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Original Research Article

Histopathological Findings in Kidney with Reference to PAS Stain in Medicolegal Autopsy Cases: A Cross-Sectional Study at a Tertiary Care Centre

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Abstract

Background: The kidney is a vital organ that is frequently involved in both pre-existing chronic disease and acute terminal events identified at medicolegal autopsy. Routine hematoxylin and eosin (H&E) staining, while useful, often fails to highlight subtle changes in glomerular and tubular basement membranes. The Periodic Acid-Schiff (PAS) stain, by selectively staining carbohydrate-rich structures, provides superior contrast for evaluating renal microanatomy, making it indispensable in forensic renal pathology.

Aims and Objectives: To elucidate and characterise histopathological changes in kidney tissue using the PAS technique in medicolegal cases, and to investigate and document proliferative changes in glomeruli, basement membrane, and Bowman's capsule while correlating histopathological findings with medicolegal aspects of renal disease.

Materials and Methods: This cross-sectional study was conducted at the Department of Pathology, Rajendra Institute of Medical Sciences (RIMS), Ranchi over 18 months (July 2024–2026). A total of 110 consecutive medicolegal autopsy kidney specimens were processed using standard tissue preparation protocols and stained with the PAS technique. Glomerular and tubular changes were graded using the Arzec and Blanchette grading system. Statistical analysis was performed using SPSS v.22; chi-square test was applied, and $p < 0.05$ was considered significant.

Results: The predominant age group was 31–40 years (30.0%) with a strong male preponderance (74.55%). Cardiorespiratory failure was the most common certified cause of death (65.45%). Diabetes mellitus (21.82%) and hypertension (15.45%) were the leading comorbidities. On PAS staining, moderate-to-severe GBM thickening was present in 45.0% of cases, mesangial expansion in 56.36%, and Bowman's capsule thickening in 44.55%. Tubular atrophy was noted in 57.27%, severe tubular damage in 35.0%, and interstitial fibrosis in 50.0%. Chronic pyelonephritis (26.36%), glomerulosclerosis (16.36%), and chronic glomerulonephritis (14.55%) were the most common final diagnoses. No statistically significant association was found between PAS-detected changes and cause of death.

Conclusion: Significant renal histological changes are common in medicolegal autopsy cases, many of which remain undetected during life. PAS staining is a reliable, cost-effective tool for detecting chronic basement membrane disease, tubular injury, and incidental lesions in forensic settings. Systematic histopathological examination of renal tissue should be considered routine in medicolegal autopsies.

Keywords: PAS Stain; Medicolegal Autopsy; Renal Histopathology; Glomerular Basement Membrane.

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Introduction

The kidney is essential to systemic homeostasis. Blood filtration, metabolic waste elimination, fluid and electrolyte balance regulation, acid–base equilibrium, and blood pressure control are crucial processes. Besides these functions, the kidney generates erythropoietin, renin, calcitriol, and klotho and aids glucose metabolism. Any dysfunction in these processes can lead to systemic effects, many of which remain clinically silent until the disease progresses [1].

Due to its intricate microanatomy and high metabolic requirement, the kidney is prone to several diseases. These include chronic systemic disorders like diabetes and hypertension; acute situations like shock and sepsis; and toxic and ischaemic injuries from poisoning, trauma, or environmental exposure [2]. Structured kidney damage typically does not correlate well with functional tests like serum creatinine or estimated glomerular filtration rate, a common renal medicine issue. Even with a severely damaged contralateral kidney, a healthy kidney can disguise early illness. This separation of shape and function is why microscopic renal tissue evaluation is the gold standard in clinical and forensic diagnosis [3, 4].

Medicolegal autopsies are performed on sudden, inexplicable, or suspicious deaths. Pathological investigation is crucial in such cases since the clinical history is sometimes lacking. Gross examination generally misses subtle or early pathological alterations in the kidney, which is involved in many systemic diseases and acute terminal events. Histopathological examination of renal tissue might reveal pre-existing diseases, acute traumas, and contributing events that may have caused death [5, 6]. Multiple Indian and worldwide investigations have detected kidney lesions in a considerable percentage of medicolegal corpses, many of which are incidental and would not have been found without thorough histological examination [7].

Routine H&E staining has been used to evaluate renal tissue histopathologically. H&E gives a broad morphological picture but fails to reveal carbohydrate-rich structures such as basement membranes, mesangial matrix, and tubular brush boundaries. The Periodic Acid–Schiff (PAS) stain, a vital renal pathology stain, gives glycogen, glycoproteins, glycolipids, and mucins a magenta hue. PAS improves kidney imaging of the GBM, Bowman's capsule, mesangial expansion, tubular basement membranes, and proximal tubular brush boundary [8, 9]. Acute tubular damage is indicated by the absence of a PAS-positive brush border in proximal tubules, while chronic diseases such as diabetic nephropathy and hypertensive nephrosclerosis are characterised by GBM thickness and mesangial expansion [10].

Renal pathology in medicolegal autopsy is varied. Acute tubular necrosis (ATN), tubulointerstitial, glomerulonephritis, nephrosclerosis, and toxic nephropathies are common [11]. Histological examination of shock, poisoning, sepsis, burns, and trauma deaths often shows tubular epithelial necrosis, flattening, luminal debris, and loss of PAS-positive brush-border boundaries [12, 13]. On PAS-stained sections, chronic hypertension-related nephrosclerosis appears as vascular hyalinisation, glomerulosclerosis, and interstitial fibrosis. These pathological alterations can cause death or impair physiological reserve and aggravate acute events [16]. Critically, many kidney abnormalities found at postmortem go undetected during life. According to Perrone et al., roughly one-third of adult postmortem cases had undiagnosed renal illness [17].

In India, snakebite envenomation causes haemorrhagic, acute tubular, and cortical necrosis, which PAS staining can identify. Hypovolaemic shock-induced renal ischaemia and tubular injury are common in burns and trauma deaths [18, 19, 20]. PAS staining is important in renal diagnosis; however, medicolegal autopsies from Indian tertiary care hospitals rarely capture renal histological abnormalities. The present study was therefore designed to evaluate histopathological changes in kidney tissue using the PAS stain in medicolegal autopsy cases at Rajendra Institute of Medical Sciences (RIMS), Ranchi, correlating findings with clinical history and cause of death, and contributing valuable data to forensic pathology literature from India.

Materials And Methods

This was a cross-sectional study conducted over a period of 18 months, from July 2024 to 2026, in the Department of Pathology at Rajendra Institute of Medical Sciences (RIMS), Ranchi. The study included renal tissue specimens obtained from medicolegal autopsy cases referred to the department. Sample size was calculated using the formula $N = 4PQ/d^2$, where prevalence (P) was estimated at 84% based on a reference study by Duduyemi et al. on special stains in postmortem kidney assessment, yielding a minimum sample of 110 cases.

Cases included were renal tissue specimens from all age groups and both sexes of medicolegal cases with available clinical history and adequate glomeruli for light microscopy. Excluded were specimens considered inadequate (too small for processing, absence of glomeruli on serial sections), or those lacking required clinical and histopathological details.

Kidney tissue received along with autopsy requisition forms was processed using standard histological protocols. Fixation was carried out in 10% neutral buffered formalin for a minimum of 4–6 hours, with overnight fixation preferred for optimal morphological preservation. Tissue was subsequently dehydrated through ascending concentrations of ethanol (70%, 80%, 90%, 100%), cleared in xylene, and embedded in molten paraffin wax. Paraffin blocks were sectioned at 3–5 μm thickness using a microtome, and sections were mounted on glass slides.

For PAS staining, deparaffinized sections were rehydrated through descending alcohol

concentrations. The glycol groups of polysaccharides were oxidized using 1% periodic acid solution for 5–10 minutes. After rinsing in distilled water, sections were treated with Schiff's reagent for 15–30 minutes, producing a magenta colour in carbohydrate-rich structures. Counterstaining was performed with haematoxylin for 3–5 minutes, followed by dehydration, clearing, and mounting in DPX. Stained sections were examined under light microscopy. All normal and pathological renal structures were systematically evaluated including the glomerular basement membrane (GBM), mesangial matrix, Bowman's capsule, tubular basement membranes, proximal tubular brush border, and interstitial architecture.

Glomerular and tubular changes were graded using the Arzec and Blanchette grading system. Glomerular basement membrane thickening and mesangial matrix expansion were each graded 0–3 (Grade 0: normal; Grade 1: mild; Grade 2: moderate; Grade 3: severe). Similarly, tubular basement membrane thickening and tubular damage were graded 0–3. Additional glomerular parameters such as Bowman's capsule thickening and glomerulosclerosis were recorded as present or absent. Tubular atrophy, glycogen accumulation, and interstitial changes including fibrosis and inflammatory cell infiltration were also recorded systematically.

Statistical Analysis

All relevant data were collected in structured case record forms and entered into Microsoft Excel for analysis using SPSS version 22.0 (IBM, USA). Descriptive analysis was performed using mean ± standard deviation, proportions, and range. Data were presented in the form of frequency distribution tables. Univariate, bivariate, and multivariate analyses were performed where appropriate. The chi-square test was used to assess associations between histopathological findings and cause of death. A p-value < 0.05 was considered statistically significant.

Results

A total of 110 medicolegal autopsy kidney specimens were evaluated over 18 months. The findings are presented in the following tables.

Table 1: Age Distribution of Medicolegal Autopsy Cases (n = 110)

		No. of Cases (n)	Percentage (%)
Age Group (years)	≤20	3	2.73
	21–30	12	10.91
	31–40	33	30.00
	41–50	13	11.82
	51–60	22	20.00
	>60	27	24.55
	Total	110	100.00

The 31–40 year age group was most frequently represented (30.0%), followed by individuals above 60 years (24.55%). Only 2.73% of cases were aged ≤20 years, indicating that renal histopathological changes in medicolegal autopsies are predominantly found in adults (Table 1).

Table 2: Sex Distribution and Cause of Death in Study Subjects (n = 110)

		n	%
Sex	Male	82	74.55
	Female	28	25.45
Cause of Death	Cardiorespiratory Failure	72	65.45
	Septic Shock	21	19.09
	Acute Renal Failure	11	10.00
	Haemorrhagic Stroke	6	5.45

Males constituted 74.55% of cases. Cardiorespiratory failure was the leading certified cause of death (65.45%), followed by septic shock (19.09%) and acute renal failure (10.00%) (Table 2).

Table 3: Relevant Clinical History of Study Subjects (n = 110)

Clinical Condition	n	%
Diabetes Mellitus	24	21.82
Hypertension	17	15.45
Chronic Kidney Disease	13	11.82
Uremic Encephalopathy	6	5.45
Epilepsy	6	5.45
Jaundice	6	5.45
PPH	6	5.45
Meningitis	4	3.64

Subdural Haemorrhage	4	3.64
Tuberculosis	4	3.64
Chronic Myeloid Leukaemia	1	0.91
Renal Cell Carcinoma	1	0.91
None	18	16.36

Diabetes mellitus (21.82%) and hypertension (15.45%) were the predominant comorbidities. Notably, 16.36% had no documented clinical history, highlighting the value of postmortem histology in uncovering silent disease (Table 3).

Table 4: PAS Stain Grading of Glomerular Basement Membrane Thickening and Mesangial Matrix Expansion (n = 110)

Finding	Grade	Description	n	%
GBM Thickening	0	Normal	52	47.27
	1	Mild	6	5.45
	2	Moderate	44	40.00
	3	Severe	8	7.27
Mesangial Expansion	0	Normal	33	30.00
	1	Mild	15	13.64
	2	Moderate	32	29.09
	3	Severe	30	27.27

Moderate-to-severe GBM thickening (≥Grade 2) was present in 47.27% of cases. Mesangial expansion was widespread, with Grades 2 and 3 together accounting for 56.36% of all cases. These chronic glomerular changes strongly reflected underlying metabolic and vascular comorbidities (Table 4).

Table 5: Other Glomerular Findings on PAS Staining (n = 110)

Finding	n	%
Bowman Capsule Thickening	49	44.55
Glomerulosclerosis	52	47.27
Chronic Glomerulonephritis	16	14.55
Chronic Pyelonephritis	29	26.36
Cloudy Swelling	10	9.09
Inflammatory Cell Infiltration	6	5.45
Granulomatous Lesion	5	4.55
Interstitial Nephritis	5	4.55
Leukemic Infiltration	5	4.55
Renal Cell Carcinoma	5	4.55
Autolysis	5	4.55
No Significant Pathology	6	5.45

Chronic pyelonephritis (26.36%) and glomerulosclerosis (47.27%) were the most frequent final diagnoses. Incidental findings including granulomatous lesions, leukemic infiltration, and renal cell carcinoma were each noted in 4.55% of cases, underscoring the value of histological examination in revealing unsuspected pathology (Table 5).

Table 6: Tubular and Interstitial Findings on PAS Staining (n = 110)

Parameter	Finding	n	%
Tubular BM Thickening	Grade 0 (Normal)	47	42.73
	Grade 1 (Mild)	0	0.00
	Grade 2 (Moderate)	52	47.27
	Grade 3 (Severe)	11	10.00
Tubular Damage	Grade 0 (Normal)	37	33.64
	Grade 1 (Mild)	10	9.09
	Grade 2 (Moderate)	23	20.91
	Grade 3 (Severe)	40	36.36
Tubular Atrophy	Present	63	57.27
	Absent	47	42.73
Glycogen Accumulation	Present	5	4.55
	Absent	105	95.45
Interstitial Fibrosis	Present (focal)	55	50.00

	Diffuse	13	11.82
	Normal	20	18.18
	No Abnormality	11	10.00

Moderate tubular BM thickening (Grade 2) was the most common tubular finding (47.27%). Severe tubular damage was found in 36.36% of cases and tubular atrophy in 57.27%. Interstitial fibrosis was the dominant interstitial pattern (50.0%), reflecting chronic injury burden. Glycogen accumulation was infrequent (4.55%), consistent with its association with specific metabolic disorders (Table 6).

Discussion

The present study examined histopathological findings in 110 medicolegal autopsy kidney specimens using the PAS stain at a tertiary care institution in Jharkhand, India. The results confirm that significant renal lesions are commonly encountered in medicolegal autopsies and that PAS staining adds meaningful diagnostic information beyond routine H&E assessment.

The predominance of cases in the 31–40 year age group (30.0%) and the over-60 cohort (24.55%) is consistent with the known epidemiology of medicolegal deaths, where adults bear the highest burden of both trauma-related and chronic disease-associated mortality. Similar findings were reported by Garg et al. [21], who noted the highest autopsy caseload in the 41–50 year group, and by Neha et al. [27], who found most lesions in the 41–60 year bracket, underscoring that renal pathology becomes more manifest with advancing age due to cumulative metabolic and vascular insults. In contrast, some studies from trauma-heavy settings such as Pathan et al. [24] and Jain et al. [25] reported more cases in younger adults (20–40 years), attributable to a higher proportion of accidental and traumatic deaths in those populations.

Male predominance in the present study (74.55%) is consistent across virtually all published autopsy series. Neha et al. [27] reported males in 79.4% of cases, while Garg et al. [21] and Basra et al. [26] recorded male predominance exceeding 70%. This pattern likely reflects greater male exposure to outdoor occupational hazards, accidents, substance use, and lifestyle-related chronic diseases such as hypertension and diabetes, all of which predispose to both medicolegal deaths and renal pathology detected on PAS staining.

Cardiorespiratory failure was the certified cause of death in 65.45% of our cases, followed by septic shock (19.09%) and acute renal failure (10.00%). This distribution is important in interpreting PAS findings: kidneys in cardiorespiratory failure deaths often exhibit chronic comorbid disease footprints such as vascular sclerosis, glomerulosclerosis, and interstitial fibrosis, while septic shock typically superimposes acute tubular injury on pre-existing disease. Neha et al. [27] and Pathan et al. [24] similarly highlighted that acute tubular necrosis (ATN) is the commonest renal lesion in autopsy series dominated by shock- and sepsis-related deaths, and that gross renal examination frequently appears normal even when significant microscopic damage is present, reinforcing why histology—including PAS staining—adds irreplaceable diagnostic value in forensic settings.

The finding that diabetes mellitus (21.82%) and hypertension (15.45%) were the leading comorbidities, and that 16.36% of subjects had no recorded clinical history, has important forensic implications. This substantial proportion of individuals with occult metabolic disease constitutes the very population in whom PAS-based evaluation is most rewarding: GBM thickening, mesangial expansion, and tubular basement membrane changes attributable to diabetic nephropathy or hypertensive nephrosclerosis are often the only evidence of pre-existing systemic disease at autopsy. Mulay et al. [22] reported similar findings in a tertiary centre autopsy study, noting that non-glomerular lesions reflecting tubulointerstitial and vascular injury constituted a major hidden disease burden in the community.

Glomerular basement membrane thickening of Grade 2 or more was present in 47.27% of our cases, with the highest prevalence in the CKD/DM/HTN group (51.28%). Although higher-grade GBM thickening was less frequent in septic shock cases (33.33%), the chi-square analysis showed no statistically significant association with cause of death ($p = 0.341$), indicating that GBM thickening is a marker of chronic metabolic and vascular disease burden rather than a reflection of the terminal event. Mesangial expansion of Grade 2 or more was even more prevalent, affecting 56.36% of all cases. This high frequency of mesangial disease is consistent with reports from Basra et al. [26] and further supports the notion that chronic glomerular injury represents a substantial, often silent, community burden in India.

Tubular changes were prominent in the current study. Moderate-to-severe tubular basement membrane thickening (≥Grade 2) was present in 57.27% of cases, and severe tubular damage (Grade 3) was noted in 36.36%. Tubular atrophy was the most frequent individual tubular finding, present in 57.27% of specimens. These results align with autopsy series in which tubular lesions consistently predominate: Garg et al. [21] found ATN and cloudy swelling as the leading lesions (28.6% and 13.3%

respectively), and Neha et al. [27] reported tubular necrosis as the single most common diagnosis (51.8%). Interestingly, the association between tubular damage grade and cause of death did not reach statistical significance ($p = 0.847$), suggesting that while acute tubular injury occurs in all terminal scenarios, its severity is shaped by the interaction of pre-existing chronic injury and terminal physiology.

Interstitial fibrosis was the dominant interstitial pattern (50.0%), with diffuse fibrosis in an additional 11.82%. Final diagnosis of chronic pyelonephritis was the most frequent (26.36%), followed by glomerulosclerosis (16.36%) and chronic glomerulonephritis (14.55%). These chronic tubulointerstitial diagnoses are a recurring feature in autopsy renal pathology. Pathan et al. [24] described chronic tubulointerstitial nephritis as a major category (29.78%), and Mulay et al. [22] similarly documented chronic pyelonephritis among the leading lesions. The PAS stain is particularly valuable in recognizing the thickened tubular basement membranes, "thyroidization" of tubules, and interstitial scarring that characterize chronic pyelonephritis, features that may be subtle on H&E alone.

A particularly valuable aspect of routine renal histopathology in medicolegal autopsies is the identification of incidental but clinically significant lesions. In the present study, granulomatous lesions, leukemic infiltration, and renal cell carcinoma were each detected in 4.55% of cases. These findings are consistent with reports from Gouda et al. [23] and Mulay et al. [22], who also documented incidental renal tumours at autopsy. Such discoveries carry important medicolegal significance, potentially altering the understanding of a decedent's medical background and the circumstances surrounding death.

The study has several limitations. It was conducted at a single tertiary centre with a sample of 110 cases, limiting generalizability. Comprehensive clinical history and premortem laboratory data were unavailable in many cases. The duration and progression of renal disease before death could not be determined. Only PAS staining was employed; complementary techniques such as immunofluorescence, silver staining (Jones methenamine), Masson's trichrome, and electron microscopy were not applied, which constrains the full characterisation of some lesions.

Conclusion

This study demonstrates that significant renal histological changes are a frequent finding in medicolegal autopsy specimens, the majority of which remain undiagnosed during the patient's lifetime. The PAS stain is a reliable, cost-effective, and highly informative tool that clearly delineates glomerular basement membrane thickening, mesangial expansion, tubular injury, and interstitial fibrosis. Chronic conditions such as diabetes mellitus, hypertension, and chronic kidney disease leave a distinctive histopathological signature in renal tissue that is readily identifiable on PAS-stained sections even in the absence of a clinical history.

The absence of a statistically significant association between PAS-detected changes and the immediate cause of death further reinforces that most observed lesions reflect underlying chronic disease burden rather than acute terminal events. Systematic histopathological examination of kidneys using PAS staining should be considered a standard component of medicolegal autopsy protocols, as it aids in the detection of concealed renal pathologies, helps accurately determine the degree of renal dysfunction, and provides vital information for both medical and forensic purposes.

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