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Original Article

Correlation of Time Since Death with Morphological Changes in Neutrophils

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ABSTRACT

Background: Death is the end of dying. It is a process rather than an event except in the exceptionally rare situations where death may be almost instantaneous [1]. **Materials & Methods:** In this study morphological changes in neutrophils were studied in autopsy cases. Blood samples from 57 different autopsy cases were taken and smears were prepared after staining them with Leishman stain. The samples were examined using light microscope under 40X and 100X magnification. **Results:** Among 57 cases no degenerative changes were seen in 8. Mild degenerative changes were seen in among 23, Moderate among 12 and Marked among 14 cases. Among 57 cases while observing degenerative changes in neutrophils nuclear changes were seen in 47 cases in form of pyknosis among them 40 reached to level of nuclear fragmentation. Cytoplasmic changes were seen in form of cytoplasmic vacuolation seen in 49 cases. Among them loss of cytoplasm was seen in 36 and cytoplasmic degeneration seen in 26. There was only 8 case in which no change was seen. No changes were seen upto 6 hrs. **Conclusion:** The present study proves that changes in the morphology of neutrophils can be helpful as supplementary procedure for estimating time since death.

Keywords: Morphological changes, White blood cells, Lysis, Time since death

INTRODUCTION

The proper estimation of time since death sometime gives important hint for solving the crime for the investigating agencies & punishing the true offender & proper administration of justice [2].

Changes after death vary with time since death and can be discussed under three headings. Immediate changes are insensitivity, respiratory arrest & circulatory arrest. Early change are postmortem cooling (algor Mortis), Eye Changes, Skin changes, Post Mortem Lividity (Livor Mortis) & Muscular Changes (Rigor Mortis). Late Changes are Putrification, Adipocere Formation, Mummification & skeletonization [3]. Pathological examination of viscera has been an integral part of diagnosis ever since the inception of forensic medicine [4]. Numerous cells in blood shows varying degree of post-mortem changes and these occurring cellular changes could be utilized to estimate death interval [5]. Following cessation of the circulation,

ischemia in organs and tissues leads to reversible, then unrecoverable changes affecting their structure and function. The cellular death arises by the irreversible change in the internal environment of body consequent to death. Neutrophils show deep purple nucleus composed of 2-5 lobes connected by a thin filamentous strand of nuclear material and show light pink cytoplasm. Fine granules are seen in cytoplasm. Numerous cells in blood show varying degree of post-mortem changes and these changes vary with regards to the post-mortem interval [6]. During degeneration these cells pass through the series of changes in chronology. Thus, the study of these changes in neutrophils after death provide useful information in determination of time passed since death. In blood cells variation in morphology can be noted in integrity, shape, central pallor. Nuclear changes assume one of three patterns, all resulting from a breakdown of DNA and chromatin. Pyknosis is characterized by nuclear shrinkage and increased basophilia; the DNA condenses into a dark shrunken mass. The pyknotic nucleus can undergo fragmentation; this change is called karyorrhexis. Ultimately, the nucleus may undergo karyolysis, in which the basophilia fades because of digestion of DNA by deoxyribonuclease (DNase) activity. In 1 to 2 days, the nucleus in a dead cell may completely disappear.[7] 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. In this study, Morphological changes in neutrophils and gross changes in slides will be studied from blood films in autopsy cases and these would be correlated with time since death. This may be helpful in estimating time since death and thus it may complement other methods for time since death estimation.

MATERIALS AND METHODS

The present study was conducted in Department of Forensic Medicine B.P.S. Government Medical College for Women, Khanpur Kalan, Sonipat with assistance from Department of Pathology, for preparation and analysis of samples after obtaining due clearance from research and review board. It was a one year study of the cases brought to the mortuary of B.P.S. Government Medical College for Women, Khanpur Kalan, Sonipat for Post-Mortem examinations. All the known cases of hospital death of all age group coming for medico legal post-mortem examination within 96 hrs of death without any history or microscopic picture of haematological malignancies were included. The blood samples were collected from heart. Slides were prepared by push technique, stained by Leishman's stain method and were examined using compound microscope under 400x and 1000X magnifications.

RESULTS

In our study, a total number of 57 (43 Males and 14 females) cases were studied. There were 75.4% males and 24.6% females (Table 1).

Table 1: Distribution of cases according to Sex

Sex	Frequency	Percentage
Male	43	75.4%
Female	14	24.6%
Total	57	100.0%

Among 57 cases 15 (26.3%) were from urban locality and 42 (73.7%) were from rural locality (Table 2).

Table 2: Distribution of cases according to residence

Locality	Frequency	Percentage
Urban	15	26.3%
Rural	42	73.7%
Total	57	100.0%

Table 3 is showing distribution of autopsy cases with respect to post-mortem interval. In our study, highest numbers of cases were examined in post-mortem time interval of 19 to 24 hours as 20 out of total 57 cases taken in the study.

Table 3: Distribution of cases according to Time since death

Time since death	Frequency	Percentage
0 to 6 hours	8	14.0%
7 to 12 hours	11	19.3%
13 to 18 hours	18	31.6%
19 to 24 hours	20	35.1%
Total	57	100.0%

Among 57 cases, overall no degenerative changes were seen in 8 (14.0%), mild degenerative changes were seen in among 23 (40.4%), moderate among 12 (21.1%) and marked among 14 (24.6%) cases (Table 4 and Figure 1).

Table 4: Distribution of cases according to overall total degenerative changes

Degenerative changes	Frequency	Percentage
Unremarkable	8	14.0%
Mild	23	40.4%
Moderate	12	21.1%
Marked	14	24.6%
Total	57	100.0%

While studying degenerated changes in neutrophils in correlation with time since death no changes were seen upto 6 hrs. In time since death between 7-12 hrs, 11 (100.0%) were showing cytoplasmic vacuolation 9 (81.8%) were showing pyknosis, 8 (72.7%) were showing nuclear fragmentation, 7 (63.6%) were showing loss of cytoplasm and 4 (36.4%) were showing cytoplasmic degeneration. In time since death between 13-18 hrs, 18 (100%) were showing pyknosis and cytoplasmic vacuolation, 14 (77.8%) were showing nuclear fragmentation and loss of cytoplasm and 11 (61.1%) were showing cytoplasmic degeneration. In time since death between 19-24 hrs, 20 (100%) were showing pyknosis and cytoplasmic vacuolation, 18 (90.0%) were showing nuclear degeneration (Karyolysis), 15 (75.0%) were showing loss of cytoplasm and 11 (55.0%) were showing cytoplasmic degeneration (Table 3, 5, Figure 1, 2 and 3).

DISCUSSION

In the present study, male were more preponderant as compared to female, male to female ratio was 3:1. Males were 75.4% and female 24.6%. Similar results

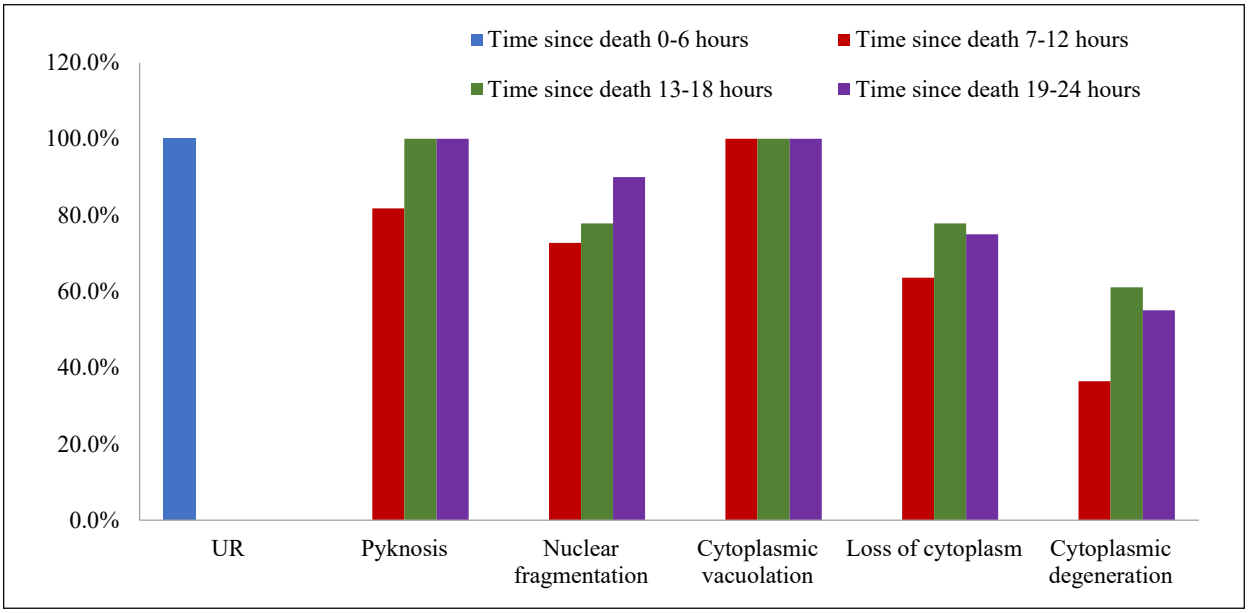

Figure 1: Distribution of study population according to degenerative changes in neutrophil

Table 5: Distribution of study population according to degenerative changes in neutrophil

Neutrophil Changes	Time since death					p-value
	0-6 hours (n=8)	7-12 hours (n=11)	13-18 hours (n=18)	19-24 hours (n=20)	Total cases (n=57)	
Unremarkable	8	0	0	0	8	0.101
	100%	0.0%	0.0%	0.0%	14 %	
Cytoplasmic vacuolation	0	11	18	20	49	0.041*
	0%	100.0%	100.0%	100.0%	86 %	
Pyknosis	0	9	18	20	47	0.037*
	0%	81.8%	100.0%	100.0%	82.5 %	
Nuclear degeneration (Karyolysis)	0	8	14	18	40	0.039*
	0%	72.7%	77.8%	90.0%	70.2%	
Loss of cytoplasm	0	7	14	15	36	0.048*
	0%	63.6%	77.8%	75.0%	63.2 %	
Cytoplasmic degeneration	0	4	11	11	26	0.045*
	0%	36.4%	61.1%	55.0%	45.6 %	

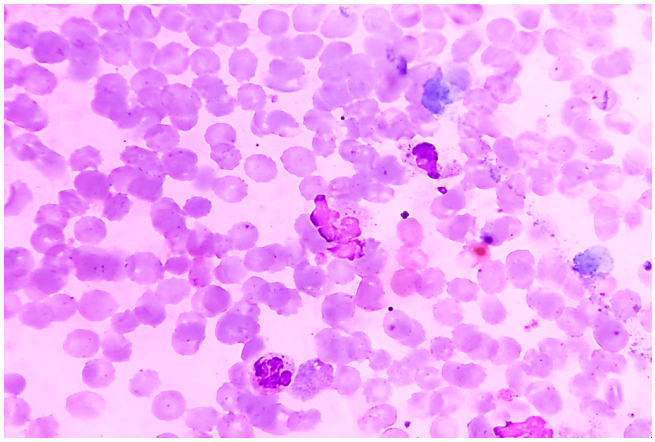

Figure 2: showing microphotograph showing neutrophils with cytoplasmic degeneration and nuclear degeneration (Karyolysis)

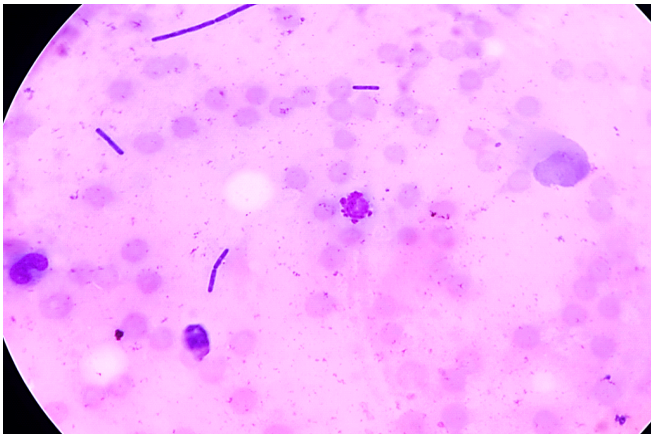

Figure 3: showing microphotograph showing neutrophils with nuclear degeneration (Karyolysis)

were observed by Bardale [8] who observed an almost equal male to female percentage of 75% for male and 25% for female and Ahmad [9] who observed 80 percent for male and 20 percent for female. In the present study 26.3% of cases were from urban locality and 73.7% of cases were from rural locality. This big deviation towards rural locality could be due to a big proportion of population in India are living in villages which falls under rural area.

In the present study, within 6 hrs of time since death, no neutrophils in the samples show degenerative changes. This observation is in concordance with previous studies by Thabet [5], Ahmad [9], Bishnoi [10], Babapule [11], Jat [12] and Bardale [8] who observed almost equal results as our study except some cytoplasmic changes in some neutrophils and lymphocytes in late hrs. In the present study, it was observed that cytoplasmic changes were seen in form

of cytoplasmic vacuolation seen in 86.0% of cases. These changes were observed in all cases examined after 6 hours of time since death.

In present study, degenerative changes in neutrophils (Pyknosis) were observed in 82.5% (47 cases out of 57 cases examined). Within 7-12 hours time since death, 81.8% cases show pyknosis. It is also observed that Pyknosis is observed in all cases with time since death greater than 12 hours.

In present study, overall Karyolysis (nuclear degeneration) was observed in 70.2% (40 cases out of 57 cases examined). No Karyolysis is observed within 6 hours. It varies from 70.2% to 90% from 7 to 24 hours time since death.

In present study, loss of cytoplasm in neutrophils was seen in 63.2% and cytoplasmic degeneration was seen in 45.6% of cases. Although no study matched with the results our above study but it was similar with other studies in the way that in almost all the studies cases with no degenerative changes were of within 6 hrs of time since death and they had more load cases within that period of time as compared to above study.

In present study correlation of time since death with degenerative changes in neutrophils was observed as no changes were seen up to 6 hrs. In time since death between 7-12 hrs, 81.8% were showing pyknosis, 72.7% were showing nuclear fragmentation, 100.0% were showing cytoplasmic vacuolation, 63.6% were showing loss of cytoplasm and 36.4% were showing cytoplasmic degeneration. In time since death between 13-18 hrs, 100% were showing pyknosis, 77.8% were showing nuclear fragmentation, 100.0% were showing cytoplasmic vacuolation, 77.8% were showing loss of cytoplasm and 61.1% were showing cytoplasmic degeneration. In time since death between 19-24 hrs, 100% were showing pyknosis, 90.0% were showing nuclear fragmentation, 100.0% were showing cytoplasmic vacuolation, 75.0% were showing loss of cytoplasm and 55.0% were showing cytoplasmic degeneration. Pyknosis, Nuclear fragmentation, Cytoplasmic vacuolation, Loss of cytoplasm and Cytoplasmic degeneration were significantly more among subjects with Time since death 19-24 hours. Similar results were observed by Bishnoi R[10] who observed almost equal results as no changes were seen within post-mortem interval of 6 hrs. Within duration between 7 to 12 hrs 81.25% of cases were showing dysmorphic changes. In within 12 to 24 hrs of post-mortem interval 100% of cases were showing gross dysmorphic changes. Similar results were observed by Ahmad [9] who observed almost equal results as no changes were seen within post-mortem interval of 6 hrs. Within duration between 7 to 12 hrs 91.66% of cases were showing dysmorphic changes. In within 12 to 24 hrs of post-mortem interval 100% of cases were showing gross dysmorphic changes. Similar results were observed by Thabet [5] in his study who observed almost equal results as no changes were seen within post-mortem interval of 6 hrs and pyknotic changes are seen in 12 to 18 hrs. Similar results were observed by Jat [12] who observed that no degenerative change was observed in time since death within 6 hrs and dysmorphic changes were seen after 6 hrs and severity of dysmorphism increases with time. Similar results were observed by Bardale [8] in his study that changes in neutrophils starts after 4 hrs. Cytoplasmic changes starts after 6 hrs and completely lysed neutrophils are also seen after 18 hrs.

CONCLUSION

The present study suggests that there are significant changes in White Blood Cells and platelets with time since death and these morphological changes in blood cells may add another parameter and may be a complimentary method to the already established methods. It may help in determining time since death in association with other parameters and further studies are recommended.

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