A Histological Study of Some Important Visceral Organs for Estimating Time Since Death

Kapoor AK*, Sinha US, Pathak YK***, Singh Sanju******

* Professor & Head, Dept. of Pharmacology, MLN Medical College, Allahabad, UP
** Professor & Head, Dept. of Forensic Medicine, MLN Medical College, Allahabad, UP
***Ex-Lecturer, Dept. of Forensic Medicine, MLN Medical College, Allahabad, UP
****Senior Resident, Department of Hospital Management, A.I.I.M.S. New Delhi.

ABSTRACT

Histological changes following death follow a characteristic fixed pattern and these can be utilized to estimate time since death within a definite time brackets as is reflected by a high percentage of positive cases conforming to definite brackets. A clear-cut approximation in time brackets following death can be arrived at with some adjustment for seasonal variations. In the present study, histological changes occur much earlier than reported by earlier workers in the field. Moreover, histological changes in the visceral organs (heart and liver) may offer an improvement over other conventional methods for estimating time since death.

INTRODUCTION

The accurate estimation of time since death is an onerous duty of autopsy surgeon in all medico-legal cases concerned with life or death of accused involved.

Various physical methods such as rigor mortis[1,2], cooling of cadavers[3,4], etc, chemical and biochemical methods such as changes in intraocular fluid [5,6], enzymatic estimation[7,8] etc, decomposition changes, histopathological changes[9-14] and pupillary reaction to drug[15] have been tried for an accurate estimation of time since death. Since a variety of external factors including environmental and climatic conditions, cause and place of death, age, sex, built, nutrition, nature of clothing etc, can influence the above changes hence only a broad estimate of time since death can be arrived at within a reasonable wide range of time.

Rigor mortis because of its easy detection and simple demonstration has been used an indicator. Modi[2] has observed that is India rigor mortis usually starts within 1-2 hrs after death and may take 2-3 hrs to develop and its duration is 24-48 hrs in winter and 18-35 hrs in summer.

Cooling of Cadavers has also been considered by a number of worders[3,4,16] as an important indicator for estimating time since death but has limitation pertaining to constants and no universal formula has been floated.

Kapoor and Saksena[15] have observed that a positive pupillary reaction (miosis) with drugs is quite valuable for ascertaining a post mortem interval within 24 hrs of death. Chemical changes observed in various body fluids like blood, spinal fluid, aqueous and vitreous humour of the eye may also help to estimate time since death[5,6]. Garg et al[17] have reported that potassium concentration in vitreous humour increased in a linear fashion up to 104 hrs time since death.

Although Modi2, Boyd[18], Anderson[19], and have mentioned about degenerative changes occurring after 24 hrs, 72 hrs, 96 hrs and 7 days following death in different organs of the body yet these

authors have not described the time of onset of various degenerative changes. Klier et al.[20] have noted that immunohistochemical evidence of caspase 8 can be used for detection of damaged tissue as caspase 8 participate in different stages of apoptosis. Thus, caspase 8 activity has been described in heart muscle, liver and skeletal muscles.

Very few workers[9-13] have tried to explore histological features at different time intervals to accurately determine the time since death. Janseen[14] and Chandra et al.[13] have studied histological changes in various visceral organs e.g. cardiac tissues, skeletal muscles, Kidneys, liver and spleen and have tried to correlate these findings with estimation of time since death.

The aim of the present study is to observe histological changes following death in various visceral organs and to correlate these changes with actual estimation of time since death.

MATERIAL AND METHODS

The present study included a total of 100 properly selected dead bodies brought to the mortuary of S.R.N. Hospital attached to M.L.N. Medical College, Allahabad for medico-legal postmortem examination. Cadavers of either sex belonging to different age groups, of different strata of the society, and both from the urban and rural areas were taken into consideration. Mostly hospital deaths, where time of death was precisely clear, were preferred so that time of death could be correlated with histological changes.

Those bodies where the cause of death was burn, drowning or poisoning or where body was severely mutilated decomposed or in which parts of the body were not intact were excluded for the purpose of study. Prior to collection of samples, a record of age, sex, mode of death, seasonal variation and stage of rigor mortis was kept. Stage of rigor mortis was used as a parameter for corroborating the results of histology for estimating time since death. Presence or absence of

stage of rigor mortis was recorded under following heads. (a) Started, (b) fully developed, (c) partially passed off, (d) passed off completely.

Further a record of maximum and minimum temperature as well as humidity was also maintained during the study. It was observed that temperature in summer ranged between 35^0C to 45^0C, in rainy season it ranged between 25^0C to 35^0C whereas in winter season temperature ranged between 18^0C to 25^0C.

The present study included almost equal number of cases in each season namely summer, rainy and winter was purposely done to rule out discrepancies due to seasonal variations.

For histological examination approximately one inch of the tissue from the heart and liver were dissected. Due care was exercised to cut macroscopically healthy tissue from the myocardium and liver tissue from both lobes. Tissues were collected in four wide mouthed glass tubes and were fixed in 10% formaldehyde. The paraffin embedded tissues were sectioned at 3-5 microns thickness and were studied by a pathologist. The following stains were utilized e.g. Haematoxylin and Eosin staining for detailed histological study, Gorden and Sweet's Silver impregnation stain for visualization of reticulin fibre in the liver and Periodic acid Schiff's staining for visualization of autolytic structural changes in liver epithelium.

RESULTS

A season wise breakup of dead bodies in different age groups and sex distribution was depicted in Table -1. The maximum number of 46 cases (46%) were in the age group of 21-40 yrs and least number of 3 cases (3%) were in the elderly age group of 71-80yrs. Males were 62 and female 38 with male/female ratio of 1.63:1.

Table -2: Depicted the mode of death and their season wise breakup. Maximum numbers of cases were accidents 44% followed by homicide cases 42% and non-medicolegal cases were only 6%.

Distribution of cases as per actual time since death in different seasons was presented in table -3. Maximum number of 25 cases (25%) belonged to 19-24 hrs bracket followed by 22% in 25-48hrs and minimum 7% belonged to the period where death occurred beyond 72 hrs.

Distribution of cases as per stages of rigor mortis and actual time since death was presented in table-4. In 31 cases (31%) rigor mortis was fully developed whereas in 29 cases (29%) it passed off. In only 2 cases (2%) it did not start.

Distribution of cases as per stages of rigor mortis and actual time since death in the summer, rainy and winter seasons was presented in table 5,6 and 7. In summer a fully developed rigor mortis was observed in 8 cases (24.24%), in 12 cases (36.37%) it passed off, whereas in 1 case (3.03%) it did not start. In rainy season, rigor mortis was fully developed in 11 cases (32.35%) whereas in 9 cases (26.47%) it passed off. In winter season, rigor mortis was fully developed in 12 cases (36.37%) whereas it passed off in 8 cases (24.24%) and in 1 case (3.03%) rigor mortis did not start.

Of the 100 cases, selected initially for the purpose of study, 6 cases on histological considerations were further excluded based on the following criteria; in liver tissue 3 cases exhibited cirrhosis and 1 case exhibited marked fatty changes, and in myocardial tissue 2 cases showed marked ischaemia and did not reveal clear cut structural findings. Thus, the present study comprised of histopathological findings of only 94 cases and their season-wise breakup included summer 31 cases, rainy 32 cases and winter 31 cases. It may be mentioned that those cases where histological finding of tissue corresponded to tabular details as per time bracket were

labeled as +ve cases and where histological findings were variable not conforming to relevant time brackets were labeled as -ve cases.

Table-8: Depicts histological changes in the cardiac tissue in different seasons as per actual time since death. It was visualized that during first 6 hrs, no light microscopic changes were detected during all of the three seasons. During 7-12 hrs since death, beginning of pyknosis and hyperchromatism in the nuclei of muscle cell fibres was well marked during summer and rainy seasons, whereas in winter season though pyknosis was seen, but hyperchromatism in the nuclei of muscle cell fibre was less marked. During 13-18 hrs since death fragmentation of heart muscle fibres was seen in summer, rainy as well as winter seasons. During 19-24 hrs, intensification of nuclear pyknosis and fragmentation of more muscle fibres were seen during summer. In rainy season, intensification of nuclear pyknosis and fragmentation of some muscle fibres were seen whereas in winter season, intensification of nuclear pyknosis and fragmentation of only few muscle fibres were observed, thus fragmentation of maximum muscle fibres were noticed during summer season.

During 25-48 hrs since death there was diffuse increase in number of fragmented fibres both in summer and rainy seasons however in winter months diffuse increase was not seen in number of fragmented fibres. During 49-72 hrs there was a partial loss of transverse and longitudinal striations in all three seasons. In cases in which more than 72 hrs had elapsed since death in all the three seasons there was loss of transverse and longitudinal striation, lysis of muscle fibres with homogenization. It may be clearly inferred that post mortem histological changes in the heart appeared beyond 6 hrs, these were early in summer season followed by rainy season and were slightly delayed in winter season, though beyond 48 hrs following death a similar pattern of histological changes were observed in all the three seasons.

It was observed that out of 31 cases in the summers 29 cases (93.55%) exhibited histological changes as per Table-9 and were labeled as +ve cases whereas 2 cases (6.45%) were -ve cases. Similarly out of 32 cases in rainy season, 30 cases (93.75%) were +ve and 2 cases (6.25%) were -ve whereas in winter months out of 31 cases, 28 cases (90.32%) were +ve and 3 cases (9.68%) were -ve.

Table-9: Displays histological changes occurring in the liver at different post mortem intervals in all three seasons. During the initial 6 hrs since death light microscopic changes were not detected in all the three seasons. During 7-12 hrs, breaking of reticulin fibres started in all the three season. During 13-18 hrs since death distension of nuclei of hepatocyte were well marked both in summer and rainy seasons, whereas in winter season distension of nuclei of hepatocyte were only recognizable. During 19-24 hrs, both in summer and rainy seasons, structure of elastic fibres was markedly altered whereas structure of elastic fibres was slightly altered in winter. During 25-48 hrs since death no other changes were visualized both in the summer and rainy seasons whereas in the winters, structure of elastic fibres were markedly altered. During 49-72 hrs most of the reticulin and elastic fibre were broken in all the three seasons. Beyond 72 hrs since death the cell margins and nuclei of hepatocyte were not visible, reticulin fibres and other elastic fibres were not detectable and the nuclei of Kupffer's cells were pyknotically condensed during summers. In rainy season, the cell margins of hepatocytes were not visible and nuclei almost disintegrated, reticulin fibres and other elastic fibres were not detectable. The nuclei of Kupffer's cell were pyknotically condensed. In the winter season the cell margin of hepatocytes were not visible but nuclei were still recognizable to some extent. The reticulin fibres and elastic fibres were not detectable and the nuclei of Kupffer's cell were pyknotically condensed.

It can be clearly deciphered that qualitatively the histological changes follow the same pattern throughout in the all the three seasons but the onset of these changes were slightly delayed in winter months. It was observed that in summer 28 cases (90.32%) were +ve and 3 cases (9.68%) were -ve, in rainy season 29 cases (90.63%) were +ve whereas in winter 29 cases (93.55%) were +ve.

DISCUSSION

In all medico legal cases, the accurate estimation of times death is of vital importance since it concerns the life or death of criminal accused. The determination of exact time of death is fairly difficult to clinch as various changes occurring in the body following death are influenced by numerous factors.

Physical methods for estimation of tine since death provide only an "estimate" with in a reasonable wide range of time. Cooling of cadaver is studded with limitations pertaining to constants and no universal formula has yet been arrived at. Apart from enzymatic studies, chemical changes particularly estimation of K^+ in vitreous humour helps in estimation of time since death up to 72 hrs of post mortem.

Human tissues and organs are decomposed and broken down in a certain sequence. Thus, the post mortem interval can be determined from degree of decomposition found. Histological changes in various interval organs have been studied both in human beings as well as in animal [9-13]. Histological findings from the heart, liver, kidneys and skeletal muscles have been utilized for estimating time since death[14]. In this randomly selected study histological changes in the dead bodies were studied in the heart and liver at different time intervals and in different seasons and these changes were then correlated with actual time since death. Attempts were also made to

correlate the stages of rigor mortis with histological changes and to assess the impact of seasonal variation on histological changes.

A maximum of 46% of cases were in the age group of 21-40 years and accidental and homicidal cases together accounted for 86% of cases.

The present study indicated that rigor mortis gave only a rough idea of time since death up to 24 hrs. When it was in stage of start it corresponded to postmortem interval of 6 hrs; fully developed rigor mortis indicated post mortem interval of 12 hr, partially passed off rigor mortis corresponded to post mortem interval of 18 hrs in summer and rainy seasons and 24 hrs in winter season. Completely passed of rigor mortis testified a post mortem interval of more than 24 hrs in summer and rainy seasons and more than 36 hrs in winter season. In the present study rigor mortis passed off in 29 cases, existed in 69 cases (summer 20 cases, rainy 25 cases and winter 24 cases) and did not start in 2 cases.

Regarding various histological changes occurring in cardiac tissues at different time intervals in different seasons, it was observed that histological changes in heart provided a reliable estimation up to 72 hrs postmortem and the changes appeared early in summer as compared to winter. It was also observed that during first 6 hrs no light microscopic changes were present, during 7-18 hrs post mortem, changes appeared early in summer and rainy seasons. During 25-48 hrs since death, a diffuse increase in number of fragmented fibre was observed both in summer and rainy seasons but not in winter. Our findings are in agreement with those of Nicolas et al.[21] However, these authors did not mention seasonal variations in histological changes of cardiac tissue.

Regarding histological changes in the liver, it was observed that during the first 6 hrs, no light microscopic changes were detectable, during 7-12 hrs, breaking of reticulin fibres started and

these were common in all three seasons. In an experimental study on rats and in human beings Chandra et al.[9] reported that in both rat and human bodies no significant changes were noticed during first 13 hours since death. These findings did not corroborate those of ours. Moreover, these authors did not observe seasonal variations. Further, Mueller et al.[22] in an experimental study in guinea pig liver observed that distension of nuclei of liver cell occurred 6 hrs post mortem. In the present study it occurred during 13-18 hrs post mortem in human beings and that up to 24 hrs histological changes were similar in summer and rainy seasons, and were delayed in winter.

Chandra et al[9] observed cloudy swelling at 14 hrs in both rats and human beings, at 17 hrs the lobular pattern was disturbed and Kupffer's cell started showing signs of degeneration, at 20 hrs hepatocytes began degenerating and were found arranged irregularly in both rats and human beings. At 23 hrs, chromatolysis in nuclei of hepatocyte and vacuolation in cytoplasm of hepatocytes were noticed and Kupffer's cell disappeared completely in both rats and human beings. Their findings were at variance with those of ours.

Chandra et al.[9] observed no further histological changes at 36 hrs and that liver tissue become very soft and it was not possible to prepare sections. This study reports no other visual changes during 25-48 hrs but we were able to process the tissue further. Thus during 49-72 hrs and beyond pattern of histological changes in all the three seasons were same. Moreover, Mueller[22] observed that after 4 days of post mortem the cell margins were no longer visible and nuclei to some extent were still recognizable, nucleoli had almost disintegrated. The reticulin fibres were no longer detectable and nuclei of Kupffer's cells were pyknotically condensed. In the present study these changes were observed after 3 days of post mortem probably due to environmental conditions prevailing here. Further Mueller[22] did not mention anything about seasonal variation

in respect to histological changes. Furthermore, it was observed that classical changes as listed in the table were observed in 28 cases (90.32%) out of 31 cases in summer, 29 cases (90.63%) out of 32 cases in rainy season and 29 cases (93.5%) out of 31 cases in winter season.

In this study no difference was observed between male and female liver tissue. Our findings are in conformity with those of Chandra et al[9] who also reported that no difference has been noticed between male and female liver tissues in cases of both rat and human beings. In brief, the reliability of histological changes in liver tissue for an accurate establishment of time since death upto 3-5 days was a matter of variance, though an approximate idea of time since death could be achieved.

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Table No.1 : Season wise distribution of Cases as per Age

S.No.	Age (Yrs)	Summer	Rainy	Winter	Male	Female	Total (%)
1.	1-10	3	2	2	4	3	7
2.	11-20	8	4	5	10	7	17
3.	21-30	9	10	7	15	11	26
4.	31-40	4	11	5	14	6	20
5.	41-50	3	4	9	9	7	16
6.	51-60	2	2	2	4	2	6
7.	61-70	2	-	3	3	2	5
8.	71-80	2	1	-	3	0	3
Total		**33**	**34**	**33**	**62**	**38**	**100 (100%)**

Table No. 2 : Season wise distribution of Cases as per Mode of Death

S.No.	Mode of Death	Summer	Rainy	Winter	Total (%)
1.	Accidental	11	21	12	44
2.	Suicidal	4	2	2	8
3.	Homicidal	15	10	17	42
4.	NonMedicolegal	3	1	2	6
Total		**33**	**34**	**33**	**100 (100%)**

Table No.3 : Distribution of Cases as per actual time since death in different seasons

S.No.	Actual time since death (hours)	Summer	Rainy	Winter	Total (%)
1.	0-6	3	3	2	8
2.	7-12	5	4	5	14
3.	13-18	6	3	5	14
4.	19-24	5	10	10	25
5.	25-48	7	9	6	22
6.	49-72	4	3	3	10
7.	> 72	3	2	2	7
Total		**33**	**34**	**33**	**100 (100%)**

Table No.4 : Distribution of Cases by stage of Rigor Mortis in different seasons

S.No.	Stage of rigor mortis	Summer	Rainy	Winter	Total No.	(%)
1.	Not started	1	0	1	2	2
2.	Started	6	5	6	17	17
3.	Fully developed	8	11	12	31	31
4.	Partially passed off	6	9	6	21	21
5.	Passed off	12	9	8	29	29
Total		**33**	**34**	**33**	**100**	**100 (100%)**

Table No. 5 : Distribution of Cases by stage of Rigor Mortis and actual time since death in summer season

		Actual Time since death								
S.No.	**Stage of Rigor mortis**	**0-6 hrs**	**7-12 hrs**	**13-18 hrs**	**19-24 hrs**	**25-48 hrs**	**49-72 hrs**	**> 72 hrs**	**Total No.**	**%**
1.	Not started	1	-	-	-	-	-	-	1	3.03
2.	Started	2	3	1	-	-	-	-	6	18.18
3.	Fully developed	-	2	4	2	-	-	-	8	24.24
4.	Partially passed off	-	-	1	3	2	-	-	6	18.18
5.	Passed off	-	-	-	-	5	4	3	12	36.37
Total		**3**	**5**	**6**	**5**	**7**	**4**	**3**	**33**	**100%**

Table No.6 : Distribution of Cases by stage of Rigor Mortis and actual time since death in rainy season

		Actual Time since death								
S.No.	**Stage of Rigor mortis**	**0-6 hrs**	**7-12 hrs**	**13-18 hrs**	**19-24 hrs**	**25-48 hrs**	**49-72 hrs**	**> 72 hrs**	**Total No.**	**%**
1.	Not started	-	-	-	-	-	-	-	0	0
2.	Started	3	2	-	-	-	-	-	5	14.71
3.	Fully developed	-	2	2	5	2	-	-	11	32.35
4.	Partially passed off	-	-	1	5	3	-	-	9	26.47
5.	Passed off	-	-	-	-	4	3	2	9	26.47
Total		**3**	**4**	**3**	**10**	**9**	**3**	**2**	**34**	**100%**

Table No. 7 : Distribution of Cases by stage of Rigor Mortis and actual time since death in winter season

		Actual Time since death								
S.No.	**Stage of Rigor mortis**	**0-6 hrs**	**7-12 hrs**	**13-18 hrs**	**19-24 hrs**	**25-48 hrs**	**49-72 hrs**	**> 72 hrs**	**Total No.**	**%**
1.	Not started	1	-	-	-	-	-	-	1	3.03
2.	Started	1	4	1	-	-	-	-	6	18.18
3.	Fully developed	-	1	3	7	1	-	-	12	36.37
4.	Partially passed off	-	-	1	3	2	-	-	6	18.18
5.	Passed off	-	-	-	-	3	3	2	8	24.24
Total		**2**	**5**	**5**	**10**	**6**	**3**	**2**	**33**	**100%**

Table No. 8 : Time Since Death and Histological changes in the heart in different seasons

S.No.	Time since death	Summer season n = 31 (100%)	Rainy season n = 32 (100%)	Winter season n = 31 (100%)
1.	0-6 hrs	No light microscopic changes were detectable	No light microscopic changes were detectable	No light microsopic changes were detectable
2.	7-12 hrs	Beginning of pyknosis and hyperchromatism in the nuclei of muscle cell fibres were well marked	Beginning of pyknosis and hyperchromatism in nuclei of mucsle cell fibres were well marked	Pyknosis is started but hyperchromatism in nuclei of muscle cell fibres were less marked
3.	13-18 hrs	Fragmentation of heart muscle fibres were started	Fragmentation of heart muscle fibres were started	Fragmentation of heart muscle fibres were started
4.	19-24 hrs	Intensification of nuclear pyknosis and fragmentation of more muscle fibres were seen	Intensification of nuclear pyknosis and fragmentation of some muscle fibres were seen	Intensification of nuclear pyknosis and fragmentation of few muscle fibres were seen
5.	25-48 hrs	Diffuse increase in number of fragmented fibres	Diffuse increase in number of fragmented fibres	More muscle fibres were fragmented
6.	49-72 hrs	Partial loss of transverse and longitudinal striation	Partial loss of transverse and longitudinal striation	Partial loss of transverse and longitudinal striation
7.	> 72 hrs	Loss of transverse and longitudinal striation. Lysis of muscle fibres with homogenization were well marked	Loss of transverse and longitudinal striation. Lysis of muscles fibres with homogenization were well marked	Loss of transverse and longitudinal striation. Lysis of muscle fibres with homogenization were well marked
8.	+ve cases	29 (93.55%)	30 (93.75%)	28 (90.32%)

Table No. 9 : Time Since Death and Histological changes in liver in different seasons

S.No.	Time since death	Summer season n = 31 (100%)	Rainy season n = 32 (100%)	Winter season n = 31 (100%)
1.	0-6 hrs	No light microscopic changes were detectable	No light microscopic changes were detectable	No light microsopic changes were detectable
2.	7-12 hrs	Breaking of reticulin fibres started	Breaking of reticulin fibres started	Breaking of reticulin fibres started
3.	13-18 hrs	Distension of nuclei of hepatocytes were well marked	Distension of nuclei of hepatocytes were well mared	Distension of nuclei of hepatocytes were recognizable
4.	19-24 hrs	Structure of elastic fibres were markedly altered	Structure of elastic fibres were markedly altered	Structure of elastic fibres were slightly altered
5.	25-48 hrs	No other changes were visible	No other changes were visible	Structure of elastic fibres were markedly altered
6.	49-72 hrs	Most of reticulin fibres and elastic fibres were broken	Most of reticulin fibres and elastic fibres were broken	Most of reticulin fibres and elastic fibres were broken
7.	> 72 hrs	The cell margins and nuclei of hepatocytes were not visible. Reticulin fibres and other elastic fibres were not detectable. The nuclei of kupffer's cells were pyknotically condensed.	The cell margins of hepatocytes were not visible and nuclei were almost disintegrated, Reticulin fibres and other elastic fibres were not detectable. The nuclei of Kupffer's cells were pyknotically condensed.	The cell margin of hepatocytes were not visible but nuclei were still recognizable to some extent. The reticulin fibres were not detectable. The nuclei of Kupffer's cells were pyknotically condensed.
8.	+ve cases	28 (90.32%)	29 (90.63%)	29 (93.35%)