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Estimation of Time since Death by Histological Examination of Proximal Convoluted Tubule in Human Kidney

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ABSTRACT

Background: Proximal tubules are highly convoluted in the cortex and become straighter toward the medulla. Proximal tubules are round to oval. Usually round nuclei are in the center or toward the base of each cell. Their wall made of simple cuboidal or low columnar epithelium; surround a central, irregularly shaped lumen. It is important for the histopathologist to differentiate postmortem autolysis from ante mortem coagulative necrosis. Histologically cells react similarly to both forms of self-digestion.

Aim: Estimation of time since death by histological examination of proximal convoluted tubules.

Materials and Methods: In this research we obtained 40 Human kidneys samples at different time intervals between 4hrs to 52.30 hr, temperature between17.3/22.3-31.3/45^{0}C, humidity between 11/36 to 75/95, different age and sex from cadavers with a known time of death where death had resulted from natural death, suffocation and trauma, dead individual health and not suffering from disease affecting kidney and excluded all kidney disease cadaver.

Result: postmortem histological changes were seen in PCT i.e. retraction as well as disruption (fragmented) of epithelium from the basement membrane although the cells were with nuclei after 4hrs (27.5/42.2^{0}C, T).

Conclusion: In Proximal convoluted tubules lumens were closed due to swelling of epithelial cells. Retraction of epithelium from the basement membrane and its disruption with individualization of cells, nuclear pyknosis, karyolysis and loss of tubular architecture with debris in the lumen were observed in Proximal convoluted tubules, This research could be used to estimation of the time after death.

Key Words: Proximal convoluted tubules, Vesicular nuclei, Ancient period, edematous, disruption.

INTRODUCTION

The renal cortex is composed of glomeruli, proximal convoluted tubules (PCT), distal convoluted tubules (DCT) in cortical labyrinth .The urinary pole of glomeruli leads into PCT that are tortuous tubules lined by pyramidal cells with basally located, round, vesicular nuclei with prominent nucleoli. The luminal surface of these epithelial cells is lined by a mucopolysaccharide brush border of microvilli. The bulk of the renal cortex is composed of these tubules. [1] Kidneys have a rich blood supply. Tubules in the renal cortex are susceptible to rapid autolytic changes. Within a few hours tubular nuclei disappear and brush borders of the proximal convoluted tubules are lost. [2] Gradual decrease of body temperature is one of the earliest sign of death. The formula for estimation of postmortem interval from rectal temperature and abdominal temperature presented by Marshal and Hoare. [3] These various gross changes in the body after death are loss of corneal reflex and changes in eye, cooling of the body, post mortem hypostasis, rigor mortis, decomposition and other putrefactive changes, mummification, adipocerous changes. [4]

Cell death occurs when cells lose all ability to perform cellular functions within minutes of death, lysosomes liberate hydrolytic enzymes into the cytoplasm.

These enzymes are activated by decreasing pH arising from decreased oxidative metabolism. Intracellular organelles and membranes are rapidly degraded in this process of self digestion. If this process occur in a living animal, it s called necrosis. If it happens after somatic death it is autolysis. [5] The time of death is sometimes extremely important. It is a question almost invariably asked sometimes with a touching faith in the accuracy of the estimate. Determining the time of death is extremely difficult, and accuracy is impossible. In this study control cannot be taken because the histological changes of tissue after death is influenced a great deal by atmospheric temperature and humidity besides other external and internal factors. [6] 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. [7,8]

The ancient period, examination the time since death based on naked eye observations of the gross changes in a dead body occurring after death to provide a rough approximation of post mortem interval, the histological changes in kidney after death have been studied in various marine and land animals but yet very few studies have been done in human kidney. That's why our present research 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.

METHODS

This present research was done in Department of Anatomy in close association with the Department of Forensic Medicine & Toxicology and Pathology, Pt. J.N.M. Medical College, Raipur (C.G.).The temperature between17.3/22.3-31.3/45^{0}C, the environmental temperature in ^{0}C [minimum/maximum] and humidity in % [minimum/maximum] was recorded from "India Meteorological Department, Meteorological Centre Raipur". Humidity between 11/36 to 75/95 and duration range was between 4hrs to 52.30hr. In the present research, each case was studied by histological H& E staining, PAS staining, 40 Human kidneys samples were of different age and sex obtained from cadavers at the time of autopsy. It was removed from cadavers with a known time of death where death had resulted from natural death, suffocation and trauma, dead individual health and not suffering from disease affecting kidney. Cases exclude dead individual those preserve in ice or ice cooler, unknown time of death, suffering from kidney disease, cases complicated by other metabolic disorders like Diabetic mellitus, renal oesteodystrophy, secondary hyperparathyroidism nutritional disturbance (PEM).

OBSERVATION AND RESULTS

The present research, post mortem histological changes of kidney was done to estimation of the time of death in 40 random samples of human kidneys at different time intervals after death and in different temperature conditions. Kidneys were studied under the light microscope after staining with Harris haematoxylin and eosin in routine manner & with PAS. We were found following result -

STUDY NO:-1. Post mortem interval (PMI) - 4 hrs Temperature-27.5/42.2^{0}C, humidity-14/41%, PCT (Proximal convoluted tubule) - retraction of epithelium from the basement membrane, edematous epithelial cell with well stained cytoplasm and dark stained nuclei. PCT is PAS+.(Fig – 1, Fig – 11).

STUDY NO:-2. PMI -5 hrs Temp-18.1/34.6 ^{0}C humidity-20/42%, PCT-retraction of epithelium from basement membrane at few places with maintained structure of epithelial cells with vesicular nuclei.(Fig- 8).

STUDY NO:-3. PMI 6hrs Temp-18.5/33.3^{0}C humidity-41/72% PCT-dilated with retraction of epithelium from the basement membrane at places& disruption at few places with dark stained nuclei and pyknotic changes.

STUDY NO:-4. PMI 6.20 hrs Temp-25.5/31^{0}C humidity 85/87%, PCT- mostly dilated with wide lumen disruption of epithelium at places along with the presence of debris in the lumen with dark stained nuclei.

STUDY NO:-5. PMI 7.35 hrs Temp - 26.8/37.1°C humidity-41/71%, PCT- disruption and retraction of epithelium at places having vesicular and dark stained nuclei but enucleated epithelial cells are also seen at places.

STUDY NO:-6. PMI 8.30hrs Temp-9.8/26.3^{0}C humidity-33/65%, PCT-expanded, edematous with nuclei are vesicular and dark stained. PCT is PAS+. (Fig – 2).

STUDY NO:-7, PMI 12.30 hrs Temp-24.5/38.1^{0}C humidity-22/37%, PCT-retraction, distruption of epithelium having vesicular & dark stained nuclei, at places enucleated epithelial cells and oedematous epithelial cells are also seen.

STUDY NO:-8. PMI 12.30hrs Temp-28.4/44.2^{0}C humidity-16/27%, PCT-retraction of epithelium with dark stained nuclei mostly but enucleated epithelial cells are also present at few places and in some of the places PCT are well maintained.

STUDY NO:-9. PMI 13hrs Temp-23.9/38.7^{0}C humidity-31/70%, PCT-expanded retraction and disruption of epithelium, mostly enucleated epithelial cells but at places having dark stained nuclei. Vesicular nuclei hardly visible. Debris present in lumen.(Fig – 3).

STUDY NO:-10. PMI 13hrs Temp-23.9/38.7^{0}C humidity-41/72%, PCT - expanded with disruption of epithelium having dark stained nuclei but enucleated also at places.

STUDY NO:-11. PMI 13hrs Temp-25.5/31^{0}C humidity-85/87%, PCT-expanded, disruption of epithelium with dark stained nuclei, enucleated epithelial cells are also present.

STUDY NO:-12. PMI 13hrs Temp-8.2/23.9^{0}C humidity-26/83%, PCT - retraction of epithelium with vesicular nuclei mostly but enucleated epithelial cells are also present.

STUDY NO:-13. PMI 13.10hrs Temp-27.3/42.2^{0}C humidity-24/52%, PCT - expanded, retraction of epithelium at places with vesicular and dark stained nuclei.

STUDY NO:-14. PMI 13.10hrs Temp-27.3/42.2^{0}C humidity-24/52%, PCT-retraction and disruption of epithelium with vesicular and dark stained nuclei.

STUDY NO:-15. PMI 13.30hrs Temp-29.6/43.1^{0}C humidity-23/38%, PCT-retraction and disruption of epithelium with dark stained nuclei but enucleated epithelial cells are also present.PCT partly PAS+.

STUDY NO:-16. PMI 16hrs Temp-11/23.7^{0}C humidity-44/77%, PCT - retraction disruption of epithelium with dark stained nuclei mostly.

STUDY NO:-17. PMI 16hrs Temp-24.6/38.1^{0}C humidity-36/46%, PCT-retraction &disruption of epithelium, epithelial cells are mostly enucleated.

STUDY NO:-18. PMI 16hrs Temp-27.5/42.2^{0}C humidity-14/41%, PCT-expanded with presence of debris in the lumen, retraction and disruption of epithelium with dark stained nuclei but enucleated epithelial cells are also present.(Fig – 42.2 / 27.5).

STUDY NO:-19. PMI 16.30hrs Temp-21/39^{0}C humidity-11/36%, PCT- retraction and disruption of epithelium having dark stained nuclei but enucleated epithelial cells are also seen.(Fig – 9).

STUDY NO:-20. PMI 17hrs Temp-12.8/23.5^{0}C humidity-44/82%, PCT-expanded with disruption of epithelium having dark stained nuclei but also enucleated at places.(Fig – 10).

STUDY NO:-21.PMI 17.30hrs Temp-27.3/42.2^{0}C humidity 24/52%, PCT-disruption of epithelium having dark stained nuclei but enucleated epithelial cells is also seen. Debris present in the lumen.

STUDY NO:-22. PMI 17.30hrs Temp-27.5/42.2^{0}C humidity-14/41%, PCT-retraction and disruption of epithelium having dark stained nuclei with pyknotic

changes in some of the nuclei while at some places epithelial cells are enucleated.

STUDY NO:-23. PMI 17.30hrs Temp-18.6/28.2^{0}C humidity-79/98%, PCT-disruption of epithelium with absence of nuclei. Debris present in the lumen. PCT is PAS+.

STUDY NO:-24. PMI 19hrs Temp-27.3/41.4^{0}C humidity-18/37%, PCT-retraction and disruption of epithelium with dark stained nuclei.

STUDY NO:-25. PMI 19hrs Temp-19.5/35^{0}C humidity-18/35%, PCT-expanded and disruption of epithelium with dark stained nuclei but enucleated epithelial cells are also present.

STUDY NO:-26. PMI 19.30hrs Temp-31.3/45^{0}C humidity-25/51%, PCT- dilated and disruption of epithelium at places with dark stained nuclei mostly but enucleated epithelial cells are also present. PAS+ spots in the interstitium of cortex at few places, PAS positivity in the basement of PCT is rarely visible.

STUDY NO:-27. PMI 20hrs Temp-25.5/31^{0}C humidity-85/87%, PCT-retraction and disruption of epithelium with dark stained nuclei epithelial cells are enucleated also at places. Debris present in the lumen.

STUDY NO:-28. PMI 20hrs Temp-27.5/42.2^{0}C humidity-14/41%, PCT-disruption of epithelium with dark stained nuclei but enucleated epithelial cells is also present. Debris present in the lumen.

STUDY NO:-29. PMI 20hrs Temp-26.3/40.6^{0}C humidity-16/46%, PCT-retraction and disruption of epithelium with dark stained nuclei and in most of the places enucleated epithelial cells and individualization of epithelial cells.

STUDY NO:-30. PMI 21hrs Temp-25.5/31^{0}C humidity-85/87%, PCT-retraction and disruption of epithelium with dark stained nuclei and at few places enucleated epithelial cells. Individualization of epithelial cells at some places. Debris present in the lumen.

STUDY NO:-31. PMI 21 hrs Temp-25.5/31^{0}C humidity-85/87%, PCT-retraction and disruption of epithelium with debris in the lumen, enucleated epithelial cells are seen at places.

STUDY NO:-32. PMI 21hrs Temp-29.6/43.1^{0}C humidity-23/38%, PCT-retraction and disruption of epithelium with dark stained nuclei having pyknotic changes at places, enucleated epithelial cells and oedematous epithelium with maintained structure in some places, at places, debris present in the lumen.

STUDY NO:-33. PMI 21.30hrs Temp-26/40.8^{0}C humidity-45/50%, PCT-disruption of epithelium with dark stained nuclei, debris present in the lumen. Basement membrane of PCT is PAS+.(Fig = 5).

STUDY NO:-34. PMI 28hrs Temp-23/33.4^{0}C humidity-65/90%, PCT- dilated having wide lumen, debris in the lumen, at few places disruption of the epithelium, nuclei are dark stained mostly in the peripheral part, and enucleated epithelial cells are also present at places.

STUDY NO:-35. PMI 28hrs Temp-26.7/38.7^{0}C humidity-44/64%, PCT-retracted and disrupted epithelium. Enucleated epithelial cells are seen in most of the places. Debris present in the lumen. Basement membranes of PCT are partly PAS+.

STUDY NO:-36. PMI 40hrs Temp-25.3/32.2^{0}C humidity-68/87%, PCT – retraction and disruption of epithelium with individualization of cells mostly, cellular debris in the lumen with pyknotic nuclei.

STUDY NO:-37. PMI 41hrs Temp-24.3/25.9^{0}C humidity-84/87%, PCT-expanded and epithelium with dark stained nuclei as well as vesicular nuclei, retraction hardly seen, Debris present in the lumen. Interstitium prominent in the cortex.(Fig - 6)

STUDY NO:-38. PMI 45hrs Temp-24.3/25.9^{0}C humidity-84/87%, PCT - oedematous, retraction, disruption of epithelium at places having nuclei dark stained at their periphery. Debris present in the lumen.

STUDY NO:-39.PMI 46hrs Temp-24.3/25.9^{0}C humidity-84/87%, PCT-

retraction and disruption of epithelium having pyknotic nuclei but enucleated epithelial cells are also present. Debris present in the lumen. Interstitium prominent in the cortex.

STUDY NO:-40. PMI 52.30hrs Temp-24.5/32^{0}C humidity-75/95%, PCT- shrunk with retraction and disruption of epithelium at places with individualization of cells having darkly stained nuclei with pyknotic changes. Debris present in the lumen. PAS+ substances are not seen.(Fig – 52.30).

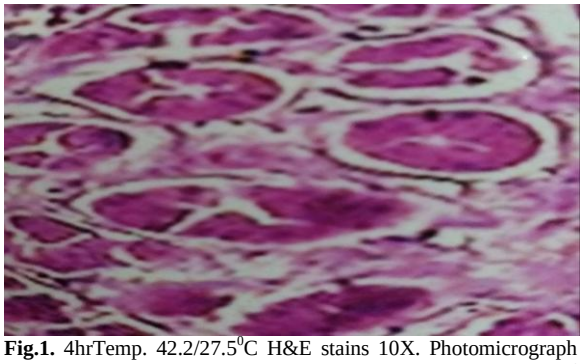

Fig.1. 4hrTemp. 42.2/27.5^{0}C H&E stains 10X. Photomicrograph showing in PCT (P) oedematous epithelial cells, retraction of epithelium from the basement membrane with stained cytoplasm. Prominent intestitium in the cortex.

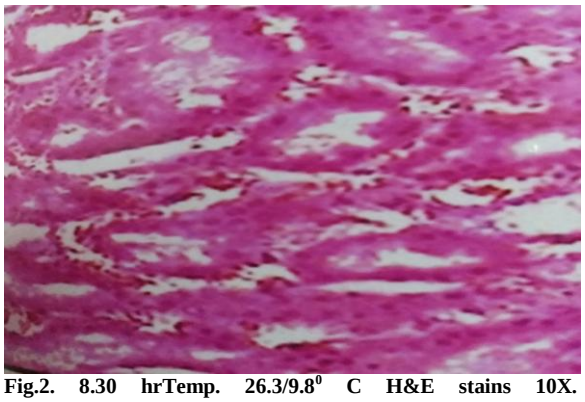

Fig.2. 8.30 hrTemp. 26.3/9.8^0 C H&E stains 10X. Photomicrograph showing in PCT (P) red blood cells, expanded with oedematous.

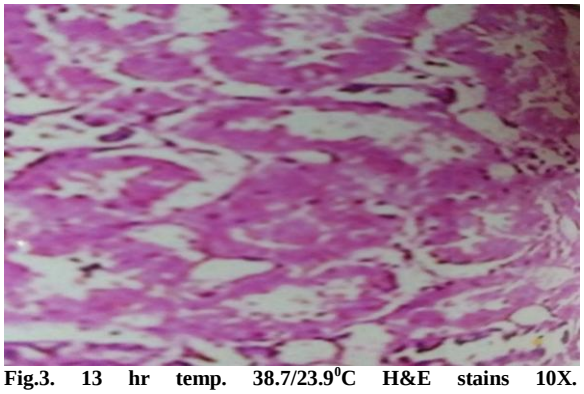

Fig.3. 13 hr temp. 38.7/23.9^{0}C H&E stains 10X. Photomicrograph showing in PCT (P) expanded, debris presence in the lumen.

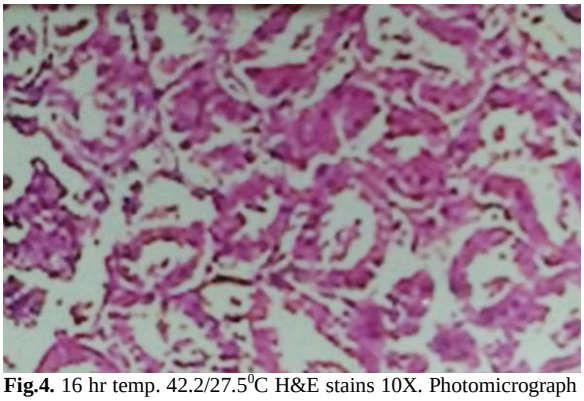

Fig.4. 16 hr temp. 42.2/27.5^{0}C H&E stains 10X. Photomicrograph showing in PCT (P) expanded with debris presence in the lumen, retraction, disruption of epithelium. Individualization of cells at places.

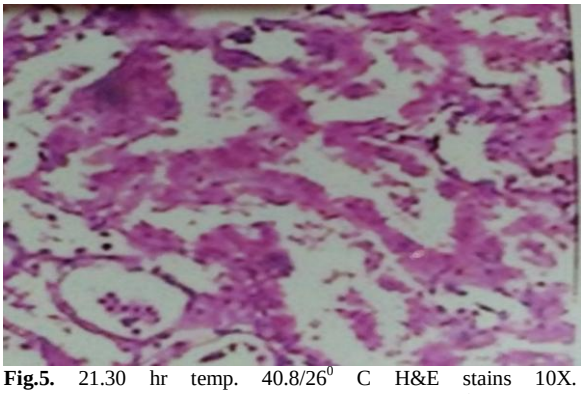

Fig.5. 21.30 hr temp. 40.8/26^0 C H&E stains 10X. Photomicrograph showing in PCT (P) disruption of epithelium, debris presence in the lumen, individualization of cells at places.

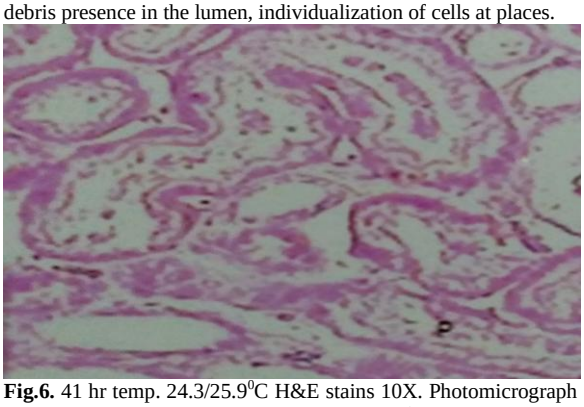

Fig.6. 41 hr temp. 24.3/25.9^{0}C H&E stains 10X. Photomicrograph showing in PCT (P) expanded, retraction of epithelium debris presence in the lumen.

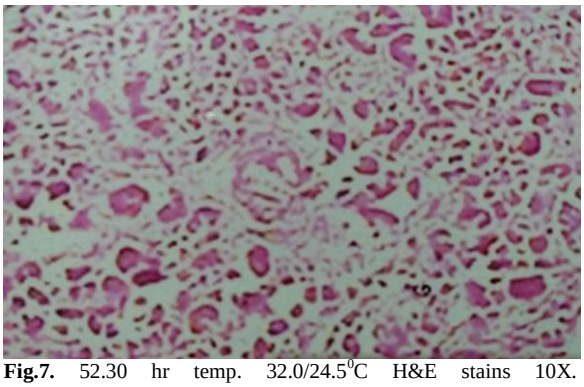

Fig.7. 52.30 hr temp. 32.0/24.5^{0}C H&E stains 10X. Photomicrograph showing in PCT (P) shrunk with retraction, disruption of epithelium at places, architecture disturbed.

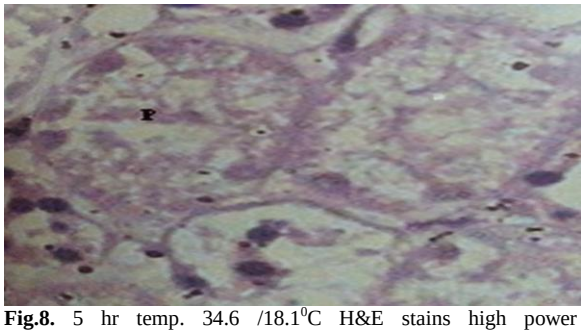

Fig.8. 5 hr temp. 34.6 /18.1^{0}C H&E stains high power Photomicrograph showing in PCT (P) maintained structure of epithelial cells with vesicular nuclei seen.

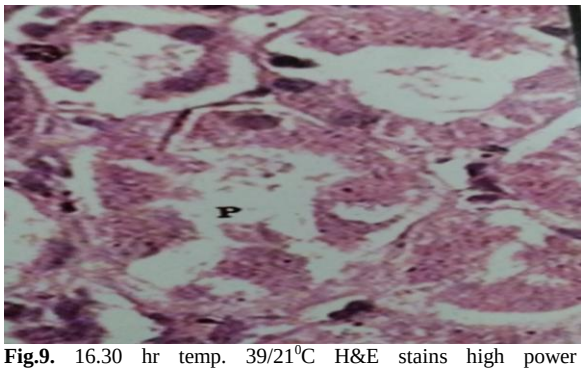

Fig.9. 16.30 hr temp. 39/21^{0}C H&E stains high power Photomicrograph showing in PCT (P) retraction and disruption of epithelium, mostly enucleated epithelial cells dark stained nuclei mostly and vesicular nuclei are rarely seen.

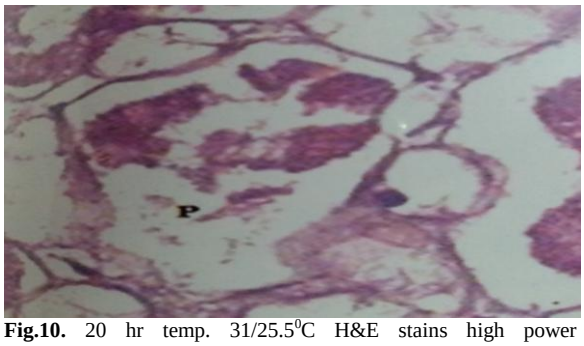

Fig.10. 20 hr temp. 31/25.5^{0}C H&E stains high power Photomicrograph showing in PCT (P) retraction and disruption of epithelium, enucleated epithelial cells at places, debris present in lumen.

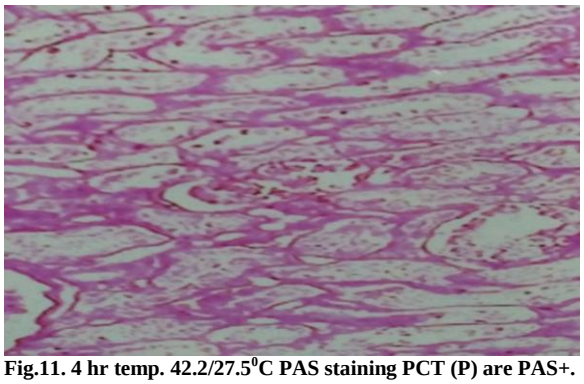

Fig.11. 4 hr temp. 42.2/27.5^{0}C PAS staining PCT (P) are PAS+.

DISCUSSION

Estimation of time since death was 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. [9] Determination of time since death was not only important in criminal cases but also in civil cases where they have legal implications in issue of insurance and inheritance. [10,11] The present research postmortem histological changes of kidney were observed in 40 cases of different age and sex. Average environmental temperature ranges between17.3/22.3-31.3/45^{0}C, humidity between 11/36 to 75/95and duration range was between 4hrs to 52.30hrs.

Pratibha Ravindra et al [12] was found, histological examination of renal slice in kidney showed different alteration including acute tubular necrosis marked dilatation of proximal convoluted tubules with sloughing of almost entire epithelium morphological alteration observed in brush border, presence of dense chromatin in the nucleus of the tubular lining and Lumen showed presence of degenerative debris. M.S. Prashanth [13] was found in the kidney of fresh water fish, deformation of brush border, gradual atrophy of basal cytoplasm and focal degeneration of tubular cells, necrosis of nephron, the area of interstitial tissue containing leucocyte and macrophages seemed to be increased as the tubules become reduced. Khaled M.A. Hassanein [14] was found in kidney, swelling, and thickening of basement membrane and proliferation of renal tubular epithelium. Salam Z AL-Agha [15] was found in rat kidney, distension of capillaries, distortion of renal architecture in most of the cells, destruction of cytoplasm organelles, thickening of the basement membrane of the PCT and irregularly shaped nuclei. Deborah Barber [16] was found in Porcine kidney with the periodic acid Schiff (PAS) reaction, the brush

borders (PCT)were observed to be mildly disrupted but still present after 48 hours at 4^0C and 12 hours at 24°C.These findings differed from earlier studies which found the borders to be extremely sensitive to autolytic destruction. In the chicken, the brush border was only occasionally seen after 6 hours at 20^0C.Persistence of the brush borders throughout the experimental period. PCT had swollen cells and occluded lumens in all except the perfused control specimens, resembling findings in the rat. Refluxed debris from the PCT was seen in all except the per fused control specimens, after 3 Hours at 4^0C, PCT-the number of obliterated lumens was minimal, After 3 Hours 24^0C, PCT-a moderate number of lumens of were obliterated due to cellular swelling. In the present study after 4hrs postmortem interval (PMI) at temperature (T)27.5/42.2^{0}C prominent interstitium in the cortex was observed. Retraction of epithelium from the basement membrane was seen in proximal convoluted tubule (PCT) while epithelial cells were oedematous with well stained cytoplasm and basal nuclei. Tomita et al [17] was found in wistar rats 5 hours after death, PM changes were clumping of nuclear chromatin in proximal tubules. Deborah Barber [16] was found in porcine kidney after 6 hours at 4^0C in PCT-the number of obliterated lumen became marked, after 6 hours at 24^0C in PCT-nearly all tubular lumens were obliterated by cellular swelling. Brush borders were indistinct but still visible. Nuclei were vesicular and enlarged. In the present study after 4 hrs PMI at (27.5/42.2^{0}C T), Retraction of epithelium was rarely seen in PCT, however structure of the epithelium was maintained. After 6 hrs PMI (18.5/33.3^{0}C, T), at places dilatation of PCT with retraction of epithelium was seen while disruption of epithelium was in few tubules. After 6.20 hrs PMI (25.5/31^{0}C, T), Most of the PCT were dilated and epithelium disrupted at places along with the presence of debris in the lumen. Tomita et al [17] was found in Wistar rats at 10 hours after death oedema in proximal tubules condensation of nuclear chromatin and edema in distal tubules. Deborah Barber [16] found in porcine kidney at 12 hours 24^0C in PCT-Patent lumens were again visible and all contained some non cellular debris. Brush borders were present. The cytoplasm was finely granular and was starting to retract off the basement membranes. Nuclei were hyper chromatic to pyknotic. Vinita Kushwaha et al. [9] was found in first 12 hrs(26-30^{0}C T) all 5 cases showed mild degenerative changes, architecture maintained, mild cloudy swelling and disruption of tubular epithelium. Dr. Rakesh Tadon [18] was found at 30^0C cloudy swelling of PCT, 12 hrs after death. Samar Omar Rabah [19] was found in12 hrs proximal tubules showed histological changes in the form of widening of tubular lumen, marked atrophy and degeneration of distal tubules cells was also observed. S.H. Garba et.al [20] was found in 12 hrs, severe multifocal congestion and tubular degeneration in cortex. In the present study after 7.35hrs PMI (26.8/37.1°C, T), in the present study after PMI 8.30 hrs (9.8/26.3^{0}C, T), PCT dark stained nuclei and vesicular nuclei were almost equal. After 12.30 hrs PMI (24.5/38.1^{0}C, T), in PCT-disruption, retraction of epithelium with vesicular as well as darkly stained nuclei and enucleated epithelial cells at places, oedematous epithelial cells were also found. After12.30hrsPMI (28.4/44.2^{0}C, T), retraction of epithelium was seen in PCT but were well maintained few places.

Vinita Kushwaha et al [9] found in their study after 13- 18 hrs PMI with increasing temperature of up to 31 to 35^{0}C, moderate & severe changes. Architecture maintained, more cloudy swelling and disruption of epithelium, glomeruli swollen. Only 2 out of 13 cases showed severe changes (G-3), architecture disturbed, cloudy swelling and disruption of epithelium was prominent and collapse of glomeruli. In the present study after 13 hrs at 23.9/38.7^{0}C,T, PCT were expanded with debris in the lumen. Retraction and

disruption of the epithelium was observed. Epithelial cells were mostly enucleated. At places dark stained nuclei were seen but vesicular nuclei were hardly visible. After 13 hrs PMI (23.9/38.7^{0}C, T) PCT expanded with disrupted epithelium having dark stained nuclei but at places enucleated also. Vesicular nuclei were rarely seen. After13hrsPMI (25.5/31^{0}C, T) PCT were expanded with disrupted epithelium having dark stained nuclei but enucleated epithelial cells were also present. Red blood cells were seen in the interstitium of cortex. After13hrsPMI (8.2/23.9^{0}C, T) PCT were seen with retraction of epithelium having vesicular nuclei but most of the places it was enucleated. In two cases of 13.10hrs PMI (27.3/42.2^{0}C, T), PCT were expanded with retraction of epithelium at places in one case while retraction, disruption of epithelium in another case with vesicular and dark stained nuclei in the epithelium of PCT in both cases. Interstitium was visible in the cortex in both. After13 hrs PMI temp 23.9/38.7^{0}C and13.10hrs PMI (27.3/42.2^{0}C, T), visible interstitium was observed in the cortex. After 13.30hrs PMI (29.6/43.1^{0}C, T), PCT were seen with retraction and disruption of epithelium having dark stained nuclei. Enucleated epithelial cells were also present. Red blood cells were present in PCT, after 8.30 hrs PMI (9.8/26.3^{0}C, T), after13 hrs PMI (8.2/23.9^{0}C, T). In the present study after 16hrsPMI (11/23.7^{0}C, T), In PCT retraction and disruption of epithelium with dark stained nuclei were seen. After 16hrs PMI (24.6/38.1^{0}C, T), In PCT retraction and disruption of epithelium without nuclei mostly. After 16hrs PMI (27.5/42.2^{0}C, T), PCT expanded with presence of debris in the lumen having retraction and disruption of epithelium with dark stained nuclei as well as enucleated epithelial cells were also seen. After16.30hrs (21/39^{0}C, T), PCT- retraction and disruption of epithelium having dark stained nuclei, nucleated epithelial cells were also seen. In the present study after17hrsPMI (12.8/23.5^{0}C, T), PCT- expanded with disruption of epithelium having dark stained nuclei but epithelial cells were also enucleated at places. After17.30hrs PMI (27.3/42.2^{0}C, T), PCT disruption of epithelium having dark stained nuclei but enucleated epithelial cells were also seen, debris in the lumen. After 17.30hrsPMI (27.5/42.2^{0}C, T), PCT showed retraction and disruption of epithelium having dark stained nuclei with pyknotic changes in some of the nuclei. At some places epithelial cells were enucleated. Vinita Kushwaha et.al [9] was found in their study after 19- 24 hrs PMI, with increasing temperature of up to 31to 35^{0}C, severity increases, architecture disturbed, cloudy swelling and disruption of epithelium was prominent, collapse of glomeruli. In the present study after19hrs PMI (27.3/41.4^{0}C, T), PCT showed retraction and disruption of epithelium with dark stained nuclei. After 19hrs PMI (19.5/35^{0}C, T), PCT were expanded with disrupted epithelium. After 19.30hrs PMI (31.3/45^{0}C, T), PCT were dilated with disruption of epithelium at places having dark stained nuclei but most of the places without nuclei, after 20hrs PMI (25.5/31^{0}C, T), PCT showed debris in the lumen, retraction and disruption of epithelium with dark stained nuclei but epithelial cells enucleated also, after20hrs PMI (27.5/42.2^{0}C, T), PCT showed disruption of epithelium with dark stained nuclei and debris in the lumen. Enucleated epithelial cells were also present. After 20hrs PMI (26.3/40.6^{0}C, T), PCT showed retraction and disruption of epithelium with dark stained nuclei but in most of the places enucleated, after 21hrs PMI (25.5/31^{0}C, T), In PCT retraction and disruption of epithelium with dark stained nuclei and debris in the lumen were seen. At few places enucleated epithelial cells and individualization of epithelial cells were present. After 21 hrs PMI (25.5/31^{0}C, T), retraction and disruption of epithelium with debris in the lumen of PCT. After 21hrsPMI (29.6/43.1^{0}C, T), PCT showed retraction and disruption of epithelium with debris in the lumen but at places oedematous with maintained structure and nuclei were dark

stained with pyknotic changes at places. Epithelial cells were also seen enucleated in some of the places. After21.30hrs PMI (26/40.8^{0}C, T), Disruption of epithelium with dark stained nuclei were seen in PCT with debris in the lumen.

Deborah Barber [16] was found in Porcine kidney after24 hours 4^{0}C in PCT-swollen cells partially occlude some of the lumens and no cellular debris was present within some lumens. Cells were in contact with the basement membrane, vesicular nuclei were basally located within cells. Disruption of the brush border was evidence but most PAS+ brush border. After 24 Hours 24^{0}C in PCT Nuclei within the tufts were small and hypercromatic PCT-patent lumen were again visible and all contained some noncellular debris. Brush border were present. The cytoplasm was finely granular and was starting to retract off the basement membranes. Nuclei were hyper chromatic to pyknotic. Dr. Rakesh Tandon [18] was found by24 hrs there was diffuse cloudy swelling of the cells of renal tubules and this also involved the blood vessels, at 20^{0}C mild and focal autolytic changes could be seen 24 hrs after death. At 40^{0}C the changes became diffuse by 24 hrs, advanced autolytic changes so that only vogue outline of tubules. Piyanut Peebua et.al. [21] 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 [19] was found in mice kidney after 24 hrs PMI, both necrotic and apoptotic changes in the renal tubules, focal apoptotic changes involved proximal 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. Marcelo leite da Veiga [22] was found at 24 hrs onwards the kidney showed, the basal membrane presented loss of cells content, the tubular cells appeared swollen, vacuolated and thick relatively regular from with a condensed chromatin on its central region, while other showed themselves relatively small and pyknotic and necrosis focus. In the present study after 28hrsPMI (23/33.4^{0}C, T), PCT were dilated having wide lumen with debris at few places, disruption of the epithelium was seen with nuclei having dark stained mostly in the peripheral part, after 28hrs PMI (26.7/38.7^{0}C,T), retracted, disrupted epithelium were seen in PCT with debris in the lumen. Epithelial cells were enucleated in most of the places. Vinita Kushwaha et al [9] was found in their study only mild and moderate changes after 31-34hrs PMI with increasing temperature of up to 31to35^{0}C.Mild changes were maintained architecture, mild cloudy swelling and disruption of tubular epithelium while moderate changes were more cloudy swelling and prominent disruption of epithelium with maintained architecture. Dr. Rakesh tendon [18] 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 and blood vessels could be made out. Ali Asghar [23] was found in Rat kidney, there were also severe atrophy and necrosis of the tubules, some tubules showed marked thinning of the epithelial cells, which were transformed into a thin layer of cytoplasm covering the basement membrane. There was a substantial degree of oedema and numerous cells in the interstitium. The inflammatory cells were mostly in the vicinity of the interlobular blood vessels, some place mesangial thickening with shrinkage of capillary tuft. In the present study after 40hrs PMI (25.3/32.2^{0}C, T), retraction and disruption of epithelium with pyknotic nuclei were seen in PCT, after 41hrs PMI (24.3/25.9^{0}C, T), epithelium was oedematous with dark stained nuclei in PCT with debris in the lumen. Retraction was seen occasionally, after 45hrsPMI (24.3/25.9^{0}C, T), oedematous PCT were seen. While retraction and disruption of epithelium with darkly stained nuclei at

their periphery with debris in the lumen at places was observed in PCT, after 46hrs PMI (24.3/25.9^{0}C, T), retraction, disruption of epithelium with enucleated epithelial cells as well as pyknotic nuclei and debris in the lumen were seen in PCT. Deborah Barber [16] was found in Porcine kidney after 48 hours (24^{0}C), cells were retracting off the basement membrane in single units in PCT with no cellular individualization. Non cellular debris was present in all lumens. Brush borders were still evident, after 48 hours at 4^{0}C all lumens in PCT contained debris. PAS+ brush border were disrupted, but some were still present. Cells were beginning to retract from the basement membranes, but most were still in contact. Some nuclei were hyper chromatic. Marcelo leite da Veiga [22] was found at 48 hrs the kidney showed the tubular cells appeared swollen vacuolated and with thin and thick cytoplasmic granulations. Some of the cells nuclei kept relatively regular from with a condensed chromatin on its central region while other showed themselves relatively small and pyknotics advancing necrosis focus. Samar Omar Rabah [19] found in mice kidney after 48 hrs PMI, tubules had dilated lumina and marked atrophy was observed. At cortico-medullary junction showed massive degeneration of vascular elements perivascular odema. In the present study after 52.30hrs PMI (24.5/32^{0}C, T) PCT were shrunk with retraction, disruption of epithelium and at places with individualization of cells. Debris was present in the lumen. Nuclei were darkly stained with pyknotic changes.

Samar Omar Rabah [19] was found in mice kidney PAS+ material in the basement membrane of renal tubules, the brush border of proximal tubules lining cells showed highly PAS positive staining. Deborah Barber [16] was found in Porcine kidney (perfused control) brush borders (PCT) were present and strongly PAS+. In the present study after 4 hrs PMI (27.5/42.2^{0}C, T) & 8.30hrsPMI (9.8/26.3^{0}C,T, basement membrane of PCT were PAS+, after 13.30hrs PMI (T 29.6/43.1^{0}C) basement membrane of PAS+ completely of PCT, after17.30hrs PMI (18.6/28.2^{0}C,T) basement membrane of PCT were PAS+, after 19.30hrs PMI (31.3/45^{0}C,T) basement membrane of PCT were rarely seen, PAS+ debris in the lumen. After 21.30hrs PMI (26/40.8^{0}C,T) basement membrane of PCT were PAS+ ,after 28hrs PMI (26.7/38.7^{0}C T) Basement membranes of PCT were partly PAS+, after 52.30hrs PMI (24.5/32^{0}C, T) PAS+ substances were not seen.

CONCLUSION

In the present research earliest remarkable postmortem histological changes were seen in PCT i.e. retraction as well as disruption (fragmented) of epithelium from the basement membrane although the cells were with nuclei after 4hrs (27.5/42.2^{0}C, T). PCT were PAS+, PAS+ substances were in the form of small spots at places after4 hrs PMI (27.5/42.2^{0}C,T) & 8.30hrs PMI(9.8/26.3^{0}C,T). After 52.30hrs PMI (24.5/32^{0}C,T) PAS+ substances were not seen. Further studies using large number of cases & environmental conditions such as age, sex, humidity, body built, clothings & surrounding of the body etc. in different seasons should be done.

List of abbreviations: None declared.

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Author's contribution: Dr. Rajni Thakur has made to conception, all histological procedure, drafting the manuscript, covert images in JPG file. Dr. Anjana Tiwari has made collection of sample, revising manuscript, arrange the image.

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