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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

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Abstract

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. Numerous cells in blood show varying degree of post-mortem changes and these changes vary with regards to the post-mortem interval. Therefore, this hospital based descriptive observational study were carried out at the Department of Forensic Medicine, SMS Medical College, Jaipur to study the estimation of time since death by morphological changes in red & white blood cells. The present study proposes that lymphocytes are the most resistant blood cells as regards to degeneration after death. Other white blood cells and also red blood cells also show certain pattern of degradation which can be correlated to the time passed since 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. Thus we concluded that the present study proves that changes in the morphology of red blood cells & white blood cells can be helpful as supplementary procedure for estimating time since death.

Keywords: *Time since death, W.B.C., R.B.C, Cellular changes, Degeneration.*

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. 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]

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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.[2]

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. The time course of these phenomena is, however, very different, depending on the tissues; for example, brain cortex structures undergo definitive alterations after a few minutes, whereas other tissues kidney, skeletal muscle

may tolerate prolonged ischemia for up to several hours.[2]

Numerous cells in blood show varying degree of post-mortem changes and these changes vary with regards to the post-mortem interval. 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. 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 i.e. Neutrophils; Lymphocytes, Eosinophils can be noted as normal, slightly dysmorphic, grossly dysmorphic, mixture of dysmorphic & lysed with the passage of time.[1]

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. Therefore, this study was undertaken to study the estimation of time since death by morphological changes in red & white blood cells.

Material & Method

This is a hospital based descriptive observational study were carried out at the Department of Forensic Medicine, SMS Medical College, Jaipur with assistance from Department of Pathology, for preparation and analysis of samples after obtaining due clearance from research and review board of SMS Medical College and Hospital, The morphological changes observed in RBCs and WBCs 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.

Inclusion criteria:

Dead individuals of either sex and any age

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

Above cases where attendants consent to participate in the study

Exclusion criteria:

Cases who have received blood transfusion during hospitalization prior to death

Cases with any haemolytic disorder as per available history and documents.

Cases satisfying the inclusion criteria but whose attendants did not consent for participation in the study.

Method

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. 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, SMS Medical College, Jaipur.

Observations & Results

A total of 8865 medico-legal autopsies were conducted during the study period out of which 150 cases were included in the study. . The majority of cases were seen in 21-40 years of age groups, followed by 41-60 years and minimum cases were seen in 0-10 years of age in our study (table 1). males were more preponderant as compare to female, male to female ratio was 4.35:1. We found that lymphocytes are the most resistant blood cells as regards to degeneration after death. Other white blood cells and also red blood cells also show certain pattern of degradation which can be correlated to the time passed since death.

Although uniformity was desired in the number of cases with different times since death but due to restraint of post-mortem after legal formalities, it was not absolutely achieved. Thus, the time since death of majority of cases included in the study were 12-18 hours & more than 48 hours (22% each respectively), followed by 21.33% cases of 0-6 hours and 16% cases of 6-12 hours (table 2).

Morphology changes in of RBC:

All cases examined after death the integrity of RBC was intact till 18-24 hours and mixture of lysed and intact after 24-36 hours. Complete lysis of RBCs was observed in all cases after 36 hours had elapsed after death. The shape of RBCs became slightly dysmorphic in 6-12 hrs and grossly dysmorphic after12-24 hrs of death. Mixture of grossly dysmorphic with microcytic cells was seen at 24-36 hrs after death and completely lysis was seen after 36 hours of death. The central pallor was retained until 0-6 hrs, reduced during 6 hrs to 18 hrs and loss of central pallor was seen after 18 hrs in this study. Hemoglobinized periphery of cell was seen at 0-18 hrs, which got pale during 18hrs to 36 hrs and was not recognizable after 36 hrs after death in majority of smears (table 3).

Morphological Change in of WBCs:

In this study during the first 6 hours after death, in all cases morphology of all WBCs were found to be normal till 6 hours until death. After 6 hours of death, dysmorphic changes started in all types of WBCs. During 6 to 12 hours after death, Monocytes and Eosinophils became grossly dysmorphic. Neutrophils and Large Lymphocytes started exhibiting gross dysmorphism after 12 hours, whereas Small Lymphocytes after 18 hours. Complete lysis was an observed in most cells after 18 hours and all WBCs were lysed after 36-48 hours' time period (table 4)

Table 1 : Distribution of cases according to age

Age group (yrs.)	No. of cases	Percentage
0-10 yrs	05	03.33%
11-20 yrs	14	09.33%
21-30 yrs	41	27.33%
31-40 yrs	40	26.66%
41-50 yrs	25	16.66%
51-60 yrs	14	09.33%
>60 yrs	11	07.33%
Total	**150**	**100%**

Graph 1 : Distribution of cases according to gender wise

Table 2: Distribution of cases according Time since death

Time since death	No. of cases	Percentage
0-6 hrs	32	21.33%
6-12 hrs	24	16%
12-18 hrs	33	22%
18-24 hrs	08	05.33%
24-36 hrs	13	08.66%
36-48 hrs	07	04.66%
>48 hrs	33	22%
Total	**150**	**100%**

Table 3 : Morphological changes in RBCs

Time since death	RBC			
	Integrity	**Shape**	**Central Pallor**	**Periphery of cell**
0-6 hrs	Intact	Normal	Intact	Hemoglobinized
6-12 hrs	Intact	Slightly dysmorphic	Reduced	Hemoglobinized
12-18 hrs	Intact	Grossly dysmorphic	Reduced	Hemoglobinized
18-24 hrs	Intact	Grossly dysmorphic	Lost	Pale
24-36 hrs	Mixture of lysed and intact	Grossly dysmorphic with microcytic	Lost	Pale
36-48 hrs	Completely Lysed	Lysed	Lost	Not recognized
>48 hrs	Completely Lysed	Completely Lysed	Lost	Not recognized in all

Table 4 : Morphological Change in of WBCs

Time since death	Neutrophils	Small lymphocyte	Large lymphocyte	Monocytes	Eosinophil
0-6 hrs	Normal	Normal	Normal	Normal	Normal
6-12 hrs	Slightly dysmorphic	Slightly dysmorphic	Slightly dysmorphic	Grossly dysmorphic	Grossly dysmorphic
12-18 hrs	Grossly dysmorphic	Slightly dysmorphic	Grossly dysmorphic	Lysed	Lysed
18-24 hrs	Grossly dysmorphic	Grossly dysmorphic	Grossly dysmorphic	Lysed	Lysed
24-36 hrs	Lysed with grossly dysmorphic	Lysed	Lysed	All Lysed	All lysed
36-48 hrs	All Lysed	Lysed	Lysed	All Lysed	All Lysed
>48 hrs	All Lysed	All Lysed	All Lysed	All Lysed	All Lysed

Discussion

This study showed that the majority of medico-legal deaths (54%) were seen in 21-40 years of age, which are the years of active life. Similar results were found by **Shah K, et al**.(2015)[3] with maximum cases (75%) seen in21 – 50 years of age group. Similar results were observed by **Kundu SS, et al** (2017)[4] who reported majority of cases (46.43%) were seen in 21-40 years of age group.

In the present study, male were more preponderant as compared to female, male to female ratio was 4.35:1. Similar results were observed by **Kundu SS, et al (2017)[4]** & contradictory to those of **Shah K, et al (2015)**[3] who observed an almost equal M:F ratio being 14:15.

The majority of cases in the present study comprised of those where time since death at the time of autopsy was 12-18 hours & >48 hours (22% each respectively). **Penttila A** and **Laiho K (1981)[5]** have observed corpses with post-mortem interval ranging from 1.7 to 270.4 hours in their study. **Babapulle CJ** and **Jayasundera NP (1993)[1]** observed corpses from 0 to 84 hour period in their study. **Dokgoz H, et al (2001)[6]** had observed in vitro and post mortem changes in morphology of blood cells ranging from 0 to beyond 120 hours of post mortem interval. **Bardale R and Dixit PG (2007)[7]** have observed the changes in corpses only up to 24 hours' time period after death, probably for reasons that their study was conducted on non-refrigerated cadavers. **Kumar B, et al (2014 & 2015)[8,9]**observed blood cells in dead bodies up to more than 48 hours of time since death. **Manohar WS, et al (2015)[2]** had observed corpses similar to **Dok Goz H, et al (2001)[6]** for a period up to 120 hours. **Shah K, et al (2015)[3]** observed the blood cells in corpses with time since death varying from 2.5 to 19 hours.

The present study showed that the integrity of RBCs was intact till 18-24 hours after death in all cases and a mixture of lysed and intact cells was observed after 24-36 hours of death in most cases. Beyond the 36 hours' time period following death, they were completely lysed losing their integrity. A study done by **Bardale R and Dixit PG, et al.(2007)[7]** who reported that up to two-hour post-mortem interval (PMI), the shape and morphology of Red blood cells (RBC) was found to be normal. Whereas, **Bardale R** and **Dixit PG (2007**)[7]found that a rise in temperature hastens decomposition of living substances.

The results of the present are quite in accordance of those of **Penttila A** and **Laiho K (1981)[5]**. **Mukherjee JB (2007)**[10] found that as fluid exudes out of vessels in dependent body parts in hypostasis, haematocrit value vary from place to place, hence not dependable. The results of the present study are also quite in coherence to those of **Shah K, et al (2015)[3]** 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.

In this study during the first 6 hours after death, in all cases morphology of all WBCs were found to be normal till 6 hours until death. After 6 hours of death, dysmorphic changes started in all types of WBCs. During

6 to 12 hours after death, Monocytes and Eosinophils became grossly dysmorphic. Neutrophils and Large Lymphocytes started exhibiting gross dysmorphism after 12 hours, whereas Small Lymphocytes after 18 hours. Complete lysis was an observed in most cells after 18 hours and all WBCs were lysed after 36-48 hours' time period.

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.[5] **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)**[6] 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. **Arican N, et al (2000)**[11] found that the changes of total WBC counts and differential counts together may be used for evaluation of PMI**. Kumar B, et al (2014**[8]**& 2015**[9]**)** reported normal morphology of neutrophils in 91% cases among the deaths autopsied within the first 6 hours after death and in rest 9% cases, they were slightly dysmorphic. In 6 to 12 hours after death they were normal in 60.7% and slightly dysmorphic in 39.2% cases. **Manohar WS, et al (2015)**[2] found that in Lymphocytes showed degenerative changes even after 84 hours, but could be easily identified even after that period.

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 proposes that lymphocytes are the most resistant blood cells as regards to degeneration after death. Other white blood cells and also red blood cells also show certain pattern of degradation which can be correlated to the time passed since 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. Thus we concluded that the present study proves that changes in the morphology of red blood cells & white blood cells can be helpful as supplementary procedure for estimating time since death.

Conflict of Interest: None Declared.

Ethical Clearance: Taken from the Research , Review and Ethical Committee of SMS Medical College and Hospital.

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