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Original Research Article.

To Assess the Time Since Death on Morphological Changes of Neutrophils, Lymphocyte, Monocytes & Eosinophils: An Autopsy Based Study

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ABSTRACT

Background: In Medico-legal work the determination of the accurate time passed since Death has always been important. Therefore, this study was undertaken to study the estimation of time since death by morphological changes in neutrophils, lymphocytes & monocytes.

Materials & Methods: The hospital based observational study was carried out on 150 cases in Department of Forensic Medicine, RNT Medical College, Udaipur. All the corpses were kept in deep freezer at 4^0 C after certified death in the attached hospital. The morphological changes observed in neutrophils, lymphocytes, Monocytes & eosinophils were observed in terms of change in their appearance, shape, central pallor, integrity and lytic activity in the cells and their internal structures.

Results: In this study, males were more preponderant as compare to female, male to female ratio was 4:1. Neutrophils were found to be recognizable latest by 30 hours & Lymphocytes were found recognizable latest by 24 hours after death in present study. There was no disintegration observed in monocytes during the 1st six hours after death in any case.

Conclusion: The present study proposes that lymphocytes are the most resistant blood cells as regards to degeneration after death. Although a single cell change viewed in isolation may not do wonders in framing opinion of time elapsed since death but a study of morphological appearance of various cells at the time of examination may lead to meaningful inferences.

Keywords: Autopsy, Neutrophils, Monocytes, Lymphocytes, Eosinophils.

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INTRODUCTION

The proper estimation of time since death sometimes gives important hints for solving the crime to the investigating agencies and punishing the true offender and proper administration of justice. Death is an irreversible cessation of circulation, respiration and brain activity, which is termed as somatic death. It is a known fact that different cells of the body die at different times after somatic death, which is termed as cell death.[1]

Following cessation of the circulation, ischemia in organs and tissues leads to reversible, then irreversible changes affecting their structure and function. The cellular death arises by the irreversible change in the internal environment of body consequent to death. This irreversible change in the internal environment is due to non-availability of oxygen, accumulation of carbon dioxide, pH change and accumulation of toxic products.[1]

Numerous cells in blood show varying degree of post-mortem changes and these changes vary with regards to the post-mortem interval. Studies reveal that cells that are likely to be affected in blood by any irreversible altered internal environment are the normal blood cells i.e. red blood cells, white blood cells and platelets.[2]

During degeneration these cells pass through the series of changes in chronology study of which may prove useful in determination of time passed since death. Estimation of time elapsed since death can be done by studying the changes in red blood cells and white blood cells which is easy and cheap to perform.

Therefore, this study was undertaken to study the estimation of time since death by morphological changes in neutrophils, lymphocytes & monocytes.

MATERIALS & METHODS

The hospital based observational study was carried out on 150 cases in Department of Forensic Medicine, RNT Medical College, Udaipur with assistance from Department of Pathology, for preparation and analysis of samples after obtaining due clearance from research and review board of RNT Medical College and Hospital, Udaipur.

Inclusion Criteria

1. Deaths occurring at the institute wherein time of death is certified in hospital record.

Exclusion Criteria

1. Cases with any haemolytic disorder as per available history and documents.
2. Cases satisfying the inclusion criteria but whose attendants did not consent for participation in the study.

Methods

All the corpses were kept in deep freezer at 4^0 C after certified death in the attached hospital. Time of death in included cases was taken as the time of death declaration officially recorded treatment record in International Death Certificate format. Autopsy Examination was performed as per norms after fulfilling all medico-legal formalities. All details pertaining to relevant external and internal findings, pattern of injuries and cause and manner of death were observed and recorded as per Performa. Sample collection was done from each case included in study group at the time of Medico-legal autopsy. Time since death was the controlled factor in the present study as all deaths included in the study were declared in the hospital. This time was then correlated to the morphological changes observed in different blood cells at different post mortem periods.

Sample Collection & Blood Smear Preparation

After initial dissection of the dead body, 2 ml of blood was collected from the heart chambers in EDTA vial using aseptic precautions. Thin blood smear was prepared and 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 under the supervision of Department Of Pathology, RNT Medical College, Udaipur.

The morphological changes observed in neutrophils, lymphocytes, Monocytes & eosinophils were observed in terms of change in their appearance, shape, central pallor, integrity and lytic activity in the cells and their internal structures. The observations were categorized on the basis of findings documented in earlier researches and available literature.

Table 1: Distribution of cases according to gender

Gender	No. of cases	Percentage
Male	120	80%
Female	30	20%
Total	150	100%

Table 2: Descriptive statistics of Morphological Changes in Neutrophils

Time since death	Normal	Recognizable but slightly dysmorphic	Grossly dysmorphic	Mixture of dysmorphic and Lysed	Lysed	Total
0-6 hrs	32 (100%)	00	00	00	00	32
6-12 hrs	02 (8.33%)	22 (91.66%)	00	00	00	24
12-18 hrs	00	00	33 (100%)	00	00	33
18-24 hrs	00	00	08 (100%)	00	00	08
24-36 hrs	00	00	00	09 (69.23%)	04 (30.76%)	13
36-48 hrs	00	00	00	00	07 (100%)	07
>48 hrs	00	00	00	00	33 (100%)	33

Table 3: Descriptive statistics of Morphological Changes in Lymphocytes

Time since death	Normal	Recognizable but slightly dysmorphic	Grossly dysmorphic	Mixture of dysmorphic and Lysed	Lysed	Total
0-6 hrs	32 (100%)	00	00	00	00	32
6-12 hrs	02 (8.33%)	22 (91.66%)	00	00	00	24
12-18 hrs	00	00	33 (100%)	00	00	33
18-24 hrs	00	00	08 (100%)	00	00	08
24-36 hrs	00	00	00	00	13 (100%)	13
36-48 hrs	00	00	00	00	07 (100%)	07
>48 hrs	00	00	00	00	33 (100%)	33

Table 4: Descriptive statistics of Morphological Changes in Monocytes

Time since death	Normal	Recognizable but slightly dysmorphic	Grossly dysmorphic	Mixture of dysmorphic and Lysed	Lysed	Total
0-6 hrs	32 (100%)	00	00	00	00	32
6-12 hrs	02 (8.33%)	00	22 (91.66%)	00	00	24
12-18 hrs	00	00	00	00	33 (100%)	33
18-24 hrs	00	00	00	00	08 (100%)	08
24-36 hrs	00	00	00	00	13 (100%)	13
36-48 hrs	00	00	00	00	07 (100%)	07
>48 hrs	00	00	00	00	33 (100%)	33

Table 5: Descriptive statistics of Morphological Changes in Eosinophils

Time since death	Normal	Recognizable but slightly dysmorphic	Grossly dysmorphic	Mixture of dysmorphic and Lysed	Lysed	Total
0-6 hrs	32 (100%)	00	00	00	00	32
6-12 hrs	02 (8.33%)	00	22 (91.66%)	00	00	24
12-18 hrs	00	00	00	00	33 (100%)	33
18-24 hrs	00	00	00	00	08 (100%)	08
24-36 hrs	00	00	00	00	13 (100%)	13
36-48 hrs	00	00	00	00	07 (100%)	07
>48 hrs	00	00	00	00	33 (100%)	33

RESULTS

In this study, males were more preponderant as compare to female, male to female ratio was 4:1. Males being active members of the society generally comprise a higher proportion of medico-legal deaths as compared to females (table 1).

Morphological Changes in Neutrophils (table 2)

In our study among the cases examined during the first 6 hours after death in all cases (100%) morphology of neutrophils were found to be normal. In 6 to 12 hours after death they were normal in 8.33% and slightly dysmorphic in 91.66% cases. Whereas in 12 to 18 hours and 18 to 24 hours after death they were grossly dysmorphic in all cases (100%).

A mixture of grossly dysmorphic cells was seen in 69.23% cases and complete lysis in 30.76% cases after 24 to 36 hours of death. Neutrophils were recognizable latest by 30 hours in present study.

Morphological Changes in Lymphocyte (table 3)

Out of all the cases examined during the first 6 hours after death lymphocytes were found to be normal in 100% cases and they were normal in 8.33% cases & slightly dysmorphic in 91.66% cases during 6 hours to 12 hours after death. In 12 to 18 hours & 18 to 24 hours after death cells were grossly dysmorphic was in 100%. They were found lysed in all the cases examined beyond 24 hours after death.

Morphological Changes in Monocytes (table 4)

In first 6 hours after death in 100% cases morphology of the monocytes were found to be normal. Whereas they were normal in 8.33% case and grossly dysmorphic in 91.66% of cases examined during 6-12 hours after death. They were found lysed in all the cases examined beyond 12 hours after death.

Morphological Changes in Eosinophils (table 5)

In first 6 hours after death in 100% cases morphology of the Eosinophils were found to be normal. They were normal in 8.33% case and grossly dysmorphic in 91.66% of cases examined during 6-12 hours after death. They were found lysed in all the cases examined beyond 12 hours after death.

DISCUSSION

Numerous cells in blood show varying degree of post-mortem changes and these changes vary with regards to the post-mortem interval.

In the present study, male were more preponderant as compared to female, male to female ratio was 4:1. Similar results were observed by Shah K, et al (2015)[3] who observed an almost equal M:F ratio being 14:15 and Kundu SS, et al (2017)[4] reported 58.33% males as compared to 41.67% females in their study.

Regional and socio-cultural variations in the different places of study are probable explanations for such notable variation in the gender wise distribution of medico-legal deaths in the two studies.

Neutrophils were found to be recognizable latest by 30 hours & Lymphocytes were found recognizable latest by 24 hours after death in present study. There was no disintegration observed in monocytes during the 1st six hours after death in any case. The maximum period up to which monocytes were recognizable was 18 hours.

Same as the other White blood cells, the Eosinophils were also morphologically normal up to a period of 6 hours following death in all cases. The reason might be that degenerative cellular changes occur earlier and more rapidly in cadaveric blood than in vitro blood of controls or might be attributable to environmental and temperature difference.[5,6]

Penttila A and Laiho K (1981)[5] stated that when corpses were kept at +4°C the lymphocytes seemed to be most resistant and basophils the least resistant to the effects of autolysis.

Dokgoz H, et al (2001)[7] found that eosinophils and monocytes were identifiable up to 72 hours, neutrophils up to 96 hours and lymphocytes up to 120 hours after death in non-refrigerated cadavers.

Bardale R and Dixit PG (2007)[6] observed in their study that neutrophils up to 20-24 hrs, lymphocytes up to 30 hours, eosinophils up to 21 hrs and monocytes are identifiable up to 18 Hrs after death. This variability in results can be attributed to the climatic and temperature variations in the places of study (Rajasthan, India & Istanbul, Turkey, which is a much cooler place than ours) along with the differences in the period over which the two studies were conducted (cooler season). In refrigeration too, the temperature at which the cadavers were stored in the present study at +4^{0}C. The present study observed that Lymphocytes were the most resistant group of blood cells in view of autolytic morphological changes after death. Similar results have also been proposed by Bardale R and Dixit PG (2007).[6]

CONCLUSION

In a country like India, such types of works bear many difficulties, not always for the economic scarcity, but during the proceedings less cooperation from the legal guardian of the deceased. There is a continuous need for the development of an accurate method, by which the time of Death can be determined. For the last so many years the Blood has been considered as a rich source for this purpose.

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