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CASE SERIES

Podocyte infolding glomerulopathy: Clinical insights from two case studies in Malaysia

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Abstract

Introduction: Podocyte infolding glomerulopathy (PIG) is a rare glomerular disorder characterised by the infolding of podocytes into the glomerular basement membrane (GBM) with the presence of intramembranous cytoplasmic microspherules or microtubules. Most patients with PIG presented with nephrotic or subnephrotic proteinuria accompanied with microscopic haematuria. The condition is often associated with autoimmune diseases. *Case Report:* Recently, two cases of PIG were reported in Malaysia for the first time involving two female patients aged 25 and 36 years. The first patient had a history of systemic lupus erythematosus (SLE), peripheral neuropathy, anti-nuclear matrix protein 2 (NXP2)-positive antibody, autoimmune hypothyroidism, and primary ovarian failure. She presented with persistent nephrotic range proteinuria and haematuria. Meanwhile, the second patient with history of SLE presented at rheumatology clinic with bilateral pedal oedema, frothy urine, and haematuria. The electron microscopy (EM) analysis of the first patient revealed widespread and extensive invagination of podocyte cytoplasmic processes into the GBM, forming subepithelial clusters of microspherules and microtubules and separated by an intervening basement membrane. Likewise, extensive infolding of podocyte cytoplasmic processes into the GBM were found in the second case, and the intervening basement membrane separated the microspherules and microtubules. *Discussion:* These findings confirmed the diagnosis of PIG, and the patients were treated with Prednisolone, maintaining normal creatinine level during follow-up. In summary, PIG is a rare and new glomerular disease which has been known to be associated with connective tissue diseases, predominantly affecting young individuals with a favourable clinical outcome.

Keywords: Podocyte infolding glomerulopathy, glomerular disease, renal biopsy, glomerular basement membrane, intramembranous cytoplasmic microspherules

INTRODUCTION

Podocyte infolding glomerulopathy (PIG) is a rare glomerular disease worldwide and is recognised as a distinct clinical entity. This kidney disorder was discovered in 2002 and was officially described as a new entity in 2008. A total of 26 cases have been reported from Japan, eight from China, and one case from the USA, Germany, India, South Korea, Argentina, and Canada, respectively. Most patients with PIG are presented with nephrotic or subnephrotic proteinuria with microscopic haematuria and were often associated with autoimmune diseases, such as systemic lupus erythematosus (SLE), collagen diseases, and haematological malignancy, including multiple myeloma. The key features observed under electron microscopy (EM) in the renal biopsy of PIG patients include the infolding or protrusion of podocytes into the glomerular basement membrane (GBM) and the presence of intramembranous cytoplasmic microspherules or microtubules. This report

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describes the first two cases of PIG diagnosed in Malaysia and a review of the literature.

CASE REPORT

Clinical presentation and investigations

Case 1: A 25-year-old female with a medical history of SLE, peripheral neuropathy, anti-nuclear matrix protein 2 (NXP2) positive antibody, autoimmune hypothyroidism, and primary ovarian failure was presented with persistent sub-nephrotic range proteinuria and haematuria. The patient's 24-hour renal function indicators were as follows: urine protein: 0.5 g/day, creatinine level: 30 μmol/L, and the urine protein creatinine ratio was approximately 164 mg/mmol. Upon admission, the patient was well-oriented, and her vital signs were normal. An ultrasound of the kidney, ureter and bladder (KUB) revealed no hydronephrosis or renal thrombosis. The patient's hepatitis B, hepatitis C, and HIV were also negative. Complement levels, including C3 and C4, were low. Anti-nuclear antibodies (ANA) and double-stranded DNA (dsDNA) were positive, while the rheumatoid factor (RF) was negative (Table 1). Subsequently, the patient was subjected to a renal biopsy.

Case 2: A 36-year-old Malay female with a history of joint pain and photosensitivity diagnosed with SLE in February 2021 was presented at the rheumatology clinic with bilateral pedal oedema, frothy urine, and haematuria. A review of her other systems was unremarkable. On physical examination, her temperature was 36.2°C, respiratory rate was 16 per breath per minute, blood pressure was 167/90 mm Hg, heart rate was 62 beats per minute (bpm), and her oxygen saturation was within normal limits on ambient air. Bilateral pedal oedema was detected, but her chest, cardiac, and abdominal examinations were normal. The patient's 24-hour urine protein was 0.6 g/24 hr, with urea and creatinine levels within normal limits. Moreover, the patient's C3 and C4 levels were low, and her ANA and anti-dsDNA levels were positive. However, her infective screening for hepatitis B, hepatitis C, and HIV were non-reactive. Her albumin was also low (Table 1).

Diagnosis

Case 1: The patient's renal biopsy exhibited near-normal-looking light microscopy findings, demonstrating only subtle, patchy thickening of the capillary walls (Figures 1A & 1B) lacking in dominant immune deposits. The immunofluorescence (IF) and ultrastructural findings did not favour lupus nephritis, given the lack of full house positivity and the absence of subepithelial electron-dense deposits (EDD) or any forms of EDD. Notably, the EM analysis revealed widespread and extensive invagination and infolding of podocyte cytoplasmic processes into the glomerular basement membrane (GBM). These structures appeared as subepithelial clusters of microspherules and microtubules, separated by an intervening basement membrane (Figures 1C & 1D). The size of the membrane-bound microspherules and microtubules ranged from 45 nm to 50 nm. These findings confirmed the PIG diagnosis.

Case 2: The patient's renal biopsy revealed capillary wall thickening with membrane vacuolation and occasional red trichrome deposits. No distinctive subepithelial deposits or subepithelial spikes were observed. Mild mesangial hypercellularity was noted (Figures 2A & 2B). The IF assessment indicated trace positivity for IgG, IgM & IgA. Furthermore, the IF and ultrastructural findings did not support a diagnosis of lupus nephritis due to the lack of the full house staining pattern and the absence of subepithelial EDD. Nevertheless, there was extensive invagination and infolding of podocyte cytoplasmic processes into the GBM, and the microspherules and microtubules were separated by intervening basement membrane (Figures 2C & 2D).

Treatment

Both patients were diagnosed with PIG and were treated with Prednisolone tablets.

Follow-up and outcomes

Both patients maintained normal serum creatinine levels throughout the follow-up period.

DISCUSSION

PIG is a rare kidney disorder, and the literature on this glomerular disease remains lacking. The morphological pattern was first identified as a new and unique entity in 1992 by Sato *et al*.[1] in Japan. It is described as the diffuse thickening of the GBM with small intramembranous electron-dense deposits observed under EM and is commonly associated with autoimmune diseases. In 2008, the Japanese Society of Nephrology research working group proposed the PIG term following their study of 25 renal biopsy cases.[2] The EM findings of these cases

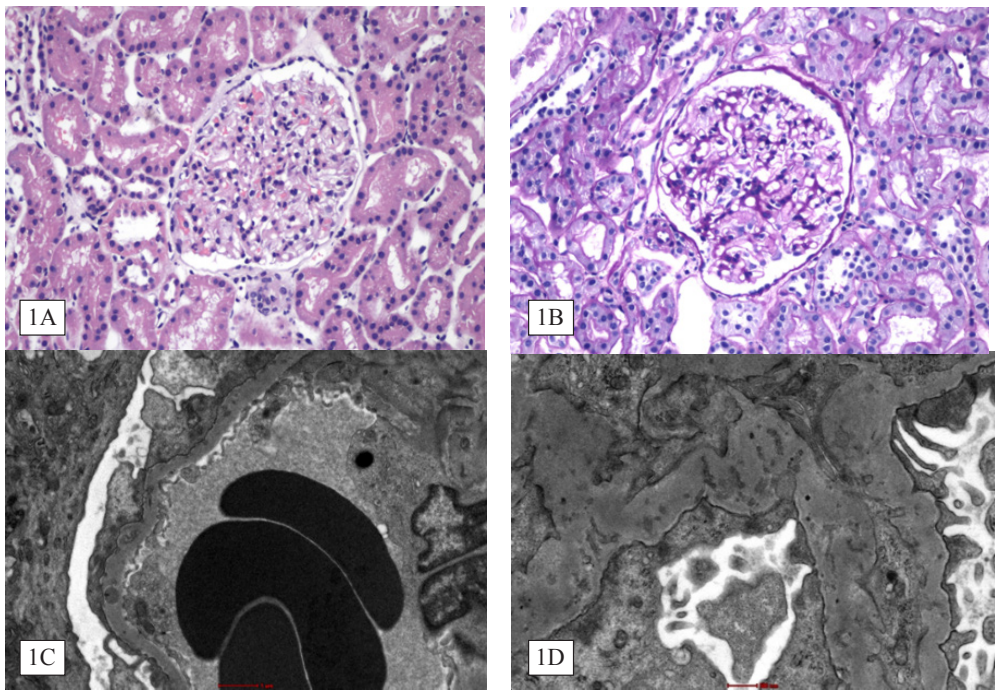

FIG. 1. (A-B) Photomicrographs show a glomerulus with minor glomerular changes and focal mesangial matrix expansion accompanied by mild capillary wall thickening, haematoxylin & eosin (H&E) and Periodic acid-Schiff (PAS) staining, 200×; (C-D) Ultrastructural findings demonstrate intramembranous microspherules and invagination of foot processes into GBM.

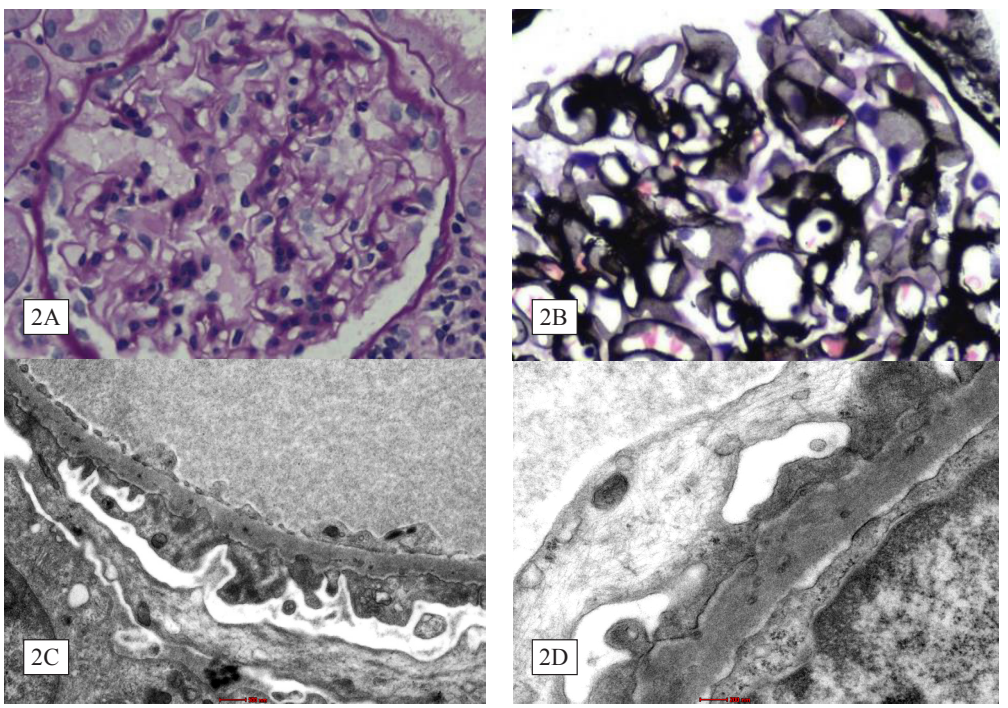

FIG. 2. (A) Photomicrographs show a glomerulus with capillary wall thickening accompanied by membrane vacuolations, Periodic acid-Schiff (PAS) staining, ×200; (B) Periodic Schiff-Methenamine Silver (PASM) staining ×600; (C-D) Ultrastructural findings demonstrate diffuse and extensive invagination and infolding of podocyte cytoplasmic processes into GBM, forming subepithelial clusters of microspherules and microtubules separated by intervening basement membrane.

TABLE 1: Clinicopathological features and laboratory investigation results of podocyte infolding glomerulopathy (PIG) patients

Parameter	Case 1	Case 2
Age	25	36
Gender	Female	Female
Race	Indian	Malay
Nationality	Malaysian	Malaysian
Presentation	Persistent subnephrotic range proteinuria and haematuria	Bilateral pedal oedema with frothy urine and haematuria
Comorbidity/ associated disease	SLE, bilateral hydronephrosis, autoimmune hypothyroidism, primary ovarian failure	SLE
24hour urine protein	0.5 g/24hr.	0.6 g/24 hr
UPCR	164 mg/mmol	-
Urea (2.8 8.1 mmol/L)	1.7–2.9 mmol/L	1.9–3.9 mmol/L
Creatinine (44–80 umol/L)	13 – 30 μmol/L.	51–62 umol/L
Albumin 35–52	22–28	29–30
ANA	Positive	Positive
dsDNA	Positive	Positive
Others	AMA, ASMA anti-LKM, anti-GPA: Negative	ENA: Anti-Ro/SSA & anti-La/SSB: Positive Anti-CCP: Positive Anti-Cardiolipin (ACL): Normal Anti-Beta 2 Glycoprotein1: Negative.
ANCA	Negative	Negative
Rheumatoid factor	Negative	Negative
C3 (0.83–1.93g/l)	0.35	0.89–1.15g/l (within normal range)
C4 (0.15–0.57g/l)	0.01	0.09–0.13g/l (low)
Hepatitis B	Non-reactive	Non-reactive
Hepatitis C	Non-reactive	Non-reactive
HIV	Non-reactive	Non-reactive
CRP < 5 mg/L	3.26 mg/L	6 mg/L
ESR0 - 20 mm/hr	78 mm/hr	61 mm/hr
Ultrasound KUB	Bilateral hydronephrosis. No thrombosis	No hydronephrosis or renal thrombosis
Light microscopy	Normal glomerular architecture with patchy thickening of capillary wall	Capillary wall thickening with membrane vacuolation and occasional red trichrome deposits
Mesangial cell hypercellularity	No	Mild
Mesangial expansion	Mild	Mild
Endocapillary hypercellularity	No	No
Crescent	No	No
Capillary wall thickening	Patchy & mild	Mild
Membrane vacuolation	Occasional	Mild

Subepithelial spikes	No	No
Subepithelial deposits	No	No
Subendothelial deposits	No	No
Necrotising lesion	No	No
Global sclerosis	No	No
Segmental sclerosis	No	No
IFTA	Focal and mild	Mild
Interstitial inflammation	Occasional	Mild to moderate
Vessels	No vessel wall thickening	No vessel wall thickening
Immunofluorescence	Fresh tissue: IgG(+/-), IgA(+/-), IgM(1+), C1q(1+); granular positivity within the mesangium. C1q(+/-); granular positivity within the capillary wall. C3, C1q, kappa and lambda were negative	Pronase digested paraffin section: Ig(+/-), IgA (+/-), IgM(+/-); fine granular positivity in mesangium. C3, C1q, kappa & lambda were negative
Electron microscopy (EM)	Diffuse invagination and infolding of podocyte cytoplasmic processes into GBM with microspherules and microtubules that are separated by an intervening basement membrane	Diffuse invagination & infolding of podocyte cytoplasmic processes into GBM featuring microspherules and microtubules separated by intervening basement membrane
Treatment	Prednisolone	Prednisolone

SLE: Systemic lupus erythematosus; AMA: Antimitochondrial antibodies; ASMA: Anti-smooth muscle antibody; LKM: Liver kidney microsome; GPA: Granulomatosis with polyangiitis; ENA: Extractable nuclear antigen; SSA: Sjögren's syndrome type A; SSB: Sjögren's syndrome type B; Anti-CCP: Anti-cyclic citrullinated peptides; ACL: Anti-cardiolipin; KUB: Kidney, ureter and bladder; GBM: Glomerular basement membrane.

exhibited microspheres and/ or microtubular structures in the GBM, leading to the recognition of PIG.[2] To date, 28 PIG cases have been reported from Japan, 10 from China, and one case each from the USA, Germany, India, South Korea, Argentina, and Canada.[3] The total of global PIG cases stands at 46, including the recently reported cases in Malaysia. To our knowledge, these cases are the first two PIG cases recorded in Malaysia.

The reported PIG cases involved patients between 4 and 69 years. Most cases originated from East Asian countries and revealed similar clinical patterns, such as a higher prevalence in females and the younger age group. Furthermore, most patients presented with subnephrotic range proteinuria with or without haematuria.[3] The current cases demonstrated similar trends to previous findings, where both patients were female and fell into the young age group (25 and 36 years). The patients presented with subnephrotic-range proteinuria and haematuria while maintaining normal renal function. Earlier cases of PIG also reported normal renal function in general, suggesting a favourable prognosis for this condition. Meanwhile, proteinuria has been identified as one of the clinical features of PIG, potentially resulting from alterations in the podocyte architecture, which allows the protein to filter into the urine. Nephrotic-range proteinuria associated with oedema and hypoalbuminemia can lead to nephrotic syndrome.

Most PIG patients were also presented with autoimmune diseases, such as lupus nephritis, rheumatoid arthritis, autoimmune thyroiditis, Sjögren's syndrome, primary biliary cirrhosis, mixed connective tissue disease, autoimmune thyroiditis or lupus nephritis. In addition, this condition has been linked to stage 3 membranous nephropathy, hepatitis B infection, and malignancies such as multiple myeloma. Nonetheless, PIG occurred independently in a minority of cases, without any association with other diseases.[4] Based on these findings, PIG can be further classified into two categories: Connection tissue disease-associated (CTD-PIG) and non-connective tissue disease-associated (NCTD-PIG). Examples of CTD-PIG include lupus nephritis, Sjögren's syndrome, rheumatoid arthritis, chronic thyroiditis, and mixed

connective tissue disease (MCTD). On the other hand, NCTD-PIG is associated with hepatitis B virus infection, ovarian mature teratoma, or vesicoureteral reflux (VUR) with Basedow's disease. Case 1 in this report was associated with SLE, autoimmune hypothyroidism, primary ovarian failure, and bilateral hydronephrosis, which falls under both the CTD-PIG and NCTD-PIG categories. Meanwhile, the second patient was diagnosed only with SLE and, thus, categorised as CTD-PIG.

Assessing PIG through light microscopy is challenging due to its overlapping morphological features with other glomerular diseases. These features can vary from normal glomerular architecture to mesangial hypercellularity and focal segmental glomerulosclerosis. In some instances, the capillary walls may appear thickened with open, rounded lumina, resembling early membranous glomerulonephritis.[3] However, uniform thickening of the GBM can also be observed in conditions such as smoking, diabetic nephropathy, obesity, and SLE. Therefore, a detailed clinical history and relevant investigations are crucial to diagnose PIG accurately. PIG can be distinguished from diffuse capillary wall thickening in class V lupus nephritis by the diffuse membranous pattern in the latter, characterised by subepithelial dense deposits along the GBM. In contrast, the capillary wall thickening in PIG may result from an imbalance between the synthesis and degradation of the podocyte matrix instead of immune deposits.[3]

In the present cases, capillary wall thickening with membrane vacuolation was evident, which initially suggested early membranous nephropathy. Despite that, the absence of subepithelial spikes and deposits indicated that a diagnosis of membranous nephropathy is unlikely. Additionally, both cases exhibited similar immunofluorescence findings, in which trace positivity for IgG, IgA, and IgM were noted. Meanwhile, only trace positivity of C1q was noted in the first case and negative in the second case, thus excluding the diagnosis of lupus nephritis. Furthermore, no tubuloreticular inclusions were visible in the ultrastructural studies to suggest lupus nephritis in either case.

The sole dependence on histological and IF findings can be challenging in differentiating PIG from other glomerular diseases. Therefore, the definitive diagnosis is reliant upon the specific EM findings, which demonstrate diffuse extensive podocytic infolding or protrusion of podocyte cytoplasm into the irregularly thickened GBM associated with microspheres or microtubule formation. The microvillous cytoplasmic extension forms the infolding into an altered GBM. Membrane-bound microtubules or microspheres are also cytoplasmic in origin. These findings are associated with diffuse effacement of foot processes. The irregular thickening of the basement membrane and neo-membrane formation, mainly at the subepithelial area, can also be appreciated in some cases.

The PIG can be categorised into three types: Type A, type B, and type C. Type A is characterised by the presence of only podocytic infolding into GBM, type B shows podocytes infolding into GBM with microstructures formation, whereas type C only reveal the formation of the microstructure in GBM. In type C, bubbles or holes formation in GBM can be observed with a PASM stain. Likewise, the spike formation of podocytes infolding into GBM in type A can also be detected via PASM.[2] In the current cases, the PIG diagnosis was characterised by the presence of diffuse extensive invagination & infolding of podocyte cytoplasmic processes into GBM with clusters of microspherules and microtubules separated by intervening basement membrane observed in EM.

Most PIG patients were treated with immunosuppressants, with many achieving complete remission. Nonetheless, the literature has reported patients achieving remission without any immunosuppressive therapy. This result suggested that PIG may represent a new glomerular disease with favourable clinical outcomes, with or without immunosuppressive treatment. Further research is necessary to better understand the characteristics and features of PIG, its prognosis, and the underlying pathogenesis of the disease to develop personalised therapies for this unique condition in the future.

The underlying pathogenesis of PIG remains unclear due to its rarity and limited reports. Podocytes are highly specialised epithelial cells distributed on the surface of capillary loops, forming parts of the filtration barrier, GBM, and endothelial cells. Precisely, podocytes produce laminin (a type IV collagen) and heparan sulphate, which are essential for GBM formation. This process is indicated by the endothelial and podocyte marker positivity and the presence of interdigitating foot process with a slit diaphragm to connect the gap.[4] Their dysfunction and specific abnormality have been implicated in various glomerular diseases, including PIG.

Clinically, PIG may be associated with connective tissue disease and other underlying glomerulopathy in some cases. It has been hypothesised that PIG is a form of podocytopathy characterised by the alteration of podocytes and basement membrane interaction, leading to the invagination of the podocyte cell membrane into the GBM. Another potential mechanism of PIG involves an exaggerated "wear and tear" response of podocytes. In some instances, a concurrent viral infection is thought to contribute to the disease's underlying pathogenesis.[4]

Additionally, the activation of complement in situ is a possible mechanism that leads to the microstructure formation within the GBM. A deficiency in the podocytes' cytoskeletal structure may also play a role in PIG development.[3] Previous studies have shown that the presence of the membrane attack complex within the GBM of PIG supports the idea of intra-GBM complement activation.[5,6] Moreover, earlier reports suggested that inverted formin 2 (INF2) mutation could be responsible for PIG development, which could be confirmed through molecular studies.[3]

The genomic and proteomic approach is critical to shed light on the pathogenesis of PIG. For instance, research has identified SWitch/Sucrose non-fermentable related matrix-associated actin-dependent regulator of chromatin, subfamily A-like protein 1 (SMARCAL1) mutation in a PIG case, which could potentially serve as a novel marker for the disease.[4] Additionally, the alpha-actinin 4 (ACTN4) protein, crucial for actin crosslinking in the cytoskeleton and for maintaining normal podocyte structure, was found to be significantly decreased in PIG cases.

This reduction implied that ACTN4 might play a role in PIG pathogenesis.[6] It was also hypothesised that injury to the podocytes would alter the cytoskeleton stability and eventually initiate the invasion of the dysfunctional podocytes in the GBM that give rise to PIG. Apart from that, the endothelial cell was found to be invaginated into GBM in the process of podocyte invagination for some PIG cases, which possibly contributed to haematuria.[2,6] The association of the disease with the potential genetic markers should be investigated via molecular studies to improve future diagnosis.

CONCLUSION

PIG is a rare and unique glomerular disease with distinct electron microscopic features. Our report highlights the first two cases of PIG in Malaysia, emphasising the importance of electron microscopy for accurate diagnosis of PIG. The findings from the 2 cases reaffirm PIG's association with autoimmune diseases and its generally favourable prognosis. While the pathogenesis remains to be elucidated, the role of podocyte-GBM interaction, complement activation, potential genetic markers like INF2 mutation and ACTN4 protein deficiency warrants further study.

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