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CORRELATION OF CELLULAR AUTOLYTIC CHANGES IN BONE MARROW WITH POST-MORTEM INTERVAL

BIRADAR GURURAJ[1], *BAKKANNAVAR SHANKAR M[2], G PRADEEP KUMAR[3], BHAT SHAILA[4], NAYAK VINOD C[2]

[1]Assistant Professor, Department of Forensic Medicine and Toxicology, Vijaynagar Institute of Medical Sciences, Bellary, India.
[2]Associate Professor, [3]Professor, Department of Forensic Medicine and Toxicology, Kasturba Medical College, Manipal University, Manipal, India.
[4]Professor, Department of Pathology, Melaka Manipal Medical College, Manipal University, Manipal, India.
*Corresponding author email: shankar.mb@manipal.edu

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ABSTRACT

Estimation of post mortem interval is of great importance in both civil and criminal disputes. Determining time since death is extremely difficult and accuracy can never be met. Most of the methods currently employed are temperature based algorithms, rigor mortis, livor mortis, thanato-chemistry etc. But the uncertainties attaching to traditional means of establishing the time since death have directed attention to the chemical changes in the body fluids like cerebrospinal fluid and vitreous humour along with various cellular changes in the body tissues. The study of the viability and morphological changes seen in the cells of various body tissues could provide useful information regarding post-mortem interval. In our study the cellular and cytoplasmic changes observed were time related up to 16 hours.

KEYWORDS: post mortem interval; cellular changes; cytoplasmic changes.

INTRODUCTION

Estimation of time since death (post mortem interval) is one of the most important and complex objective of a medico-legal autopsy. Determination of post mortem interval is an important tool in both civil and criminal disputes [1]. Determining post mortem interval is extremely difficult and accuracy is almost unattainable because of numerous factors[2] Most of the methods currently employed are temperature based algorithms, rigor mortis, livor mortis, thanato-chemistry etc. These methods are still subject to considerable inaccuracy. But the uncertainties attached to traditional means of establishing the time since death have directed attention to the chemical changes in the body fluids like cerebrospinal fluid and vitreous humour along with various cellular changes in the body tissues.

"Whatever method was adopted to calculate the estimated time since death from, all the variable factors must be taken into account to modify any basic formula, though this adjustment was very arbitrary and can only be attempted in the light of previous experience. It must be used to construct a "bracket of probability". The application of scientific methods to correlate time since death has proved to be unrewarding [3].

"No problem in forensic medicine has been investigated as thoroughly as that of determining the time since death on the basis of post mortem findings. Apart from its obvious legal importance, its solution has been so elusive as to provide a constant intellectual challenge to workers in many sciences. In spite of the great effort and ingenuity expended, the results have been meagre"[3].

The morphology & cytochemistry of the hematopoietic cells in the bone marrow of cadavers could provide useful information regarding post-mortem interval that motivated us to take up this study.

The main aim of the study is to correlate the cellular changes that occur in the bone marrow with the respective postmortem interval. To attain this aim the following objectives were framed

- *To study the various types of cells found in the bone marrow after death*
- *To study the morphological changes due to the autolysis and depletion of the cells*
- *To correlate the cellular changes in the bone marrow with the post mortem interval*
- *To correlate the cellular changes of our study with the existing literature.*

METHODOLOGY:

This prospective study was conducted in the department of Forensic Medicine, Kasturba Medical College, Manipal, South India, from 2011 to 2012. During the study period 100 autopsy cases were included in the study. The cases with history of hypothermia, cases of burns and mal nourishment were excluded from the study as these conditions can affect the cellular morphology.

The cadavers were preserved in cold chamber at 0 – 4^0 C in the mortuary of department of Forensic Medicine. The regular postmortem examination was conducted according to Letuelle's technique, during which the sternum was detached. The sternum thus obtained was cut vertically in the midline with the help of an electric saw and divided into two halves. Using a 10 ml syringe along with the needle (0.8 x 38 mm), bone marrow was aspirated from the manubrium or first or second parts of the sternum, due to the abundance of marrow in these areas[3]. Then aspirated bone marrow material was put on frosted slides and a smear was prepared. The smeared slides were air dried and stained with Leishman stain [4, 5].

The stained slides were observed under compound microscope in low power followed by high power and in oil immersion field to see the cells and cellular changes. In low power the cellularity was noted followed by observing the same in high power. Then it was observed under oil immersion 100x field for differential count. The cell count was done by using zig – zag method [5]. One thousand cells were counted per slide and various parameters like cell count, cell morphology, cell autolysis and cell depletion were noted [5, 6]. At least change in one cell was considered positive change among 1000 counted cells. These cellular changes in the bone marrow were compared with the post mortem interval.

RESULTS

The present study comprises of 100 cases autopsied at Department of Forensic Medicine, Kasturba Medical College, Manipal for a period of 2 years. All the cases have been studied to look for the changes occurring in the bone marrow after death and correlated with the available postmortem interval.

Time related changes in morphology were observed in erythroid, myeloid/granulocyte and megakaryocytic cells. The cellular morphological changes were observed in all the cells as postmortem interval increased.

In the present study, the bone marrow samples were distributed according to the gender and age wise as depicted in the Table 1.

Table 1. Gender and age wise distribution of cases

Age (Years)	Male	Female	Total
1 – 10	3	0	3
11 – 20	5	3	8
21 – 30	17	8	25
31 – 40	17	4	21
41 – 50	17	1	18
51 – 60	10	1	11
61 and above	11	3	14
	80	20	100

In our study, among the 1000 cells counted per slide of one hundred samples, the comparison with the mean cell count among the three lineages was as depicted in Table 2.

Table 2. Comparison of the mean cell count. (Mann Whitney U test)

Parameters	Mean	SD	Minimum	Maximum
Erythroid cell count	531.93	75.66	389.00	867.00
Myeloid Cell count	453.08	72.06	173.00	602.00
Megakaryocyte Cell count	16.12	11.15	00.00	43.00

The mean erythroid cell count was maximum, accounting for 531.93, followed by the mean myeloid series which was 453.08 and the mean megakaryocyte cell count accounted 16.12 respectively.

In the present study, no appreciable changes in the cellular morphology were detected in the bone marrow during the first 5 hours after death. After this period, the autolytic changes were seen in the cells which are shown in the Table 3.

Table 3. Cellular changes and Postmortem interval

PMI (hrs)	Cases	Erythroid (Nucleus)	Erythroid (Cytoplasm)	Myeloid (Nucleus)	Myeloid (Cytoplasm)	Megakaryocyte (Nucleus)	Megakaryocyte (Cytoplasm)
<5	-	-	-	-	-	-	-
5-7	4	-	+	-	+	-	+
7-9	10	+	+	+	+	+	+
9-11	6	+	+	+	+	+	+
11-13	19	+	+	+	+	+	+
13-15	17	+	+	+	+	+	+
>16	44	-	-	-	-	-	-

Based on our observations, the various autolytic cellular changes were graded as follows (Figure 1 – 6):

Figures with legends:

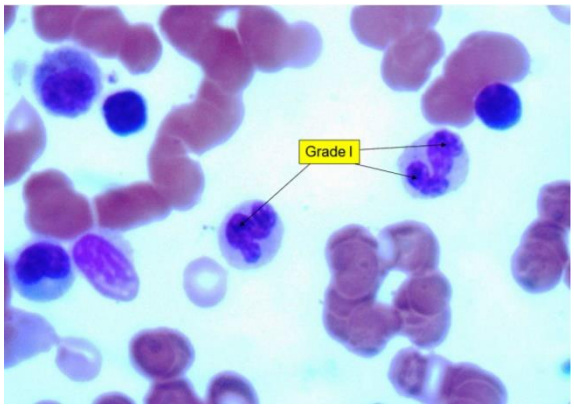

Figure 1. Nuclear change (Grade I)

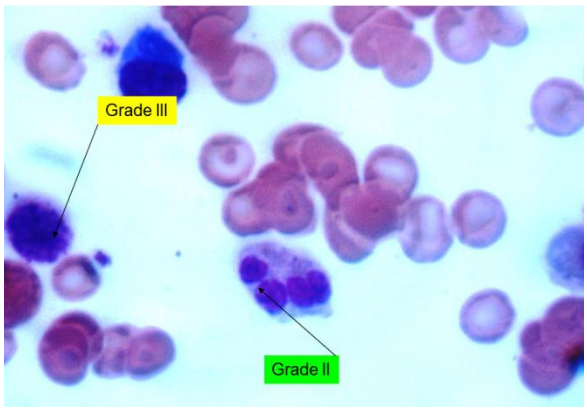

Figure 2. Nuclear change (Grade II & III)

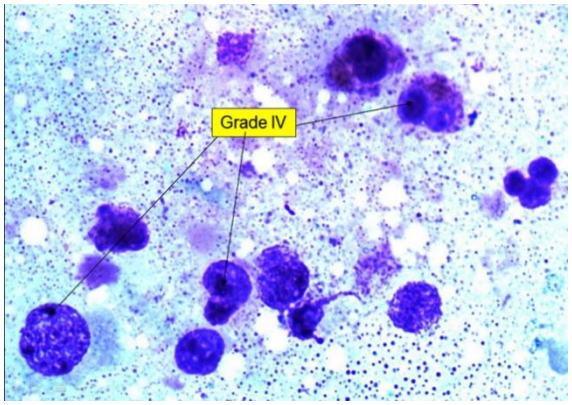

Figure 3. Nuclear change (Grade IV)

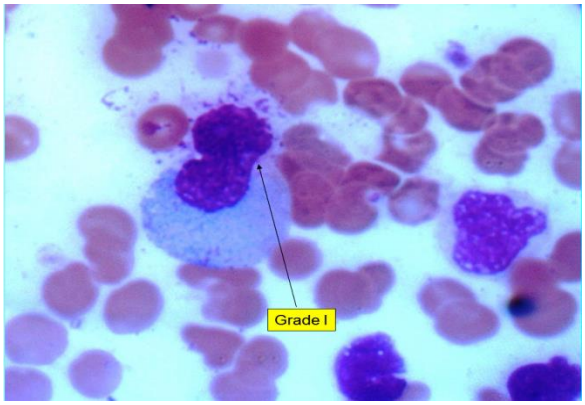

Figure 4. Cytoplasmic change (Grade I)

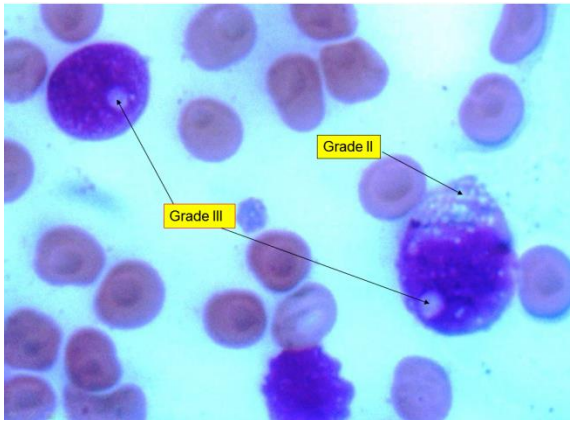

Figure 5. Cytoplasmic change (Grade II & III)

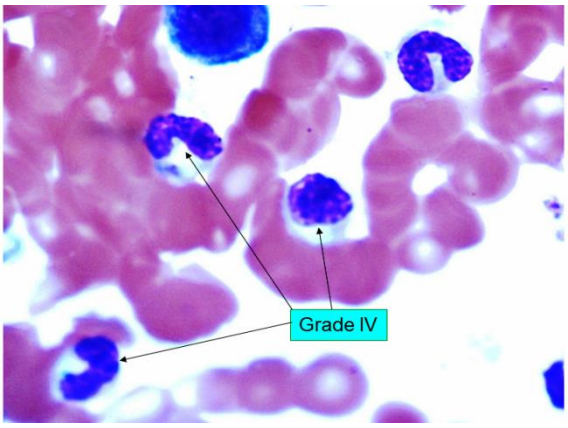

Figure 6. Cytoplasmic change (Grade IV)

Nuclear changes:

Grade I: Multilobulation of the nucleus

Grade II: Budding of the nucleus

Grade III: Syncytium of the nucleus

Grade IV: Nucleus dispersed as debris.

Cytoplasm changes:

Grade I: Break in the cytoplasmic membrane

Grade II: Small vacuoles

Grade III: Large vacuoles

Grade IV: complete loss of the cytoplasm

Among the total of 100 cases, there were 4 cases which lie in the postmortem interval of 5 - 7 hours, showed the cytoplasmic change (Grade I) without any nuclear change.

At 7 – 9 hour postmortem interval Grade I & II nuclear changes as well as cytoplasmic changes (Grade II & III) were evident in 10 cases.

At 9 – 11 hours postmortem interval Grade II & III nuclear changes and Grade III cytoplasmic changes was observed in 6 cases.

The nuclear and cytoplasmic changes were of grade III at 11 – 13 and 13 – 15 hours after postmortem in 36 cases.

After 16 hours, the complete loss of cytoplasm and nuclear debris were observed (Grade IV) which was seen in 44 cases.

STATISTICAL ANALYSIS

Statistical analysis was done by using a non-parametric test, Mann Whitney U test as no other test could be applied, because we had to compare with only two groups (changes present in the cells and not present in the cells) and the parameters observed were not normal in distribution. The present values in the study showed statistically significant result with a P value of 0.002 with Mann Whitney U test.

Table 4. Comparison of mean postmortem interval with the cellular changes. (Mann Whitney U test)

Parameters		Postmortem interval		p-value
		Mean	SD	
Erythroid Nuclear	Present (n=63)	15.28	14.41	-
	Not present (0)	-	-	
Erythroid Cytoplasm	Present (n=59)	15.85	14.73	0.002
	Not present (4)	7.00	0.82	Sig
Myeloid_nuclear	Present (n=63)	15.28	14.41	-
	Not present (0)	-	-	
Myeloid cytoplasm	Present (n=59)	15.85	14.73	0.002
	Not present (4)	7.00	0.82	Sig
Megakaryocyte nuclear	Present (n=63)	15.28	14.41	-
	Not present (0)	-	-	
Megakaryocyte cytoplasm	Present (n=59)	15.85	14.73	0.002
	Not present (4)	7.00	0.82	Sig

As evident from the statistical analysis, the mean postmortem interval for nuclear changes for all the lineages was 15.28 hours. The mean postmortem interval for cytoplasmic changes for all the lineages was 15.85 hours. Nuclear changes were not so evident till 5 – 7 hours, but break in the cytoplasm membrane

and vacuolation of the cytoplasm was visible starting from 5th to 16th hours (Table 4). In 37 cases, the cellular lines were totally not appreciable due to complete autolytic changes.

DISCUSSION

The purpose of this study is to evaluate cellular changes that occur in the bone marrow to investigate the relationship between these findings and the postmortem interval elapsed in the cases that were autopsied at department of Forensic Medicine, KMC, Manipal. The results were correlated with time of death as stated in the police inquest report of the circumstances surrounding the death.

Our study differs with that of Findlay. A. B[6]. He reported earliest change appreciated by him was that of nucleus of erythroid lineage at the time of 1-2 hour postmortem, in the form of budding. By 2-3 hour postmortem more pyknotic cells along with budding of nucleus was appreciated and by more than 3 hour postmortem multilobulation of the nucleus was seen. Among granulocytes no change was appreciated in the time interval of 1-4 hour postmortem. Later neutrophil began to lyse in few cells by 5-6 hour postmortem. By 6 – 9 hour postmortem neutrophil lysis was observed in many cells and at 9 – 12 hour postmortem advanced neutrophil lysis was observed. Among neutrophil myelocytes no change was appreciated, up to 7 hour postmortem. Then there was early myelocyte lysis by 7 – 8 hour postmortem. Later by 8 – 12 hour postmortem myelocyte lysis was observed in many of the cells. By more than 12 hour postmortem appreciable myelocyte lysis was observed.

In our study the earliest change in the cell was of that of cytoplasm in the form of vacuolation and break in the continuity of it, and the time taken for this to occur was 5-7 hour postmortem. This change was appreciable in all the erythroid, myeloid and megakaryocyte cells. As the time passed from 7 – 10 hour postmortem, nuclear changes were observed in the form of multilobulation and budding of nuclei. Advanced or severe lysis in both nuclear as well as cytoplasm, among all the three cell lineage was in between 11 – 16 postmortem. Complete loss of cellular morphology was observed at a time interval of more than 16 hours postmortem.

The differences in observations regarding findings about the cellular and cytoplasmic changes between our study and Findlay AB can be attributed the change in environmental conditions, population group, nutritional status and probably the study period. More and more studies in various parts of the world are warranted to evaluate these factors.

According to the studies reported by Stuart B. Hoffmann[7], the polymorph nuclear granulocytes began to manifest a significant decrease, dropping to ¾ of their original level at 3 hour postmortem. At 5 hour approximately ½ to ¾ of the polymorphonuclear granulocytes remained; at 7 hours less than ¼ were identified and at 13 to 15 hours only occasional ones were identified. The band cells manifested the same decrease, but they lagged behind the polymorphonuclear granulocytes by 1 to 3 hour in most instances. There was a wide variation among individual cases, but the general trend was obvious enough to be apparent, even without the differential counts. As more mature cells began to decrease, the less mature cells from the metamyelocyte through the myeloblast exhibited a slight increase at 8 – 12 hour postmortem, after which the metamyelocytes also began to decrease. It was impossible to detect a corresponding decrease of progranulocytes and the blast cells up to 15 hour postmortem. According to Rohr and Hafter the earliest changes in the polymorphonuclear leukocytes were seen at 1 hour postmortem. The nuclei began to swell, became homogenous and cloudy, and lost their nuclear membranes. At one and half hour the cytoplasm became progressively vacuolated. A significant number of ruptured cell membranes were first seen at two to two and half hours. After 10 hour postmortem the majority of the remaining and recognizable polymorphonuclear appeared as syncytium. Similar changes appeared in the more immature granulocytic cells at longer periods after death[7,8].

It must be pointed out that the observation of progressive autolysis of the erythroblasts and granulocytes is a subjective exercise, the final impression being a composite of numerous

microscopic fields. Because the cytological changes are sequential, there may be occasions when more than one bone marrow aspiration would be helpful to assist in estimation of time of death. One of the main advantages of the technique adopted by us was its simplicity. There is no need for the Forensic pathologist to have technical assistance at the time of postmortem examination. He could prepare the marrow smears himself. To ensure standard conditions however, it would be desirable that marrow be aspirated as described rather than be removed from an exposed marrow cavity. Trauma caused to the bone marrow cells during the opening procedure and also the possible contamination of the bone marrow with the non-isotonic fluids such as tap water during autopsy may sometimes invalidate the results [6,7,8].

Since the autolytic pattern did not vary much in our study, it would appear that the Sternal bone marrow is successfully shielded from seasonal variation in the temperature at least in the tropical climate of Manipal.

CONCLUSION

Very few studies have been done in the past regarding the study of cellular changes after death which can be correlated with the post mortem interval. The pyknotic erythroid, myeloid and megakaryocyte series as well as the cytoplasmic changes in the bone marrow show time related changes which gave some indication of the postmortem interval up to 16 hours after death in our study. Similar studies around the world are warranted in this field before the cellular changes could be considered in estimating the time since death.

CONFLICT OF INTEREST

None declared.

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