Original Article

Indian Journal of Forensic Medicine and Pathology
Volume 8 Number 3, July - September 2015

Estimation of Postmortem Interval based on Morphological Changes in WBC

W. Sandhya Manohar*, Vennila Vijayasree, Nishat Ahmed Sheikh*****

Abstract

Background: The estimation of time since death by whatsoever means or methods relies on changes that occur in the body after death. The changes in morphology of white blood cells are also variable depending on different factors, like other parameters used for the purpose of determination of TSD but it is less variable as compared to others. In this present study, morphological cellular changes of leukocytes had been taken into consideration, being compared with the changes with post mortem interval, these degenerative changes is being discussed here in this study. *Type of Study:* It's a cross sectional study. *Material & Method:* This prospective study was carried out at department of Forensic Medicine in collaboration with department of Pathology at Kamineni Institute of Medical Sciences, Narketpally January 2014 to December 2014. *Observation and Discussion:* In Lymphocytes identifiable degenerative changes began after 24 hours for both the group, Lymphocytes showed degenerative changes even after 84 hours, but could be easily identified even after that period. Lymphocytes nuclear swelling and indistinctness in cytoplasmic cell membrane were observed after 24 hour, while pyknosis, nuclear fragmentation and disintegration were observed after 36, 72 and 96 hour respectively. Lymphocytes were still identifiable after 120 hour and later observed in H Doghoz study. *Conclusion:* The present morphological observations suggest that the lymphocytes are most resistant blood cells to autolytic changes, the Eosinophils and Monocytes are intermediate and Neutrophils are affected early. Considering the application of these cellular changes to estimate time since death, it can be said that the result discussed thus far seems to lead disparate conclusion.

Keywords: WBC; Morphological Changes; Post Mortem Interval.

Introduction

The estimation of time since death is most important, but it is the most inaccurate and controversial topics in Forensic Medicine [1]. When calculated accurately, it has the potential to unravel many unfolded medico-legal mysteries. The estimation of time since death by whatsoever means or methods relies on changes that occur in the body after death [2]. Irreversible changes occur in the WBCs in the internal environment due to non-availability of oxygen, accumulation of carbon dioxide, PH change and accumulation of toxic products [3].

The changes in morphology of white blood cells are also variable depending on different factors, like other parameters used for the purpose of determination of time since death, but it is less variable as compared to others [4].

Forensic Pathologist continue to rely on age old subjective methods of observing the degree and chronological staging of external as well as visceral postmortem. Somatic changes like cooling of the body, rigor mortis, changes in the eyes, hypostasis, signs of decomposition, mummification, adipocere formation, maggot infestation etc. corroborated with circumstantial evidences for the same. Some clue of time of death is also gathered from the condition of food in stomach, intestine and urine in bladder [5]. Attempts have also been made to determine time

Authors Affiliation: *Associate Professor of Forensic Medicine **Associate Professor of Pathology, Kamineni Institute of Medical Sciences, Narketpally District Nalgonda. State Andhra Pradesh 508254. *** Professor of Forensic Medicine, Peoples college of Medical Sciences & Research center Bhopal.

Reprints Requests: Nishat Ahmed Sheikh, Professor of Forensic Medicine, Peoples College of Medical Sciences & Research Center Bhanpur, Bhopal. 462037. State Madhya Pradesh Country India.
E-mail: drnishatsheikh@gmail.com

passed since death by studying biochemical changes in blood, CSF and intraocular fluids [6, 7].The biochemical methods have been found to be of not much use once the decomposition changes start.

Forensic pathologist throughout the world are trying to establish time passed since death by studying degenerative changes in organs and tissues at different intervals but definitive conclusion is still awaiting [8]. An accurate PMI may aid in the identification of not only the victim, but also a suspect. Although many methods for determining time since death have been proposed, there is still a need to establish more reliable dating techniques.

Evaluation of cellular changes occurring in postmortem period is one of the methods being explored in recent times [9, 10]. Experimental studies have indicated that the cellular changes such as morphological, functional or biochemical could reliably be correlated with time since death [3, 11]. The morphological evaluation of cells in post mortem state is based on an assumption that different tissues and cells do not die at the same time or simultaneously with cardiac or respiratory arrest [12]. In this present study, morphological cellular changes of leukocytes had been taken into consideration, being compared with the changes with post mortem interval, these degenerative changes is being discussed here in this study.

Material and Method

This prospective study was carried out at department of Forensic Medicine in collaboration with department of Pathology at Kamineni Institute of Medical Sciences, Narketpally January 2014 to December 2014. The study comprises of 40 blood samples that were obtained from non-refrigerated cadavers kept at room temperature at Nalgonda General Hospital mortuary. The blood samples of 2 ml each from non refrigerated cadaver (Experimental Group) were collected from the internal jugular vein after giving the autopsy incision and identifying the vein in neck. The samples were collected into sterile glass bottles in which 0.2ml of 10% solution of Ethylene Diamine Tetra Acetic Acid (EDTA) K3 as anticoagulant had been added, with rubber bungs [13]. Blood samples from 60 Hospital patients (Control Group) were collected from their vena cava cubuiti, stored with same procedure labeled numerated and were kept at 22-28^{0}C room temperature. The blood samples from the cadavers were stored in vitro storage while a blood smear was prepared prior to in vitro storage with a label of the given postmortem interval. The blood smears of cadaveric group had been prepared immediately after being drawn, and labeled according to the time of death of the cadavers. The findings are compared with the findings of the same in vitro period of control group. Subsamples are taken from the blood samples of both groups after 0, 3, 6, 9, 12, 18, 24, 36, 48, 60, 72, 84, 96, and 120 h of storage, and peripheral blood films of both the experimental and control groups were prepared by staining Leishman's stain with standard procedure.

The peripheral smears were examined under oil immersion 1000X Objective lens, microscope in an area of 1 cm X 2 cm which was presumed to contain morphological changes in leukocytes have been recorded. The stained smears and findings were cross checked by consultant hematologist and Pathologist. The Morphological cell changes, cell membranes, cytoplasm and changes in the nucleus was studied. In Experimental group the time of death was known and the time of sample collection at autopsy was known, providing the death interval. The results were shown in tables and figures.

Inclusion Criterion

Samples from both group i.e. experimental and control group was taken from the age group of 18 to 44 years ages, experimental group cadavers whose exact time of death was known was collected with various interval of time since death up to 36 hours.

Exclusion Criterion

Samples from both group experimental and control group where any history of oncological, hematological or infectious diseases were excluded from the study.

Ethical and Legal Consideration

Sufficient permissions and consent were procured from District Coordinator of Health Services, General Hospital Nalgonda and clearance from the Institutional Ethical committee was obtained in advance.

Observation and Discussion

Table 1: Time since death and changes in WBC of Experimental group (Cadaveric Blood)

No of Cases	PM Hrs	Neutrophils	Eosinophils	Monocyte	Lymphocyte
		P C N D	P C N D	P C N D	P C N D
6	0-3	- - - -	- - - -	- - - -	- - - -

7	4-8	+ + - -	+ - - -	+ - - -	- - - -
5	9-12	+ + + -	+ + + -	+ + - -	- - - -
4	13-16	+ + + -	+ + + -	+ - - -	- - - -
6	17-20	+ + + -	+ + + -	+ + - -	- - - -
5	21-24	+ + + +	+ + + +	+ + + -	- - - -
4	25-30	+ + + +	+ + + +	+ + + -	+ - - -
3	31-36	+ + + +	+ + + +	+ + + +	+ + - -

Table 2: Morphological changes of Leukocytes of both experimental and control group samples.

Sr. No	Cell Types	Cellular Changes	Post mortem Interval (Time in Hours)
1.	Neutrophils	Pyknosis	4-8 Hrs
		Cytoplasmic and Nuclear Vacuolation	4-8 Hrs
		Nuclear Fragmentation	9-12 Hrs
		Disintegration	24-60 Hrs
2.	Eosiniphils	Pyknosis	4-8 Hrs
		Cytoplasmic and Nuclear Vacuolation	9-12 Hrs
		Nuclear Fragmentation	9-12 Hrs
		Disintegration	24-60 Hrs
3.	Monocytes	Pyknosis	4-8 Hrs
		Cytoplasmic and Nuclear Vacuolation	9-12 Hrs
		Nuclear Fragmentation	21-24 Hrs
		Disintegration	36-48 Hrs
4.	Lymphocytes	Nucleus swollen, Indistinct cell membrane	25-30 Hrs
		Pyknosis	31-36 Hrs
		Nuclear fragmentation	40- 48 Hrs
		Disintegration	60-84 Hrs

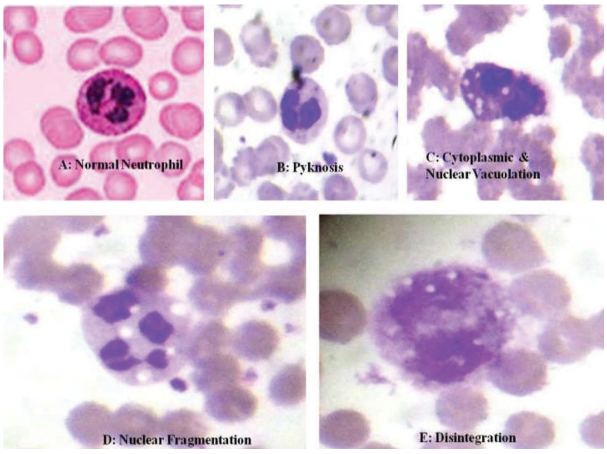

Fig. 1: Neutrophil Degenerative Morphological changes

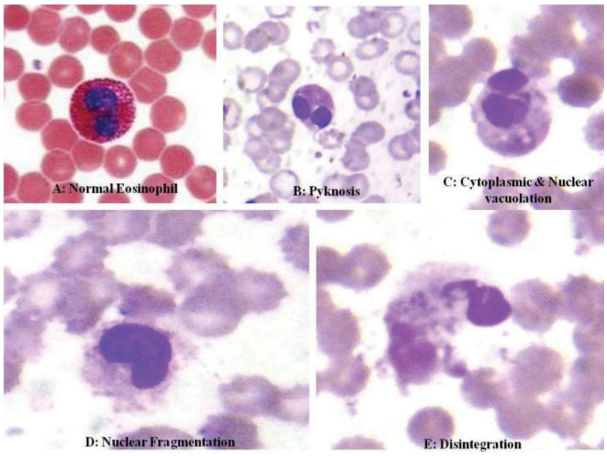

Fig 2: Eosinophil Degenerative Morphological changes

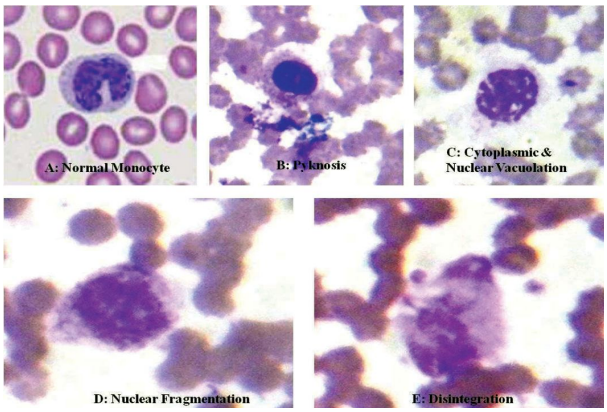

Fig. 3: Monocyte Degenerative Morphological changes

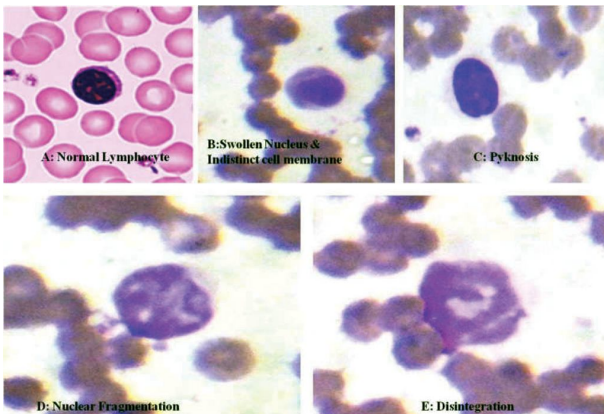

Fig 4: Lymphocyte Degenerative Morphological changes

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 environment is due to non-availability of oxygen, accumulation of carbon dioxide, pH change and accumulation of toxic products. The changing process through normal morphology to un-Identification period can be a useful criterion for estimating post mortem interval [14]. In Neutrophil identifiable degenerative changes were seen at 4 hours for both the groups and unidentifiable beyond 60 hours. In Eosinophil identifiable degenerative changes were seen at 4 hours for both the groups and it was unidentifiable beyond 60 Hours. In Monocytes identifiable degenerative changes were seen at 4 hours for both the groups and it was unidentifiable beyond 48 hours. In Lymphocytes identifiable degenerative changes began after 24 hours for both the group, Lymphocytes showed degenerative changes even after 84 hours, but could be easily identified even after that period. Similar morphological changes were seen in both control and experimental groups while examining peripheral smears prepared from the blood samples from both groups. Basophils were not taken into account, reason because of their rarely observance in peripheral smears study.

H Doghoz [13] observed pycnosis after the first 6 hour and cytoplasmic and nuclear vacuolation after 12 hour in neutrophils, eosinophils and monocytes. Nuclear fragmentation started after 18 hour in neutrophils and eosinophils and after 24 hour in monocytes. Disintegration was observed between 48 and 96 hour in neutrophils and between 48 and 72 hour in eosinophils and monocytes. In lymphocytes nuclear swelling and indistinctness in cytoplasmic cell membrane were observed after 24 hour, while pycnosis, nuclear fragmentation and disintegration were observed after 36, 72 and 96 hour respectively. Lymphocytes were still identifiable after 120 hour and later.

Babapullae and Jayasundera [3] reported that the cells of the blood samples which they examined for 144 hour were normal in first 6 hour, were normal and abnormal between the first 6 and 72 hour and were all abnormal and difficult to identify in Morphology. Nishat A Sheikh et al [15]. A Definite formula was evolved with potassium in relation to time since death, Yt=5.28+2.20 (K) there exist a significant positive co-relation for potassium ion in relation to time since death and definite equation could be evolved for computation of postmortem interval.

Wyler D [16] et al found that the postmortem cell count in cerebrospinal fluid correlates to the time after death and can be described mathematically (Polynomial curve of third order). Penttila A, Lahio K [9] 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. Platt et al [17] in their study of cerebrospinal fluid cells found that if the post-mortem duration is greater than 12 hour, the cells become vacuolated and cannot be identified.

Conclusion

There are several limitations to the present study and includes small sample size and non inclusion of pediatric age group. Another limitation is that the study include cadaver that were kept at room temperature only and no attempt was made to study the changes in respect to bodies kept at room temperature and stored in cold storage. Similarly no attempt had been made to study under varying climatic conditions. In spite of these limitations, the present study has certain advantages as blood lies relatively secure place, the procedure is simple and need usual laboratory material. From these preliminary results, at present the procedure can be utilized as useful supplementary procedure to estimate death interval.

The studies on the morphological and chemical assessment of the postmortem interval have revealed that the speed of all postmortem changes varies widely in dependence of the conditions of storage during the postmortem interval. It should be noted that, it is quite difficult to report the exact time of death because of many factors. The present morphological observations suggest that the lymphocytes are most resistant blood cells to autolytic changes, the Eosinophils and Monocytes are intermediate and Neutrophils are affected early. Considering the application of these cellular changes to estimate time since death, it can be said that the result discussed thus far seems to lead to disparate conclusion. However, meaningful inference can be obtained by considering the features of all cells in combination. Thus, it can be a useful supplementary procedure to estimate postmortem interval.

Acknowledgement

Authors acknowledge the immense help received from the scholars whose articles are cited and included in references of this manuscript. The authors

are also grateful to authors / editors / publishers of all those articles, journals and books from where the literature for this article has been reviewed and discussed.

Conflict of Interest

No conflict of interest involved so ever.

Source of Funding

The article does not have any funding issue involved in its generations.

References

1. Naumann H.N. Postmortem. Liver Function Tests. Am. J. Clin. Pathol. 1956; 26: 495-505.
2. Modi JP and Subramanium B. V. Medical Jurisprudence & Toxicology. 22nd edition, Butterworths Publication, 1998; 246.
3. Babapulle CJ, Jayasundera NPK .Cellular changes and time since death. Med Sci Law 1993; 33: 213-22.
4. Binay Kumar Determination of Time since Death from Changes in Morphology of White Blood Cells in Ranchi, Jharkhand. J Indian Acad Forensic Med. April-June 2014; 36(2): 184-187.
5. Taylor s principles and practice of medical jurisprudence. Edited by Simpson K, 1965; 70-103.
6. Coe J I. Post mortem chemistry on blood with particular reference to urea, nitrogen, electrolytes & bilirubin.1973; 18: 33-42.
7. Cumps, F.E. Gradwahl s Legal Medicine. 3 rd ed. Bristor John Wright & Sons Ltd, 1979; 78-98.
8. Berenbaum M.C. Bone marrow after death. J. Clinical Path 1956; 9: 381.
9. Penttila A, Laiho K. Autolytic changes in blood cells of human cadavers. II Morphological studies. Forensic Sci Int. 1981; 17: 121-132.
10. Bardale R, Dixit PG. Evaluation of cellular changes in blood of Human cadaver to estimate time since death. Medico-legal Update 2007; 7: 35-39.
11. Nishat A Sheikh et al. Study of sodium & Glucose levels in Cadaveric Synovial Fluid to estimate post-mortem interval Indian Journal of Forensic medicine and Pathology. July – December 2008; 1(3 / 4): 81-85.
12. Laiho K, Penttila A. Autolytic changes in blood cells and other tissue cells of human cadavers-Viability and ion studies. Forensic Sci Int. 1981; 17: 109-120.
13. Dokgoz H, Arican N, Elmas I, Fincanci SK. Comparison of Morphological changes in white blood cells after death and in vitro storage of blood for the estimation of post mortem interval. Forensic Sci Int. 2001; 124: 25-31.
14. Henssge, B. Knight, T. Krompecher, B. Madea, L. Nokes, The estimation of Death in the Early Postmortem Period. Edward Arnold, London, 1995; P.224.
15. Nishat A. Sheikh. Estimation of postmortem interval according to time course of potassium ion activity in cadaveric synovial fluid. Indian Journal of Forensic Medicine & Toxicology. July – December 2007; 1(1) : 45-49.
16. Wyler D, Marty W, Bar W. Correlation between the post-mortem cell content of cerebrospinal fluid and time of death. Int J Legal Med. 1994; 106(4): 194-9.
17. Platt M S et al. Postmortem cerebrospinal fluid pleocytosis. Am J Forensic Med Pathol 1989; 10: 209-12.