

Research Article

A study on estimation of time since death after histological examination of kidney

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ABSTRACT

Background: Estimation of time since death is one of the most important object of post-mortem examination. Many degenerative changes begin to take place in the body immediately or shortly after death. Kidney is one of the most important excretory organs of human body, its undergo series of gross as well as histological changes.

Methods: This study was performed in department of anatomy in close association with the department of forensic medicine & toxicology and pathology, Pt. J. N. M. medical college and DBRAM hospital Raipur (C.G.) India, during study period November 2012 to October 2013. Study was done on 50 human cadavers (Study subjects). Kidney was obtained from dead bodies during post-mortem examination. In each case kidney was studied histologically. Data was compiled in MS excel and checked for its completeness and correctness and then it was analyzed.

Results: In 48-60 hour increasing temperature, one case show very severe change was seen. Microscopic changes in kidney were increasing (mild to moderate, moderate to severe, severe to very severe) as temperature increases.

Conclusion: In the present study earliest remarkable postmortem histological changes were seen in DCT. Finding of present study will be useful for forensic experts.

Keywords: Chhattisgarh, Kidney, Histological changes, Time since death

INTRODUCTION

Many degenerative changes begin to take place in the body immediately or shortly after death and progress in a fairly orderly fashion until the body disintegrates. Each change has its own time, factor or rate. Unfortunately, these rates of development of post mortem changes are strongly influenced by unpredictable endogenous and environmental factors.¹

The renal cortex is composed of glomeruli Proximal Convoluted Tubules (PCT), Distal Convoluted Tubules (DCT) in cortical labyrinth and Collecting Tubules (CT) within medullary rays. Glomeruli are composed of tufts of capillaries in contact with mesangial matrix and

glomerular epithelium. Tufts are enclosed in Bowman's capsules, composed of visceral and parietal epithelia separated by a potential space Bowman's space. 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. Distal convoluted tubules are lined by low cuboidal epithelial cells, resulting in larger luminal diameters than in PCT. These cells have centrally located nuclei and no brush borders on luminal surface. Within the medullary rays the CT are lined by low cuboidal epithelium with scalloped-

edged luminal borders, pale cytoplasm, and dark, spherical centrally located nuclei.²

Body tissues are affected by autolysis at variable rates depending on sensitivity of their cells to anoxia and cellular concentration of proteolytic enzymes. Renal proximal convoluted tubules and renal medulla are tissue reported to be rapidly altered.^{3,4} it is important for diagnostic histopathologists to differentiate significant ante mortem lesions from postmortem autolysis and processing artifacts. In diagnostic histopathology, autolysis must be differentiated from coagulative necrosis. In autolysis and coagulative necrosis, cellular outlines and tissue architecture are maintained, but nuclei become pyknotic, karyorrhectic, or are lost by karyolysis.

Necrotic changes are focally distributed amid viable tissue while autolysis is generalized cell death. A zone of hyperemia and inflammation usually separates necrotic foci from viable tissue. No inflammatory or hyperemic response is elicited by autolysis.⁵

A kidney has 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.⁶

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.⁷

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.⁸

Few clue of time of death is also collected from the condition of food in stomach, intestine and urine in bladder.⁹⁻¹²

Till now the histological changes in kidney after death have been studied in various animals but yet very few studies with same view which may provide keen and fruitful results for human kidney have been done, with this background, present study was planned to estimation of time since death after histological examination of kidney.

METHODS

This study was performed in department of anatomy in close association with the department of forensic medicine & toxicology and pathology, Pt. J.N.M. medical college and DBRAM hospital Raipur (C.G.) India, during

study period November 2012 to October 2013. Study was done on 50 human cadavers (Study subjects). Kidney was obtained from dead bodies during postmortem examination. It was removed from cadavers with a known time of death where death had resulted from natural death, suffocation and trauma. The stages for which it was available were temperature between 17.3/22.3-31.3/45°C, humidity between 11/36 to 75/95% and duration range was between 4 to 52.30 hours.

In each case kidney was studied histologically.

Materials required for the study was:

1. Kidney sample of 50 dead people.
2. Plastic jars.
3. 10% formalin.
4. Scalpel, fine forcep, blunt forcep.
5. Haematoxylin and Eosin (H & E) and PAS stains.

Inclusion criteria

The selection of cases should be based on following criteria-

- Cases should be registered in department of forensic medicine & toxicology.
- Consent from department of forensic medicine & Toxicology as well as from the attendant of dead individual will be taken before kidney.
- Cases include dead individual health and not suffering from disease affecting kidney.
- The exact time of death of individual should be known.

Exclusion criteria

- Cases of unknown time of death.
- Cases suffering from kidney disease.
- Cases exclude dead individual those preserve in ice or ice cooler.
- Cases complicated by other metabolic disorders like Diabetic mellitus, renal osteodystrophy, secondary hyperparathyroidism nutritional disturbance (PEM).

For histological examination, sample was preserved in 10% formal saline solution. In each case kidney was studied histologically. Tissue sample are taken from varies site like-cortex, cortico-medullary, medullo-pelvic

region and prepared of tissue under following ways- fixation, dehydration, clearing, embedding, section cutting mounting staining with haematoxylin, eosin stain and some special stain.

The stained slides were examined under light microscope for studying the various histological changes that take place in kidney at different time intervals after death.

PAS (Periodic acid Schiff) is used for glycogen, glycoprotein (such as mucus) and basement membranes which contain glycoprotein.

First gross changes in kidney were studied. Kidneys were enlarged and swollen and cut section cortex was widened, pale and medulla is dark than microscopic changes were graded from 0-4, these are as given in Table 1.

Table 1: Grading of microscopic changes from 0-4.

Grading	Microscopic changes
G0	No change
G1	Mild, architecture maintained mild cloudy swelling and disruption of epithelium. Glomeruli-small numbers of glomerular tufts were swollen; resulting in decreased size of bowman space, A few of the space had minute amounts of regurgitated non cellular debris present within them. No nuclear pyknosis or karyolysis as present within the tufts, a moderate number of DCT had obliterated lumen due to cellular swelling, DCT cells are in contact with basement membrane, no individualization was seen, cells of CT were in connecting the basement membrane ,had no pyknotic nuclei, patent lumen free of debris.
G2	Moderate, architecture maintained, more cloudy swelling and disruption of epithelium, Glomeruli - bowman space of some glomeruli was reduced or obliterated, glomerular tufts had shrunken and prominent space was present, debris was present in some bowman spaces, pyknosis glomerular nuclei was present. PCT some degree of cellular swelling and luminal obliteration, debris accumulated in open lumen and disruption of brush border, retraction of the basement membrane. Number of pyknotic cells was minimal.DCT epithelial cells of DCT started retracting of basement membrane; number of pyknotic nuclei was individualization of cells occurred.CT slightly retracted off basement membrane, individualization of cells.
G3	Severe architecture disturbed, cloudy swelling and disruption of epithelium is prominent, collapse of glomeruli. Glomeruli had narrowed or obliterated bowman's spaces, debris was seen in bowman's space, moderate pyknotic cells, karyolysis within the glomerular tufts. PCT number of lumens were obliterated, debris in lumen disruption of brush border segmental loss of the border, cellular retraction of basement membrane no of pyknotic cells karyolysis and loss of tubular architecture. DCT these epithelial cells were extensively retracted of the basement membrane pyknotic cell, individualization of cells karyolysis and loss of tubular architecture. CT retraction of basement membrane pyknotic cells, individualization, karyolysis and loss tubular architecture.
G4	Very severe complete collapse of glomeruli, disruption of epithelium , nuclei are fragmented, PCT are fragment, and pyknotic nuclei with fragmented, DCT cells and epithelium fragmented, CT collapse epithelium cells fragmented nuclei.

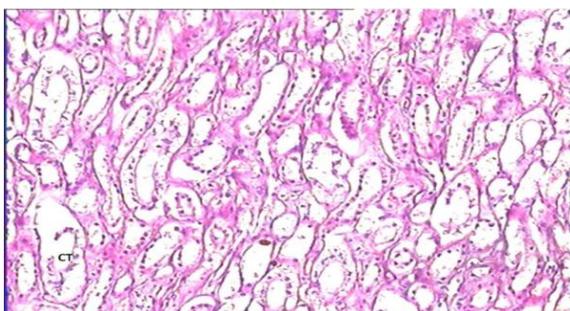


Figure 1: 21.3 hour, temperature 20/40.8°C, H&E stain 10x. Photomicrograph showing CT retraction, disruption of epithelium, individualization of cells at some places and debris present in the lumen.

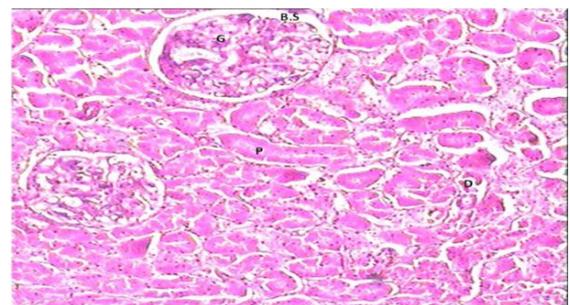


Figure 2: 46 hour, temperature 24.3/25.9°C, H&E stain 10x. Photomicrograph showing glomeruli (G) splinted, PCT (P) - oedematous, retraction of epithelium with debris in the lumen, DCT (D) - disruption of epithelium, debris present in the lumen.

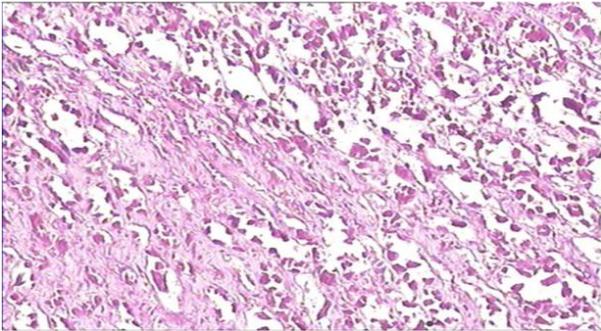


Figure 3: 52.30 hour, temperature 24.5/32.0°C, H&E stain 10x. Photomicrograph showing interstitium prominent in the medulla. CT disruption of epithelium with individualization of cells, debris present in the lumen.

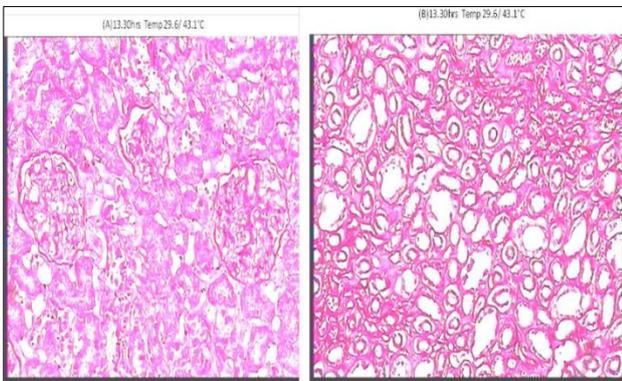


Figure 4 (A) & (B): PAS staining PMI, 30 hours, temperature 29.6/43.1°C, humidity 23/38% basement membrane of Bowman's capsules PAS+ completely while that of PCT & DCT partly PAS+, in the lumen of CT PAS+ debris.

Data was compiled in MS excel and checked for its completeness and correctness and then it was analyzed.

RESULTS

In the present study 50 cases of different age and sex were studied. Average environmental temperature ranges between 17.3/22.3-31.3/45°C, humidity between 11/36 to 75/95% and duration range was between 4 hours to 52.30 hours. Temperature and duration ranges were divided in 4 groups. After histological examination following changes were observed:

- In first 12 hour temperature 10-20°C, 6 cases shows mild to moderate degenerative changes.
- In 12-24 hour increasing temperature, 10-20°C nine case show mild to moderate, 20-30°C, 11 case show moderate to severe, 30-40°C, 13 cases shows moderate to severe and 40-50°C, 4 cases shows moderate to very severe changes

- In 24-36 hour with increasing temperature up to 30-40 and 40-50°C, 8 cases shows moderate to very severe
- In 36-48 hour with further increasing temperature 30-40°C, 4 cases shows severe to very severe
- In 48- 60 hour increasing temperature one case show very severe change was seen.

Table 2: Duration wise distribution of study subjects.

Duration range	No. of cases (n=50)
Up to 12 hours	6
12-24 hours	31
24-36 hours	8
36-48 hours	4
48-60 hours	1

Table 3: Temperature wise distribution of study subjects.

Temperature range	No. of cases (n=50)
10-20°C	15
20-30°C	12
30-40°C	10
40-50°C	13

Table 4: Degenerative histological changes in kidney according to duration.

Duration range	Grading				
	G0	G1	G2	G3	G4
	No.	No.	No.	No.	No.
Up to 12 hours (6 cases)	-	3	3	-	-
12-24 hours (31 cases)	-	7	13	11	-
24-36 hours (8 cases)	-	2	5	1	-
36-48 hours (4 cases)	-	-	2	2	-
48-60 hours (1 case)	-	-	-	-	1

(-) No case available

Table 5: Degenerative histological changes in kidney according to temperature.

Duration range	Grading				
	G0	G1	G2	G3	G4
	No.	No.	No.	No.	No.
10-20°C (15 cases)	-	9	6	-	-
20-30°C (12 cases)	-	-	8	4	-
30-40°C (10 cases)	-	-	4	6	-
40-50°C (13 cases)	-	-	6	6	1

(-) No case available

Table 6: Degenerative histological changes in kidney according to duration and temperature.

Temperature / Duration	Up to 12 hours (6 cases)	12 to 24 hours (31 cases)	24 to 36 hours (8 cases)	36 to 48 hours (4 cases)	48 to 60 hours (1 case)
10-20°C (15 cases)	Mild to moderate 6	Mild to moderate 9	-	-	-
20-30°C (12 cases)	-	Moderate to severe 11	-	-	-
30-40°C (10 cases)	-	Moderate to severe 6	Moderate to very severe 8	Severe to very severe 4	-
40-50°C (13 cases)	-	Moderate to very severe 5	-	-	Very severe 1

(-) No case available

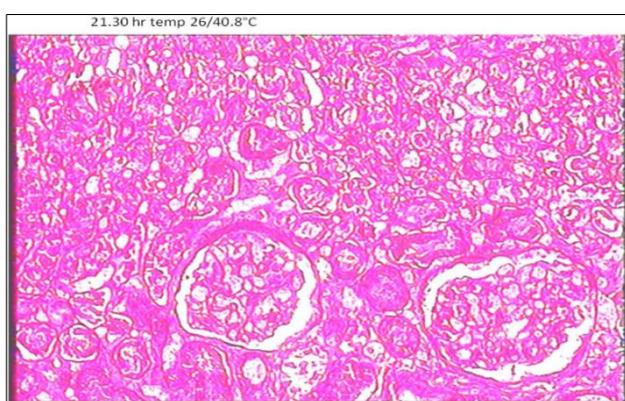


Figure 5: PAS staining PMI, 21.30 hours, temperature 26/40.8°C, basement membrane of Bowman's capsules is completely PAS +ve. Basement membranes of PCT & DCT are PAS +ve.

DISCUSSION

In study by Vinita Kushwaha et al.¹³ on 45 cases of human kidney were they observed that the rate of microscopic changes increases as the temperature and duration increase up to 24 hours and 31-35°C. But with further increase of temperature and duration mild moderate changes are observed. In this study 12-24 hour increasing temperature 10-20°C, 20-30°C, 30-40°C and 40-50°C were showed 9 (mild to moderate), 11 cases (moderate to severe), 13 cases (moderate to severe) and 4 cases (moderate to very severe) changes.

Porcine kidney, glomeruli swelled and largely obliterated Bowman's space in all except perfused specimens as observed by Deborah Barber.¹⁴ The number of glomeruli affected increasing with increased postmortem interval. Glomerular tufts became reduced in size and cellular pyknosis became conspicuous within 24 hours at 4°C and 12 hours at 24°C. PCT swelled extensively in all but the perfused specimen but showed no other morphologic changes until 48 hours after death at 4°C and 12 hours at 24°C. DCT were severely affected within 3 hours after

death. CT was moderately changed within 12 hours at 4°C and 3 hours at 24°C.

Whereas earliest remarkable postmortem histological changes were seen in DCT i.e. retraction as well as disruption (fragmented) of epithelium from the basement membrane although the cells were with nuclei after 4 hours (27.5/42.2°C temperature) in the current study. Present study also found that Basement membranes of parietal layer of Bowman capsules, PCT and DCT were PAS+ while in the interstitium of medulla PAS+ substances were in the form of small spots at places after 4 hours PMI (27.5/42.2°C, T) & 8.30 hours PMI (9.8/26.3°C, T). After 52.30 hours PMI (24.5/32°C, T) PAS+ substances were not seen.

Another study¹⁵ from India on histological changes in tissue and organ of rabbit, where they observed that kidney at 20°C mild and focal autolytic changes could be seen 24 hours after death. Between 36 & 48 hours these changes were marked and diffuse throughout the kidney substance, while after 72 hours post mortem severe autolytic changes could be seen. At 30°C Cloudy swelling of PCT & DCT was seen by 12 hours after death. By 24 hours there was diffuse cloudy swelling of the cells of renal tubules and this also involved the blood vessels and glomeruli. After 30 hours these changes became diffuse and more intense and there was serve autolysis in 48 hours. At 30°C bacterial infiltration and liquefaction of kidney after 72 hours. At 40°C, the changes became diffuse by 24 hours and observations made after 36 hours revealed advanced autolytic changes so that only vogue outlines of tubules, glomeruli and blood vessels could be made out. The finding of present study was also found comparable to above mention study. In 24-36 hour with increasing temperature up to 30-40 and 40-50°C 8 cases shows moderate to very severe, in 36-48 hour with further increasing temperature 30-40, 4 cases shows severe to very severe and in 48-60 hour increasing temperature one case show very severe change were observed in this study.

Choudhuri et al.¹⁵ were studied on kidney of goats where they observed cloudy swelling of tubular cells followed by cells of glomeruli and blood vessels in a sequence b/w 4-24 hours PM in both open air and pond water. Complete disintegration of kidney was seen at 48 hours in open air and on the 5th day in pond water. In refrigerator at 40°C the glomeruli remnants could still be identified after 5 days.

CONCLUSION

In the present study earliest remarkable postmortem histological changes were seen in DCT. Microscopic changes in kidney was increasing (mild to moderate, moderate to severe, severe to very severe) as temperature increases. Finding of present study will be useful for researchers and forensic experts.

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Ethical approval: The study was approved by the institutional ethics committee

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