes in blood cells of human cadaver for the estimation of postmortem interval. Medico-Legal Update. 2007;7(2): 56–62.

[8] Shah K, Agarwal SS, Kumar L, Chavali KH. Determining postmortem survival period and blood group antigenicity of red blood cells: a cross- sectional study. Anil Aggrawal's Internet Journal of Forensic Medicine and Toxicology. 2015;16(2):8.

[9] Kundu SD and Dutta SS. Changes in haemogram in subjects after death as a tool to estimate time passed since death. IOSR Journal of Dental and Medical Sciences. 2017;16(10):19–27.

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