Estimation of time since death from nuclei changes of bone marrow cells

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ABSTRACT

Introduction: *Estimation of time since death (post mortem interval) from chemical changes in the body fluids & cellular changes in the body tissues.*
Objective: *To correlate the cellular nuclei changes those occur in the bone marrow with the respective postmortem interval.*
Methodology: *aspirated bone marrow from sternum was stained with Leishman stain were observed under compound microscope in low power followed by high power and in oil immersion field to see the cells and cellular nuclei changes.*
Results: *At 7 – 9 hour postmortem interval Grade I & II nuclear changes were evident in 10 cases. At 9 – 11 hours postmortem interval Grade II & III nuclear changes was observed in 6 cases. The nuclear changes were of grade III at 11 – 13 and 13 – 15 hours after postmortem in 36 cases. After 16 hours, the complete losses of nuclear debris were observed (Grade IV) which was seen in 44 cases.*
Conclusion: *Nuclei changes in the bone marrow show time related changes which gave some indication of the postmortem interval up to 16 hours after death.*

Keywords: *Post mortem interval, bone marrow cells, Leishman stain, oil immersion field*

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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. Determining post mortem interval is extremely difficult and accuracy is almost unattainable because of numerous factors[1]. 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 humor 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[2].

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 present study concluded that the pyknotic erythroid, myeloid and megakaryocyte series as well as the cellular nuclei changes in the bone marrow show time related changes which gave some indication of the postmortem interval up to 16 hours after death

MATERIALS AND METHODS

The following materials were used for the purpose of this study:

- Bone marrow from sternum of cadavers
- Frosted slides
- 10 ml syringes with needles (0.8 X 38 mm – Dispovan Needle)
- Leishman stain (Merck's company)
- Staining rack
- Muslin cloth
- Tap water
- Electronic Compound microscope
- Marker pen

METHODOLOGY

The cadavers were preserved in cold chamber at $0 – 4^0$ C in the mortuary of department of Forensic Medicine, Kasturba Medical College, Manipal University, Manipal. The regular postmortem examination was conducted according to Letuelle's technique, during which the sternum was detached from its attachments and separated out.

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]. Frosted slides were cleaned with spirit on both the sides and post mortem number was labeled using a marker pen. Aspirated bone marrow material was put on frosted slides and a smear was prepared using another slide. Two slides were prepared for each bone marrow sample.

The smeared slides were air dried and stained with Leishman stain[3,4]. The slides were placed on the staining rack facing up and 8-10 drops of Leishman's stain was poured on them just to cover the slide and left for 2 minutes. During this period the alcohol in the stain fixes the cells (fixation time). Double the amount of distilled water (16 – 20 drops) was added on the smear, with the help of dropper, taking care that the water did not spill from the slide. The stain was properly mixed and water was evenly blown with the help of a pipette. A metallic shiny layer was formed on the top of this mixture. It was left for 8-10 minutes when staining occurs (staining time). Staining time was adjusted according to the reaction of the stain. The staining time is reduced if the slide is over stained, and it is increased if it is poorly stained. The stain was poured off and slide was washed under tap water. It was made sure that the stream of water did not directly fell on the smear. All the water adhering to the slide was cleaned by shaking and set the slide in an upright position to dry[4]. Using muslin cloth the back of the stained slide was cleaned to clear the back ground. 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 100 x fields for differential count. The cell count was done by using zig – zag method[4]. One thousand cells were counted per slide and various parameters' like cell count, cell morphology, and cell autolysis and cell depletion were noted[4, 5]. These cellular changes in the bone marrow were compared with the post mortem interval.

RESULTS AND OBSERVATION

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 as depicted in the Table no. 1.

In the present 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 no. 2 and Graph I

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 no. 3. Based on our observations, the various autolytic cellular nuclei changes were graded as follows (Fig. no 1 – 4):

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.

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

At 7 – 9 hour postmortem interval Grade I & II nuclear changes were evident in 10 cases.

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

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

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

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 also not normal in distribution, if we had three groups then Kruskal Walli's ANOVA test or student T test could be used. The present values in the study

showed statistically significant result with a P value of .002 with Mann Whitney U test (Table 4)

As evident from the statistical analysis, the mean postmortem interval for nuclear changes for all the lineages was 15.28 hours. Nuclear changes were not so evident till 5 – 7 hours

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

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

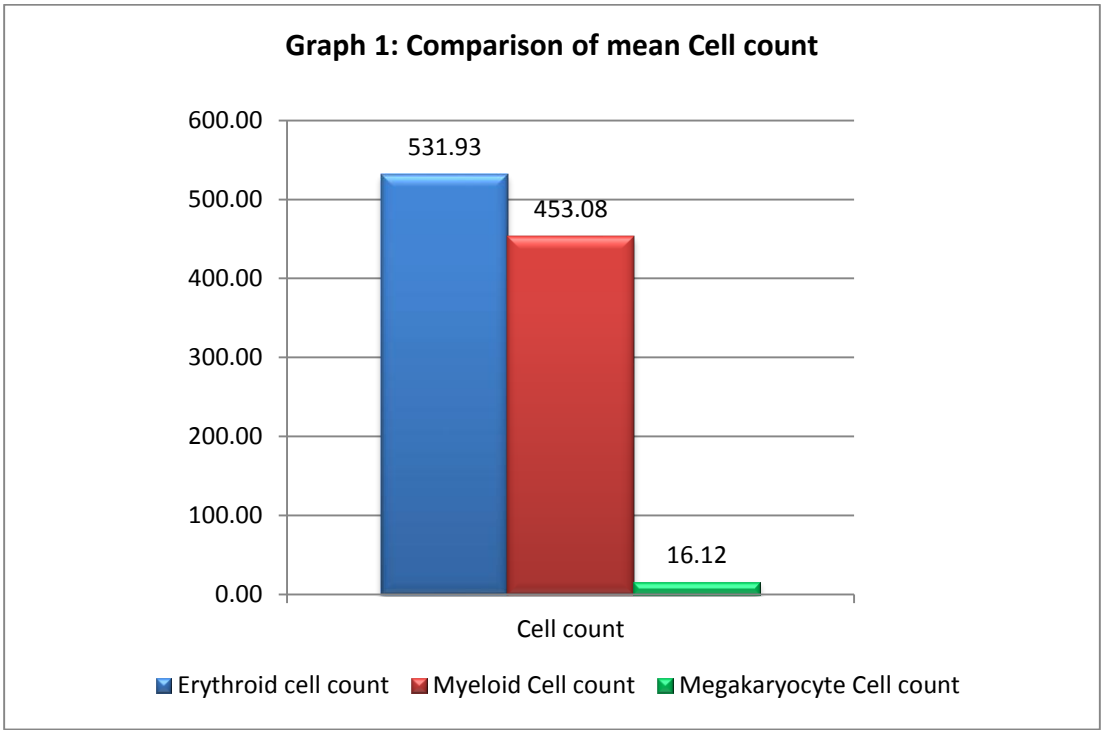

Table 3: Cellular nuclei changes and Postmortem interval

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

Table 4: Comparison of mean postmortem interval with the cellular nuclei changes. (Mann whitney u test)

parameters		Postmortem interval		p-value
		Mean	SD	
Erythroid_Nuclear	Present (n=63)	15.28	14.41	-
	Not present (0)	-	-	
Myeloid_nuclear	Present (n=63)	15.28	14.41	-
	Not present (0)	-	-	
Megakaryocyte_nuclear	Present (n=63)	15.28	14.41	-
	Not present (0)	-	-	

DISCUSSION

The purpose of this study is to evaluate cellular nuclei 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 tallies with that of Findlay. A. B[5]. 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, from 7 – 10 hour postmortem, nuclear changes were observed in the form of multilobulation and budding of nuclei. Advanced or severe lysis in nuclear, 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.

According to the studies reported by Stuart B. Hoffmann, 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 polymorpho-nuclear 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 hr postmortem. The nuclei began to swell, became homogenous and cloudy, and lost their nuclear membranes. After 10 hour postmortem the majority of the remaining and recognizable polymorpho-nuclear appeared as syncytium. Similar changes appeared in the more immature granulocytic cells at longer periods after death.[6,7]

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[5,6,7].

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

CONCLUSION

The pyknotic erythroid, myeloid and megakaryocyte series as well as the nuclei changes in the bone marrow show time related changes which gave some indication of the postmortem interval up to 16 hours after death.

CONFLICTS OF INTEREST

There is no conflict of interest since we have not gained any financial or any funds from any Agency

ETHICAL CONSIDERATION

The study was conducted after obtaining institutional ethical committee clearance. Study was autopsy based and fully anonymous. Personal details were not revealed.

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