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Original Research Article

Estimation of Post-Mortem Interval using Morphological Changes in Human Kidney Collecting Tubules

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Abstract:

Background and Objectives: The histological changes in the Collecting tubules were examined at various postmortem intervals in the deceased human. In these research histological changes of tissue after death is influenced by P.M.I. atmospheric temperature and humidity, external and internal factors.

Materials and Methods: This study was carried out, 40 cases of deceased kidney sample in between the temperature 17.3/22.3- 31.3/45^{0}C, humidity 11/36 to 75/95 and duration range 4hrs to 52.30hr. The collecting tubules were examined to establish their correlation ship with hours PMI., temperatures, humidity or time since death by studied histological (H& E, PAS staining) in Patna medical college and Hospital Patna Bihar.

Conclusion: Retraction of epithelium, disruption with individualization of cells, nuclear pyknosis, karyolysis and loss of tubular architecture with debris in the lumen were observed in collecting tubules. These criteria's presented in this study could be used to determine the time after death.

Keywords: Collecting tubules, P.M.I. (Post mortem interval), Medullary rays, Interstitium.

Introduction

The histological changes in the collecting tubules were examined at various postmortem intervals in the deceased human. This was because the postmortem changes were inevitable and varies depending on a number of intrinsic and extrinsic factors. A very precise but unreliable result might mislead and alienate the police investigations. A variable number of changes based on duration and circumstance of demise occur in the body after death[2]. Collecting tubules which join distal convoluted tubules to collecting ducts. They begin in the cortex which extends toward the medulla in medullary rays. In the medulla inner zone straight tubules join at acute angle to from the terminal papillary duct of Bellini. At tips of medullary pyramids, papillary ducts perforate the renal papilla to form the area cribrosa. Collecting tubules shows a large lumen, diameter gradually increases, lining cells bulging into the lumen from a simple epithelium, cuboidal to low columnar. Each cell has a round, central nucleus and pale cytoplasm. Peritubular capillaries are a capillary network supplied by the efferent arteriole is located around the PCT and DCT of the nephron. Vasa recta are Sets of parallel arterioles and venules supply and drain the capillary plexus around the loops of Henle and collecting ducts in the medulla[3]. Cells die by the process of enzymatic digestion. Dissolution of cells and tissues becomes apparent microscopically[4]. Estimation of time since death is one of the most important object of postmortem examination. Time passed since death continues to be a major problem for the forensic pathologist and its determination plays an important and vital issue in medico legal cases because of the fact that forensic experts are very often required to answer questions relating to time of death in the courts of law[5]. Determination of time since death is not only important in criminal cases but also in civil cases where they have legal implications in issue of insurance and inheritance[6,7]. 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[8,9]. The traditional methods of examination the time since death based on naked eye observations of the gross changes in a deceased occurring after death to provide a rough approximation of post mortem interval, the histological changes in kidney after death have been studied in various animals but yet very few studies have been done in human kidney. That's why this current study is being carried out with this hope that it will be helpful for estimation of time after death

which is very critical and one of the most important job for a forensic expert.

Materials and Methods

This study was carried done in Patna medical college and Hospital Patna Bihar. Collection of 40 cases kidneys sample of deceased, taken after all consent to relatives and done all procedure of ethic in institution. The Collecting tubules examined and to establish their correlation ship with hours PMI. , temperatures, humidity or time since death by studied histological (H& E, PAS staining). This research was done in between temperature 17.3/22.3-31.3/45^{0}C,humidity between 11/36 to 75/95 and duration range was between 4hrs to 52.30hr. We included, the exact time of death of individual should be known not suffering from any disease affecting kidney. The exact time of death of individual should be known. We Excluded, unknown time of death, suffering from kidney disease, those preserve in ice or ice cooler, metabolic disorders.

Results

Postmortem H& E and PAS staining histological changes 40 Human kidneys samples at different time intervals between 4hrs to 52.30hr., in this research increase the rate of postmortem histological changes in collecting tubules were found to be increased with rise in the temperature and duration. There were following significant changes observed.

1. STUDY NO: Post mortem interval (PMI)- 4 hrs Temperature- 27.5/42.2ºC, humidity-14/41% - CT (Collecting tubule)- disruption of epithelium with dark stained nuclei. Prominent interstitium in the medulla. PAS+ small spots are visible in the intestitium of medulla.
2. STUDY NO: PMI -5 hrs Temp- 18.1/34.6 ºC humidity- 20/42%, CT- retraction & disruption of epithelium with dark stained nuclei, debris in the lumen.
3. STUDY NO: PMI 6hrs Temp- 18.5/33.3^{0}C humidity-41/72%, CT- disruption of epithelium and retraction of epithelium are present at places with peripheral dark stained nuclei as well as dark stained nuclei.
4. STUDY NO: PMI 6.20 hrs Temp- 25.5/31ºC humidity 85/87%, CT-disruption of epithelium at places with peripheral dark stained nuclei as well as dark stained nuclei.
5. STUDY NO: PMI 7.35 hrs Temp -26.8/37.1°C humidity- 41/71%, CT- disruption and retraction of epithelium with vesicular nuclei mostly.
6. STUDY NO: PMI 8.30hrs Temp- 9.8/26.3ºC humidity-33/65%, CT- retraction and disruption of epithelium with vesicular, dark stained nuclei, individualization of cells at places, cellular debris is present in the lumen of some tubules. PAS+ small spots are present in the intestitium of medulla.
7. STUDY NO: PMI 12.30 hrs Temp- 24.5/38.1ºC humidity- 22/37%, CT- retraction and disruption of epithelium have vesicular &dark stained nuclei.
8. STUDY NO: PMI 12.30hrs Temp- 28.4/44.2ºC humidity- 16/27%, CT - retraction &disruption of epithelium with vesicular & dark stained nuclei.
9. STUDY NO: PMI 13hrs Temp- 23.9/38.7ºC humidity-31/70%, CT- retraction and disruption of epithelium, dark stained as well as vesicular nuclei are present, at places individualization of cells.
10. STUDY NO: PMI 13hrs Temp- 23.9/38.7ºC humidity-41/72%, CT- epithelium completely disrupted at places but maintained architecture of CT are also present in some places with vesicular and dark stained nuclei, some epithelial cells are enucleated.
11. STUDY NO: PMI 13hrs Temp- 25.5/31ºC humidity-85/87%, CT- retraction and disruption of epithelium with vesicular &dark stained nuclei, in the intestitium red blood cells are present.
12. STUDY NO: PMI 13hrs Temp- 8.2/23.9ºC humidity-26/83%, CT retraction and disruption of epithelium with vesicular nuclei. Red blood cells are present in the intestitium of medulla.
13. STUDY NO: PMI 13.10hrs Temp- 27.3/42.2ºC humidity- 24/52%, CT- dilated retraction & disruption of epithelium with vesicular nuclei mostly.
14. STUDY NO: PMI 13.10hrs Temp- 27.3/42.2ºC humidity- 24/52%, CT- retraction and disruption of epithelium with vesicular nuclei but enucleated epithelial cells are also present.
15. STUDY NO: PMI 13.30hrs Temp- 29.6/43.1ºC humidity- 23/38%, CT- retraction and disruption of epithelium with dark stained nuclei. PAS+ debris in the lumen of CT.
16. STUDY NO: PMI 16hrs Temp- 11/23.7ºC humidity-44/77%, CT retraction & disruption of epithelium with vesicular nuclei almost, at places cellular debris is present in the lumen.
17. STUDY NO: PMI 16hrs Temp- 24.6/38.1ºC humidity-36/46%, CT- retraction and disruption of epithelium with vesicular nuclei but enucleated epithelial cells are also present. Prominent intestitium in the medulla.
18. STUDY NO: PMI 16hrs Temp- 27.5/42.2ºC humidity-14/41%, CT retraction and disruption of epithelium with dark stained nuclei but enucleated at places.
19. STUDY NO: PMI 16.30hrs Temp- 21/39ºC humidity-11/36%, CT- dilated, epithelium retracted and disrupted with dark stained nuclei.
20. STUDY NO: PMI 17hrs Temp- 12.8/23.5ºC humidity-44/82%, CT- retraction and

disruption of epithelium with vesicular as well as dark stained nuclei. Red blood cells are present in the intestitium of medulla.

21. STUDY NO: PMI 17.30hrs Temp- 27.3/42.2ºC humidity- 24/52%, CT- retraction and disruption of epithelium have vesicular nuclei.
22. STUDY NO: PMI 17.30hrs Temp- 27.5/42.2ºC humidity- 14/41%, CT- retraction and disruption of epithelium with dark stained nuclei. Debris present in the lumen.
23. STUDY NO: PMI 17.30hrs Temp- 18.6/28.2ºC humidity- 79/98%, CT- retraction and disruption of epithelium with dark stained nuclei at places, debris present in lumen. Basement membranes of CT are PAS+.
24. STUDY NO: PMI 19hrs Temp- 27.3/41.4ºC humidity-18/37%, CT –retraction and disruption of epithelium with dark stained nuclei.
25. STUDY NO: PMI 19hrs Temp- 19.5/35ºC humidity-18/35%, CT- retraction and disruption of epithelium with dark stained as well as vesicular nuclei.

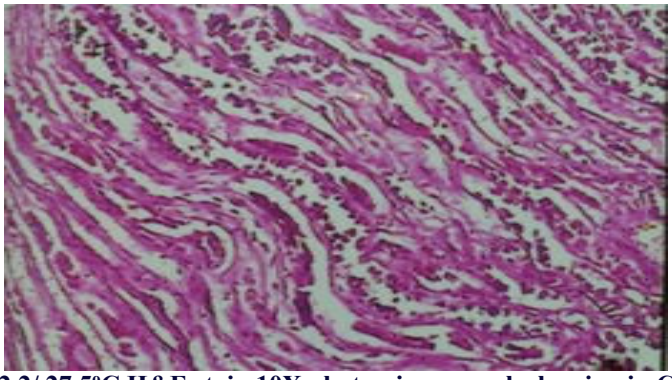

Figure 1: 4hr temp – 42.2/ 27.5ºC H&E stain 10X photomi crograph showing in CT (collecting tubule) – disruption of epithelium. Interstitium (I) prominent in the medulla.

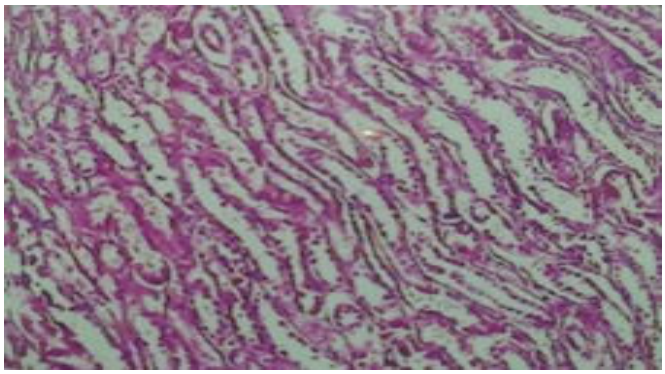

Figure 2: 13hr temp – 23.9/ 38.7ºC H&E stain 10X photo micrograph showing in CT (collecting tubule) – retraction of epithelium and individualization of cells at places.

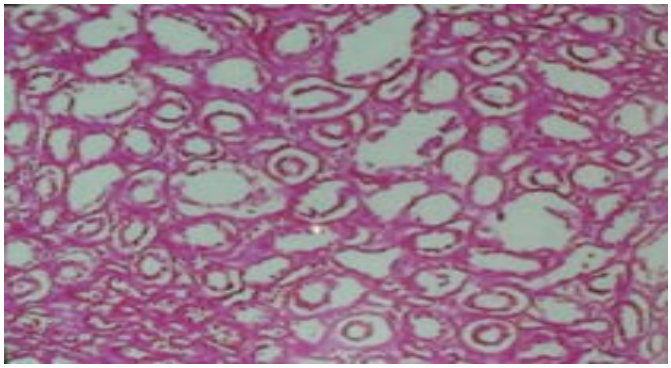

Figure 3: 16hr temp – 42.2/ 27.5ºC H&E stain 10X photo micrograph showing in CT (collecting tubule) – retraction of epithelium

Discussion

Postmortem decomposition follows the arrest of biochemical process that develops, maintains, and preserves the integrity of cellular element. During decomposition, the tissue components leak and break up releasing hydrolytic enzyme. Decomposition may vary from individual to individual, from

environment to environment and even from one part of body to another part of same individual. Process of autolysis was temperature dependent and occurs earlier in some tissue which has high level of hydrolytic enzymes such as the pancreas whereas delayed in fibrous tissue such as uterus or skeletal muscles which have fewer amounts of hydrolytic enzymes and few lysosomes. In current research postmortem histological changes in collecting tubules of kidney were observed in 40 cases of different age and sex. Deborah Barber found in porcine kidney after 3 Hours at 4ºC, epithelium of collecting tubules were slightly retracted off basement membrane. After 3 Hours 24ºC, CT-moderate numbers of CT epithelial cells started retracting off basement membranes, pyknosis was noted occasionally, individualization of sloughed cells was seen within a few tubules. In the current research after 4hrs postmortem interval (PMI) at temperature (T) 27.5/42.2ºC Intestitium was prominent in medulla and disruption of epithelium was seen in collecting tubules (CT) in most of the places, after 5hrs PMI (18.1/34.6ºC T) Retraction and disruption of epithelium and debris in the lumen was observed in CT, after 6 hrs PMI (18.5/33.3ºC, T) Disruption of epithelium was present in CT at places, prominent intestitium was present in medulla, after 6.20 hrs PMI (25.5/31ºC, T) disruption of epithelium was seen in CT at places, after 7.35hrs PMI (26.8/37.1°C,T), Disruption and retraction of epithelium was observed in CT, after PMI 8.30 hrs(9.8/26.3ºC, T) red blood cells were seen in intestitium of the medulla. Intestitium was prominent in the medulla. Disruption and retraction of epithelium with vesicular& dark stained nuclei, individualization of cells at places, cellular debris in lumen at places seen in CT. Retraction and disruption of epithelium with vesicular and dark stained nuclei were seen in CT, after 12.30 hrs PMI (24.5/38.1ºC T) ratio of dark stained nuclei and vesicular nuclei were almost same. In the present study after 13 hrs at 23.9/38.7ºC,T, in CT retraction and disruption of epithelium with dark stained as well as vesicular nuclei and individualization of cells at places were observed. After13hrsPMI (25.5/31ºC, T), CT were with retracted and disrupted epithelium along with vesicular as well as dark stained nuclei. Red blood cells were seen in the intestitium of medulla. After 13 hrs PMI (8.2/23.9ºC, T) Retraction and disruption of epithelium with vesicular nuclei were observed in CT. Red blood cells were present in the intestitium of medulla. In two cases of 13.10hrs PMI (27.3/42.2ºC, T), CT were dilated with retraction and disruption of epithelium having vesicular nuclei mostly but enucleated epithelial cells were also present, after13.30hrsPMI (29.6/43.1ºC, T), CT with retraction and disruption of epithelium having dark stained nuclei were seen. Intestitium was visible in the medulla. Red blood cells were present in CT after 8.30 hrs PMI (9.8/26.3ºC, T), after13 hrs PMI (8.2/23.9ºC,T) in CT and in the intestitium of medulla after 13.10 hrs PMI(27.3/42.2ºC,T), after 16hrsPMI(11/23.7ºC,T), CT a places cellular debris was present in the lumen, after16hrs PMI(24.6/38.1ºC,T) CT retraction and disruption of epithelium with vesicular nuclei were seen. Enucleated epithelial cells were also seen at places. Intestitium was prominent in the medulla, after 16hrs PMI (27.5/42.2ºC, T),). CT retraction disruption of epithelium with at places epithelium were enucleated, after16.30hrs (21/39ºC, T) Intestitium prominent in the medulla with the presence of red blood cells. CT- dilated epithelium retracted and disrupted with dark stained nuclei were seen. In the current research after17hrsPMI(12.8/23.5ºC,T), Red blood cells present in the intestitium of medulla's In the present study after19hrs PMI(27.3/41.4ºC,T), CT retraction were also seen, after19hrsPMI(19.5/35ºC,T), In CT retraction and disruption of epithelium with vesicular nuclei were seen in all tubules, after 19.30hrsPMI(31.3/45ºC,T), intestitum was visible in the medulla. In CT changes were disruption of epithelium mostly but a few places retraction of epithelium with maintained structure. Nuclei were darkly stained, after 20hrs PMI (25.5/31ºC, T), CT retraction of epithelium with disruption at places with dark stained nuclei was seen and in some of the tubules debris was present. Enucleated epithelial cells were also seen , after20hrs PMI(27.5/42.2ºC,T), retraction and disruption of epithelium with dark stained nuclei and red blood cells were seen in CT, after 20hrs PMI(26.3/40.6ºC,T), in CT almost disruption of epithelium with dark stained nuclei but enucleated epithelial cells also at places and individualized epithelial cells were seen, after 21hrs PMI(25.5/31ºC,T), in CT retraction, disruption of epithelium with dark stained nuclei and individualization of epithelial cells were present, after 21 hrs PMI(25.5/31ºC,T), retraction and disruption of epithelium with debris in,T) complete disruption of epithelium with individualization of cells in most of the places having dark stained nuclei was observed in CT, after21.30hrs PMI(26/40.8ºC ,T), retraction, and disruption of epithelium with individualization of cells having dark stained nuclei showing pyknotic changes at places was observed in CT with debris in the lumen. Deborah Barber[13] found in Porcine kidney after24 hours 4ºC, Disruption of the brush border was evidence but most PAS+ brush border were still intact CT-pyknosis was first observed. Epithelium had individualized within the collecting tubules lumens. All nuclei were pyknotic. Dr. Rakesh Tandon[14] was found by 24 hrs there was diffuse cloudy swelling of the cells of renal tubules and this also involved the blood vessels. of the kidney tubules, cells with small granules on its cytoplasm and little nuclear alteration, vacuoles in the cytoplasm.

Piyanut Peebua et.al. was found after 24 hrs PMI; kidney showed hydropic swelling of tubular cells, lipid vacuoles, accumulation in many tubules and nuclear pyknotic change. Samar Omar Rabah[17] was found in mice kidney after 24 hrs PMI, both necrotic and apoptotic changes in the renal tubules, the tubules showed hyaline staining. The cytoplasm was stained deeply acidophilic, the nuclei were small and deeply stained (pyknotic).There was marked loss of normal kidney parenchymatous organization. There was also hyaline degeneration or apoptosis of most tubules, unstained cells with dark stained nuclei. Ashwini kumar et.al[18] was found after 24 hrs in the kidney presence of vacuolization of the tubular lining epithelium and patchy areas heamorrhage in the interstitium. Some of the tubules toward the medullary region showed necrosis of tubular lining epithelium suggestive of early acute tubular necrosis. In the current research after 28hrs PMI (23/33.4ºC, T), intestitium was prominent in the medulla. disrupted epithelium with dark stained nuclei and individualization of cells in most of the places were seen in CT with debris in the lumen. Vinita kushwaha et al[5] was found in their study only mild and moderate changes after 31- 34hrsPMI with increasing temperature of up to 31to35ºC. Mild changes were maintained architecture, mild cloudy swelling and disruption of tubular epithelium while moderate changes were more cloudy swelling. Dr. Rakesh tandon[14] was found in the Rabbit after 36 & 48 hrs PMI changes were marked and diffuse throughout the kidney substance, revealed advanced autolytic changes so that only vogue outlines of tubules, glomeruli and blood vessels could be made out. Ali Asghar[19] found in Rat kidney, there were also severe atrophy and necrosis of the tubules, some tubules showed marked thinning of the epithelial cells,

Conclusion

postmortem histological changes in CT were observed and found some remarkable changes in different duration. In collecting tubules (CT) after 4hrs PMI (27.5/42.2ºC T) disruption of epithelium was observed in most of the place. while after 46 hrs PMI(24.3/25.9ºC,T) disruption of epithelium, debris in the lumen with enucleated epithelial cells and pyknotic nuclei were seen, after 52.30 hrs PMI(24.5/32ºC,T) disruption of epithelium, individualization of cells.

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