Original article:

To Assess the Time since Death on Morphological Changes of White Blood Cells in Human: An Autopsy Based Study

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Abstract:

Background Numerous cells in blood show varying degree of post-mortem changes and these changes vary with regards to the post-mortem interval. Therefore, this study was undertaken to study the estimation of time since death by morphological changes in white blood cells in human dead body.

Materials & Methods: The hospital based observational study was carried out on 150 cases in Department of Forensic Medicine, S.N. Medical College, Jodhpur. All the corpses were kept in deep freezer at 4^0 C after certified death in the attached hospital. The morphological changes observed in white 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.

Results: In this study, males were more preponderant as compare to female, male to female ratio was 2:1. Neutrophils were found to be recognizable latest by 30 hours & Lymphocytes were found recognizable latest by 24 hours after death in present study.

Conclusion: The present study proves that changes in the morphology of white blood cells can be helpful as supplementary procedure for estimating time since death.

Keywords: Morphological Changes, White Blood Cells, Lysis, Time Since Death.

INTRODUCTION

Determination of 'time since death' is one of the important content of the post-mortem report and is desired by the law administrating agencies. Sometimes the autopsy surgeon has to face some peculiar situations while performing autopsy where the time since death is of utmost importance for solving complex criminal matters associated with homicide. 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.[1]

Traditionally the triad of algor mortis, livor mortis and rigor mortis has been used to estimate the time since death from ages and also recently ample amount of studies have been done for time since death which are based on various chemical and physical changes that occur after death but none of them has proven to be satisfactory enough to narrow the range of time since death.

It is known that different cells of body die at different times after somatic death. The cellular death arises by an irreversible change in the internal environment of body consequent to death. This irreversible change in the internal

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environment is due to non-availability of oxygen, accumulation of carbon dioxide, pH change and accumulation of toxic products. Numerous cells in blood show varying degree of post-mortem changes and these changes vary with regards to the post-mortem interval. 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. In blood cells variation in Morphology can be noted in integrity, shape, central pallor and periphery of Red Blood Cells and changes in morphology of all types of white blood cells (WBC) i.e. neutrophils; lymphocytes, eosinophils can be noted as normal, slightly dysmorphic, grossly dysmorphic, mixture of dysmorphic & lysed with the passage of time.[2]

Literature search revealed only few studies conducted to evaluate the cellular changes occurring in blood after death for estimating time since death. The search for newer and more objective parameters and their validation continues. Keeping in mind the scarcity of expert hands and budget constraints of a developing country like India, the parameters should preferably be of such a nature that they are relatively inexpensive and can be incorporated into the routine work. 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.[3] 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, S.N. Medical College, Jodhpur with assistance from Department of Pathology, for preparation and analysis of samples after obtaining due clearance from research and review board of S.N. Medical College and Hospital, Jodhpur.

Inclusion Criteria

1. Only those cases in which TSD were known by relatives, police or doctors and verified by other post-mortem changes were included in this study.

Exclusion Criteria

1. Cases with any haemolytic disorder as per available history and documents.
2. Cases in which bodies grossly affected with septicemia, anemia, and nutritional deficiency, Malignancy of blood, blood disorders, and charred bodies were not included in this study.
3. 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

Blood samples were collected from heart chambers and slides were prepared on spot at the time of autopsy. Slides were stained by Leishman's stain and examined under light microscope. The study was based upon variation in White Blood Cells.

Morphology of all types of white blood cells (WBC) i.e. neutrophils; lymphocytes, Eosinophils and monocytes were noted in following manner:

Normal, slightly dysmorphic, grossly dysmorphic, mixture of dysmorphic & Lysed and Lysed.

For the purpose of classifying the observation systematically, the dead bodies were grouped in the following manner based on the known time elapsed since death:

RESULTS

In this study, males were more preponderant as compare to female, male to female ratio was 2:1. 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 & Lymphocyte (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 18.75% and slightly dysmorphic in 81.25% 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 66.66% cases and complete lysis in 33.33% cases after 24 to 36 hours of death. Neutrophils were recognizable latest by 30 hours in present study.

Morphological Changes in Monocytes & Eosinophils (table 3)

In first 6 hours after death in 100% cases morphology of the monocytes were found to be normal. Where as they were normal in 18.75% case and grossly dysmorphic in 81.25% of cases examined during 6-12 hours after death. They were found lysed in all the cases examined beyond 12 hours after death.

Table 1: Distribution of cases according to gender

Gender	No. of cases	Percentage
Male	80	66.66%
Female	40	33.33%
Total	120	100%

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Table 2: Descriptive statistics of Morphological Changes in Neutrophils & Lymphocytes

Time since death	Normal	Recognizable but slightly dysmorphic	Grossly dysmorphic	Mixture of dysmorphic and Lysed	Lysed	Total
0-6 hrs	22 (100%)	00	00	00	00	22
6-12 hrs	03 (18.75%)	13 (81.25%)	00	00	00	16
12-18 hrs	00	00	22 (100%)	00	00	22
18-24 hrs	00	00	05 (100%)	00	00	05
24-36 hrs	00	00	00	06 (66.66%)	03 (33.33%)	9
36-48 hrs	00	00	00	00	05 (100%)	05
>48 hrs	00	00	00	00	21 (100%)	21

Table 3: Descriptive statistics of Morphological Changes in Monocytes & Eosinophils

Time since death	Normal	Recognizable but slightly dysmorphic	Grossly dysmorphic	Mixture of dysmorphic and Lysed	Lysed	Total
0-6 hrs	22 (100%)	00	00	00	00	22
6-12 hrs	03 (18.75%)	00	13 (81.25%)	00	00	16
12-18 hrs	00	00	00	00	22 (100%)	22
18-24 hrs	00	00	00	00	05 (100%)	05
24-36 hrs	00	00	00	00	9 (100%)	9
36-48 hrs	00	00	00	00	05 (100%)	05
>48 hrs	00	00	00	00	21 (100%)	21

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 2:1. Similar results were observed by Shah K, et al (2015)[4] who observed an almost equal M:F ratio being 14:15 and Kundu SS, et al (2017)[5] 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.[6,7]

Dokgoz H, et al (2001)[8] 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)[7] 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. 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).[7]

CONCLUSION

The present study proves that changes in the morphology of white blood cells can be helpful as supplementary procedure for estimating time since death. There is a continuous need for the development of an accurate method, by which the time of Death can be determined.

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