BMC Nephrology

CASE REPORT

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An autopsy case with tubular obstruction by impacted swollen blebs due to ischemic acute kidney injury

Yukako Akiyama[1*], Mitsuhiro Sato[1] and Yoshio Taguma[1]

Abstract

Background Oliguric acute kidney injury (AKI) is one of the critical conditions which needs emergent treatment due to the lack of the capacity of excreting toxins and fluids, and plasma membrane bleb formation is considered as one of the characteristic morphologic alterations in ischemic AKI in both animal models and human. We present here an autopsy case with clear electron microscopy images capturing a definitive instance of blebbing in ischemic AKI.

Case presentation A 66-year-old man was admitted for oliguric AKI with nephrotic syndrome (NS). Because of the existence of hematuria with red blood cell casts and rapid deterioration of renal function and severe systemic symptoms such as loss of appetite and general fatigue, we started immunosuppressive therapy with steroids, considering a vasculitis-like condition with NS, and hemodialysis was also started for oliguria. However, he suddenly died of hemorrhagic shock due to gastric ulcer. Histological findings of the kidney by autopsy showed segmental sclerosis and acute tubular necrosis (ATN) in paraffin sections, which suggests that this is the case with ATN showing oliguric AKI as a clinical presentation. Interestingly, in electron microscopical study, not only apical membrane blebbing but also numerous cytoplasmic bodies were observed in proximal tubules (PT), and this bleb formation was also observed as foamy blebs in the Toluidine blue stained Epon section, where it appeared to fill the tubular lumen.

Conclusion Our distinct finding of bleb formation with tubular obstruction strongly indicates that blebbing could be related to the mechanism of oliguric AKI in human, which supports the tubular obstruction theory as a contributing factor to the pathogenesis of ischemic AKI.

Keywords Acute kidney injury, Autopsy, Bleb formation, Epon section, Toluidine blue-stain, Tubular obstruction theory

*Correspondence:
Yukako Akiyama
Yukako.Akiyama@ucsf.edu
[1]Department of Nephrology, Japan Community Healthcare Organization Sendai Hospital, 981-3281 Sendai, Miyagi, Japan

Background

Acute kidney injury (AKI) is a global public health problem because of high mortality and difficulty of treatment. According to the Kidney Disease Improving Global Outcomes (KDIGO) guidelines, AKI is defined as follows: Increase in serum creatinine (sCr) by ≥ 0.3 mg/dl (≥ 26.5lmol/l) within 48 h; or increase in sCr to ≥ 1.5 times baseline, which is known or presumed to have occurred within the prior 7 days; or urine volume < 0.5 ml/kg/h for 6 h [1]. AKI can present as non-oliguric, while on the other hand, there are cases of AKI rapidly progressing to oliguria requiring renal replacement therapy, and oliguric AKI has been reported to carry a higher risk of mortality [2]. Although the specific mechanism leading to oliguria has not fully elucidated, some factors have been advocated as the pathogenesis of the decline in GFR: a decrease of glomerular capillary permeability, back-leak of glomerular filtrate, tubular obstruction, intrarenal vasoconstriction, and generation of reactive oxygen species [3, 4]. Among these factors, the tubular obstruction by impacted swollen blebs generated in PT have been reported in ischemic AKI in animal models [5–8], and apical membrane blebbing was observed in PTs with ischemia in rats [5, 7]. The presence of microvesicles likely derived from blebs in human tubular lumens and urine has also been documented [9–11]. We here present a human case of AKI that shows prominent bleb formation in PTs which seems to cause tubular obstruction.

Case presentation

A 66-year-old man with a long-term smoking history and hypertension since the age of 40 and the history of a cerebral hemorrhage at the age of 43 resulting in left hemiplegia developed type 2 diabetes at the age of 60 and regularly visited the clinic for follow-up. He visited his previous physician with decreased appetite, severe systemic fatigue, and edema following cough and runny nose, along with a rapid weight gain of 7 kg over 10 days. His diabetes had been well controlled under medication without the development of retinopathy, and his renal function had been stable for several years with serum creatinine (sCr) levels ranging from 1.4 to 1.6 mg/dL. Although the urine test was not performed at the previous hospital, the patient had never exhibited nephrotic syndrome, nor had macrohematuria been previously identified. At the time of consultation, the patient presented with rapidly progressive renal impairment with nephrotic syndrome, accompanied by a creatinine level of 9.7 mg/dL. Because pneumonia was recognized on a plane computed tomography (CT) with pulmonary infiltrates, ceftriaxone (CTRX, 1 g/day) was started. However, since renal function exacerbated with massive proteinuria and hematuria, and urine volume decreased, the patient was transferred to our hospital three days later. At admission to our hospital, blood pressure was 150/70 mmHg, SpO_2 (room air) was 95% without fever, and physical findings showed no obvious abnormality other than edema. Laboratory investigations revealed the following: white blood cell (WBC) 15,840/μL, red blood cell (RBC) 4,440,000/mm^2, platelet 289,000/μL, blood urea nitrogen (BUN) 130 mg/dL, sCr 10.3 mg/dL, eGFR 4.51 mL/min/1.73m^2, total protein 5.5 g/dL, serum albumin 1.7 g/dL, total cholesterol 245 mg/dL, triglyceride 336 mg/dL, C-reactive protein (CRP) 12 mg/dL, HbA1c 6.4%, haptoglobulin 264 mg/dL, IgG 987 mg/dL, IgA 572 mg/dL, IgM 34 mg/dL, free immunoglobulin light chains: κ-type 206 mg/L, λ-type 189 mg/L, dip stick of urinary protein 4+, urinary protein (24 h urine collection) 5.29 g/day, urine protein/urine creatinine 13.8 g/gCr, selectivity index 0.40, urine occult blood reaction 3+, urine β2-microglobulin 17,744 ug/L, and prominent microscopic hematuria with various casts. Anti-neutrophil cytoplasmic antibody (ANCA), anti-GBM antibody, and anti-nuclear antibody (ANA), cryoglobulin were found negative, and the complement level was normal. Fundoscopic examination at the time of admission revealed no signs of diabetic retinopathy in either eye. The result of the Scheie classification was H1S1.

We considered his clinical condition to be RPGN-like condition because of the rapid deterioration of renal function, systemic symptoms such as strong systemic fatigue and loss of appetite, the presence of severe microscopic hematuria accompanied by various casts. Therefore, we treated him with high-dose intravenous methylprednisolone (500 mg for three days) followed by oral prednisone (30 mg/day) and a single dose of intravenous cyclophosphamide (500 mg). Despite the treatment, renal function was not improved, and oliguria persisted. Meanwhile, bleeding from multiple gastric ulcers was observed and successfully treated with endoscopic hemostasis, as confirmed by a subsequent endoscopy. On the 24th day after administration, the patient suddenly went into shock and died without prior signs of hematemesis or melena. An autopsy was performed one hour after death. Anatomicopathologically, hemorrhagic gastric ulcers with ruptured blood vessels were observed, which was considered as the direct cause of death. In the renal pathological section, a total of 89 glomeruli were observed. Among them, 18 glomeruli (20.2%) showed global sclerosis, and 20 glomeruli (22.5%) showed segmental sclerotic lesion (Fig. 1A).

The remaining glomeruli were hypertrophic and most of them showed subendothelial swelling with occasional double contours as shown in Fig. 1B.

Diffuse mesangial matrix prominence in addition to the thickening of the glomerular basement membrane in electron microscopy also suggested early diabetic change. Tubulointerstitial changes such as tubular atrophy,

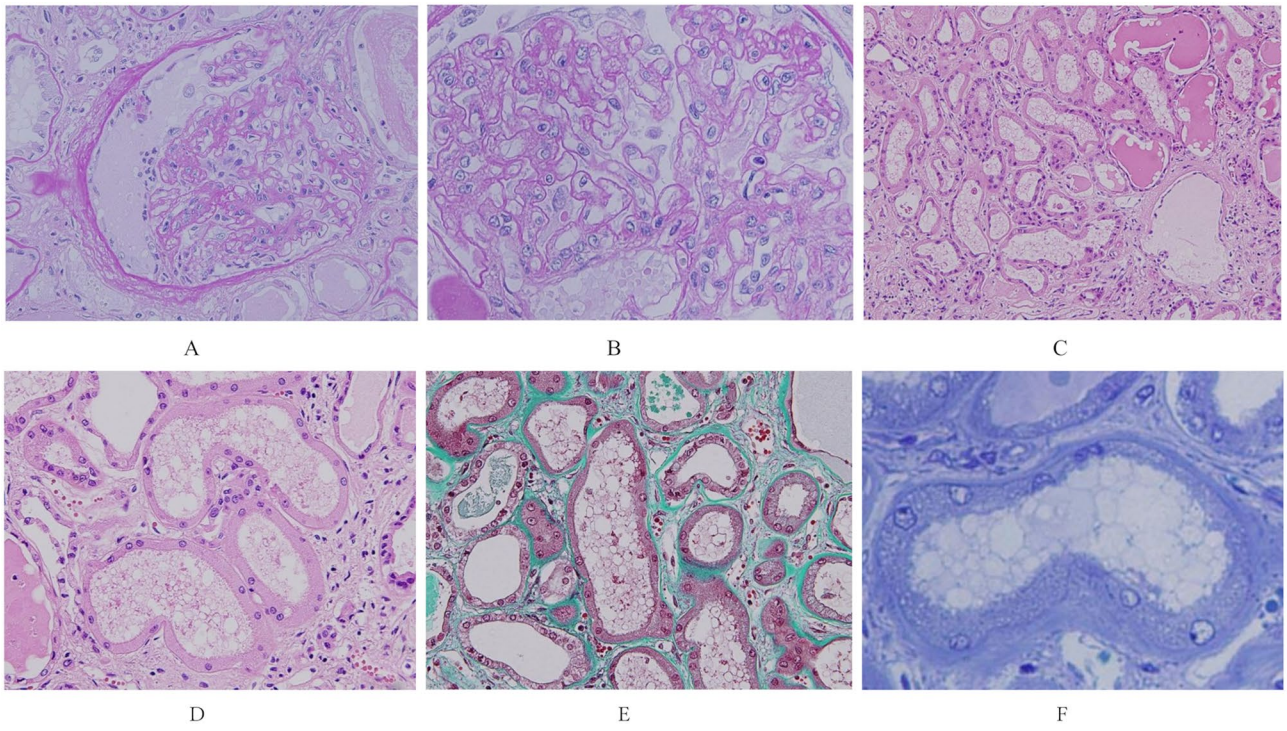

Fig. 1 Light microscopy findings of the kidney specimen. **(A)** Glomerulus with segmental sclerotic lesion. (Periodic acid Schiff staining. ×200) **(B)** Glomerulus showing subendothelial swelling with occasional double contours. The endothelial cells are swollen, and mesangial edema is observed. (Periodic acid Schiff staining. ×400) **(C)** Expansion of renal tubular lumen and simplification of epithelial cells. (Hematoxylin and eosin staining. ×100) **(D)** In Hematoxylin and eosin staining, granular or bubble-like materials are found in the tubular lumen. (×200) **(E)** Bubble-like materials are also found in Elastica-Masson staining. (×200) **(F)** In the Epon section, the renal tubular lumina is filled with foamy blebs. (Toluidine blue stained Epon section. ×200)

cell infiltration, and edema were observed in almost 60% of the tubulointerstitial area. Tubular epithelium changes like acute tubular necrosis (ATN) were also seen (Fig. 1C).

Remarkably, many dilated proximal tubules (PT) lumina were filled with eosinophilic granular materials in Hematoxylin and eosin stained and Elastica-Masson stained paraffin sections (Fig. 1D, E), and the same part exhibited foamy structures (bleb formation) in Toluidine blue-stained EPON sections (Fig. 1F).

Immunostaining revealed IgM and C3 deposition in the sclerotic areas.

In the electron microscopical study, tubular lumina were filled with impacted swollen blebs (Fig. 2A, B).

Interestingly, in addition to direct bleb formation from the apical membrane, we also observed the presence of numerous cytoplasmic bodies in cytoplasm of the proximal tubular cells, which appeared to be secreted into the tubular lumen and contributed to the bleb formation.

Discussion and conclusions

This case clearly shows that bleb formation is observed not only in animal models but also in human ischemic AKI, and bleb formation provides the morphological basis for tubular obstruction mechanism which probably leads to the reduction of urine volume and GFR as this possibility has been previously suggested [7, 9, 12, 13].

According to speculation from animal models, blebs in PT seem to be generated in three ways. (1) A part of membrane-bounded cytoplasm extrudes into the tubular lumen in varied irregular shapes and thereafter separates from the cell. (2) A part of the cytoplasm fluxes into the brush border microvilli, and the microvilli gradually swell and transform into blebs and separate. (3) So-called cytoplasmic bodies are generated intracellularly and extrude into the lumen [6, 14]. Chen et al. also reported two types of bleb formation: at the tips of microvilli and on the apical side of severely damaged cells that had lost their microvilli, with the possibility that alterations in membrane-cytoskeleton linkers may facilitate bleb formation and detachment by weakening membrane-cytoskeleton interactions [8]. Additionally, other previous studies have documented the frequent extrusion of a part of the tubular epithelial cytoplasm into the lumen under ischemic conditions [15, 16]. This time, in electron microscopy, we observed not only apical membrane blebbing but also numerous small structures that seem to be precursors of the blebs, so-called cytoplasmic bodies, in cytoplasm of PTs, and these findings may support tubular obstruction mechanism through bleb formation as a pathogenesis of ischemic AKI in human. Previous studies have

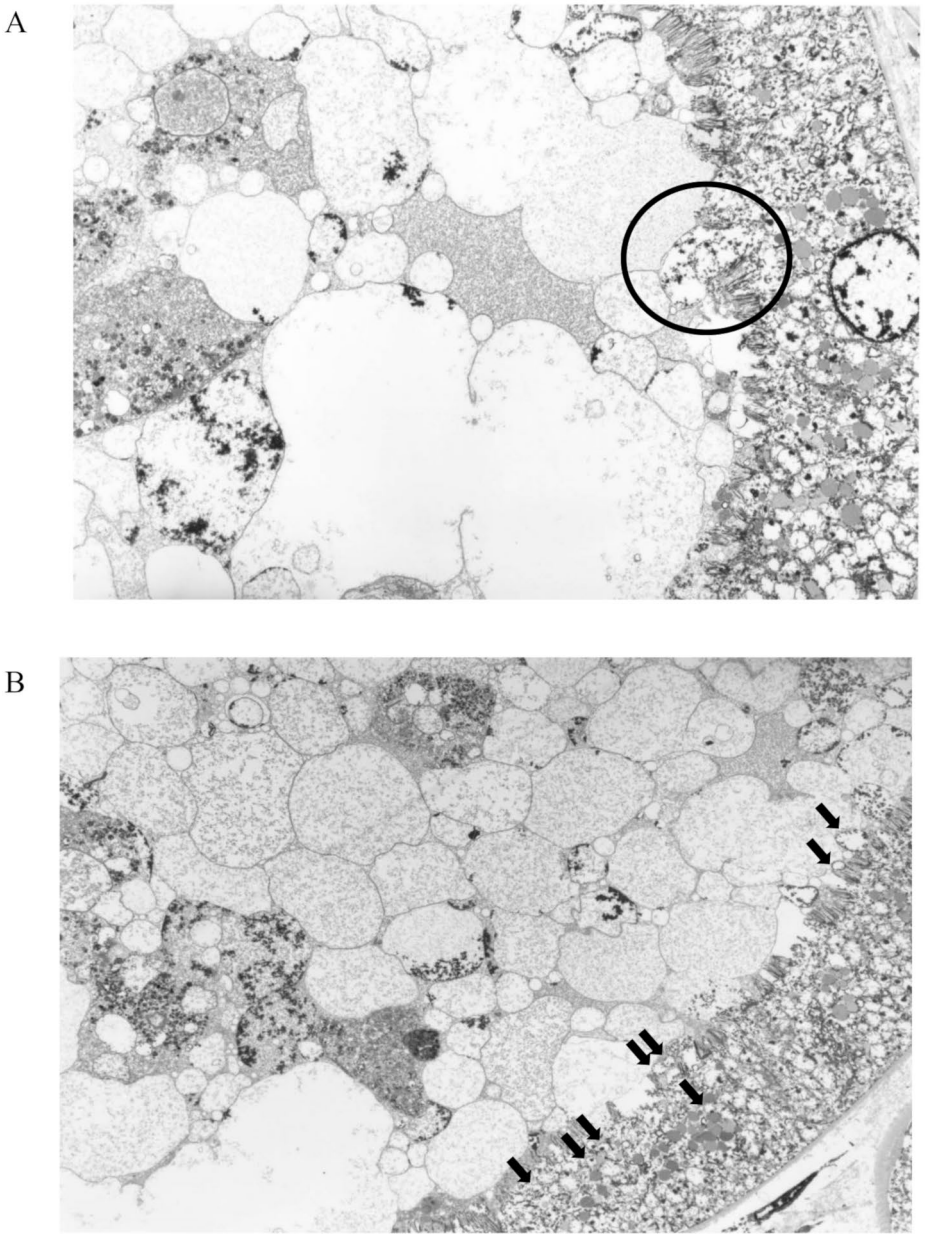

Fig. 2 Electron microscopy findings of the kidney specimen. The renal tubular lumen is filled with blebs and obstructed. (×1500). **(A)** Bleb formation from apical membrane is observed (black circle). **(B)** So-called cytoplasmic bodies are observed in cytoplasm of proximal tubular cells (black arrows)

highlighted the potential role of blebs forming on the brush border of renal tubules in causing tubular obstruction [7, 9, 12, 13]. Furthermore, several reports have documented the presence of microvesicles in human urine [10, 11], which is likely derived from tubular blebs. This supports the hypothesis that these microvesicles, once shed from the tubules, can persist without degradation until excretion in the urine, thereby suggesting a possible mechanism by which blebs could contribute to tubular obstruction.

Considering the past study reporting that bleb formation was observed within 10 min of onset of ischemia [7], this bleb formation may occur at an earlier stage than cell detachment, potentially contributing to decreased urine output and decreased GFR. This case presented with oliguric AKI along with the sudden onset of nephrotic syndrome, suggesting ischemia directly caused by severe NS seems to trigger bleb formation resulting in oliguric AKI. In addition, electron microscopic finding of apical membrane blebbing with innumerable cytoplasmic

bodies in PTs strongly suggests that this bleb formation is literally different from usual debris. While hemorrhagic shock may have further facilitated this bleb formation, given the time course that AKI appeared obviously earlier than hemorrhagic shock, almost simultaneously with the onset of severe NS, it is more likely that blebbing in PTs occurred concurrently with NS because of the intravascular dehydration, ultimately resulting in oliguric AKI as a clinical presentation.

Despite that some reports have demonstrated the bleb formation in animal models as described above [6–8], clear evidence of bleb formation, as observed in this case, has not been frequently documented in humans. We speculate that there are two reasons for this. First, kidney biopsy is sometimes clinically avoided in scenarios where systemic deterioration suggests a high risk of post-biopsy complications although a biopsy is desirable for AKI cases without an apparent cause. Studies have shown that AKI patients experience higher rates of post-biopsy complications and blood transfusions compared to non-AKI patients [17–19], and despite the KDIGO guidelines recommending kidney biopsy for AKI, its biopsy rate still remains low [18, 19]. For instance, a report from Brigham and Women's Hospital noted that among 4,903 hospitalized patients who met the laboratory criteria for AKI, only 28 underwent a kidney biopsy [18]. Another study reported that even for early AKI, the biopsy rate was merely 20.5% [20]. Therefore, the histological details of ischemic AKI are still unclear. The second is due to the difficulties of the detection of blebs since this finding is sometimes unclear in usual paraffin sections although it can be easily detected in Epon sections. Thus, we could consider this lesion as cell debris or insignificant artifacts, and they may be removed through the process of pathological preparation in paraffin sections, which could be one of the reasons to be overlooked.

In glomerular pathology, segmental sclerotic lesion was mainly observed. This is the case with pre-existing CKD, hypertension, and diabetes mellitus without diabetic retinopathy, who rapidly developed nephrotic syndrome and oliguric AKI. Although there is the possibility of secondary FSGS, the clinical course could not be fully explained by hypertension- or diabetes-related kidney damage alone and suggested the potential coexistence of MCNS or FSGS, and the histological finding of segmental sclerotic lesions in 22.5% of glomeruli and immunostaining results could not exclude the possibility of FSGS. However, given the further complexity of the case, including the fact that kidney biopsy was performed postmortem, which may have affected the histological findings such as the detachment of podocyte, determining the definitive cause of the nephrotic syndrome based on kidney pathology was challenging. Additionally, because clinical course showed rapid progressive renal dysfunction with nephrotic proteinuria accompanied by hematuria and critical systemic symptoms such as strong generalized fatigue like systematic vasculitis, it was considered different from the usual course of FSGS. Brown et al. previously reported the existence of the treatment-resistant cases of severe NS with microhematuria whose kidney biopsy showed minimal changes with FSGS and suggested the possibility of a different primarily vascular pathogenesis compared to the patients with similar histological appearances [21], and based on light microscopy and immunofluorescence findings, the coexistence of FSGS in this case remains plausible.

Another specific finding in histological analysis was observed in the tubulo-interstitium which corresponds to ATN. This lesion was considered to be caused by ischemia. The kidney of autopsied subjects was also exposed to the ischemia in the agonal stage and the period from death to the autopsy. However, the typical finding of ATN as observed in this case is not commonly observed in autopsy subjects. Therefore, we speculate that the presence of ATN, which appears to be caused by ischemia, occurred at an early stage due to severe NS, rather than changes occurring at the time of death. All this process seems to start with the onset of NS, which led to intravascular dehydration and ischemic AKI. The blebbing might have developed during the course of these processes, causing tubular obstruction and prolonged oliguria.

One of the limitations of our study is the potential influence of hemorrhagic shock on blebbing. Another limitation is that we cannot deny the effect of postmortem changes, which can affect renal histology. However, based on data demonstrating rapid bleb formation following ischemia in animal models and considering the clinical course of this case, we believe that these changes are influenced not only by postmortem alterations but also by ischemic effects caused by severe NS.

In this study, we successfully captured clear bleb formation in proximal tubular epithelial cells in humans. Many aspects of blebbing, including its contribution to tubular obstruction, remain insufficiently elucidated. Further studies are needed to clarify the role of blebbing in ischemic AKI.

Abbreviation

AKI	Acute kidney injury
PT	Proximal tubules
NS	Nephrotic syndrome
FSGS	Focal segmental glomerulosclerosis
ATN	Acute tubular necrosis
TMA	Thrombotic microangiopathy
GFR	Glomerular filtration rate
BUN	Blood urea nitrogen
ANCA	Anti-neutrophil cytoplasmic antibody
ANA	Anti-nuclear antibody

Acknowledgements
We thank Mr. Onodera for his skillful histological management.

Author contributions
YA, MS, YT diagnosed the patient. YA clinically monitored and treated the patient. YA and MS performed the histological study of the kidney. MS and YT supervised whole course of the treatment. YA and MS wrote the original draft and revised the manuscript.

Funding
This report did not recieve any funding.

Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
This study was approved by the Ethics Committee of Japan Community Healthcare Organization Sendai Hospital. The protocol number is 2024-10.

Consent for publication
Written informed consent for publication was obtained from their next of kin.

Competing interests
The authors declare no competing interests.

Received: 4 August 2024 / Accepted: 6 January 2025
Published online: 29 January 2025

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