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Case Report

Sausage on a string – two interesting case reports of dense deposit disease

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ABSTRACT

Dense deposit disease (DDD) is an ideal form of C3 glomerulopathy (C3G), with an estimated incidence of 2-3 people per million. DDD is defined by intramembranous C3 accumulation with absent or scanty immunoglobulin deposition and highly electron dense deposits visible by electron microscopy (EM). C3G is a unique and rational disease classification based on pathogenesis of dysregulated alternative complement pathway and is composed of DDD and C3 glomerulonephritis (C3GN). DDD is characterized by thickening of glomerular basement membrane by dense intramembranous deposits. Herein we present two cases of DDD diagnosed with a combined approach of clinical, pathological (light microscopy and electron microscopy) and laboratory investigations. Only EM assists definite distinction of DDD from C3 glomerulonephritis (C3GN).

1. Introduction

Dense deposit disease (DDD) is an ideal form of C3 glomerulopathy (C3G), a rare disease with an estimated incidence of 2-3 people per million.[1] It accounts for <20% of all cases of membranoproliferative glomerulonephritis(MPGN) in children and only a fractional percentage of cases in adults.[2] It is defined by C3 accumulation with an absent or scanty immunoglobulin deposition and highly intramembranous electron dense deposits visible by electron microscopy(EM). C3G is a histopathological diagnosis composed of two categories namely the DDD and C3 glomerulonephritis (C3GN). EM is necessary to distinguish between DDD and C3GN.The acute presentation of C3G represents signs and symptoms of glomerulonephritis ranging from asymptomatic hematuria, proteinuria and at times severe hypertension. The definitive cause for renal biopsy is continued hematuria and/or proteinuria with persistent low C3 levels.[3] Herein, we describe two cases of C3G who were diagnosed as DDD with combined approach of clinical and pathological (light microscopy, immunofluorescence and electron microscopic studies) correlation.

2. Case Description

Case 1 is an 18 year old male presented with complaints of facial puffiness, bilateral pedal edema grade II, hematuria, associated with history of fever decreased urine output, frothy urine and dyspnoea on exertion. History of abdominal pain present. No history of native medicine/ NSAIDS intake. No history of joint pain/skin rashes. On examination, vitals were stable and his BP was 150/90 mmHg. Systemic examination was normal. The patient was assessed by the Nephrologist, lab investigations were done (Tables 1 and 2) and a preliminary clinical diagnosis was made as

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renal failure and suspected C3 glomerulopathy. Hence renal biopsy was advised.

Native renal biopsy showed a total of 10 glomeruli, out of which 2 were segmentally sclerosed. Rest of glomeruli showed glomerular basement membrane thickening with double contouring (Figure 1 a). Mesangial and endocapillary hypercellularity was present (Figure 1 b). Periglomerular fibrosis was seen in 3 glomeruli. No evidence of crescents.

Case 2 is a 26year old female with nephrotic range proteinuria, hematuria, respiratory tract infection, bilateral leg swelling and hypertension. Past history of renal disease since childhood associated with hematuria, swelling of lower limbs and face with irregular follow up. She was on native medications for about 6 months and was symptomatically better. She delivered child in the year 2020 and on her postdelivery week 3, she was admitted to our hospital for high BP readings and deranged renal function tests (ATYPICAL PRE-ECLAMPSIA). She was on anti-hypertensives but was irregular on follow up. At the time of admission, her vitals were stable. Blood pressure was 170/110 mmHg. Systemic examination was normal. Lab investigations done (Tables 1 and 2) and clinically her preliminary diagnosis was given as chronic kidney disease, stage III and advised renal biopsy.

Renal core biopsy showed a total of 13 glomeruli, out of which 10 glomeruli showed mesangial expansion with hypercellularity and double contouring of glomerular basement membrane. 2 glomeruli showed segmental sclerosis and 1 glomerulus showed global sclerosis. Endocapillary hypercellularity was seen in few of the glomeruli. No evidence of crescents.

In both the cases, tubular atrophy was 40%. Tubular injury in the form of loss of brush border and regenerative changes present (Figure 1 c). Protein resorption droplets was noted in the cytoplasm of tubular epithelial cells. Interstitial fibrosis (40%) and patchy interstitial lymphocytic inflammation along with foam cells was seen. Blood vessels show endothelial cell prominence and medial wall hypertrophy. Luminal narrowing was 10% and 30% in case 1 and 2 respectively.

Special stains such as Periodic acid Schiff (PAS), Jones methenamine silver (JMS) and Masson Trichrome(MT) were done. PAS and JMS stains confirm glomerular basement membrane thickening and double contouring, segmental and global sclerosis. Tubular atrophy and interstitial fibrosis were highlighted by MT (Figure 1 d) and JMS stains.

2.1. *Immunofluorescence findings*

Immunofluorescence studies on both showed strong granular capillary and mesangial positivity for C3 conjugate. Figure 2 represents case 1. Rest all the conjugates (IgM, IgG, IgA, C1q, fibrinogen, kappa, lambda and albumin) were negative.

2.2. *Electron microscopic findings*

Tissue blocks were outsourced for electron microscopic studies where both the cases showed presence of wide effacement of podocyte foot process (70-80%). Mesangial interposition, neo basement membrane formation and reduplication of glomerular capillaries present. Hyperosmiophilic/extremely electron dense deposits seen in linear/continuous fashion along the glomerular capillaries and focally in mesangial areas (Figure 3 a & b-case 1 & Figure 3b & c case 2 show the deposits).

Table 1: Laboratory investigations

Lab investigations	**Case 1**	**Case 2**
Hemoglobin	10.9g/dl	11.3g/dl
Total count	14210 cells/cu.mm	24330 cells/cu.mm
Platelet	3.17 lakhs	3.47 lakhs
BUN	23 mg/dl	24 mg/dl
Serum creatinine	2 mg/dl	1.5 mg/dl
UPCR	5.19	8
24 urine protein	Not done	Not done
Total protein	4.8	Not done
Serum albumin	2.8	Not done
A : G ratio	2	Not done

BUN: Blood urea nitrogen, UPCR: Urine protein creatinine ratio, A: G ratio: albumin : Globulin ratio

Table 2: Urine analyses and antibodies test

Urine analyses	**Case 1**	**Case 2**
Glucose	Negative	Negative
Protein	4+	3+
Erythrocytes	4+	3+
RBC	20	13
Leukocytes	Negative	Negative
Pus cells	11	6-8
Epithelial cells	Negative	2-3
Bacteria	1+	Negative
Casts	Negative	Negative
Crystals	Negative	Negative
Antibodies	**Case 1**	**Case 2**
ANA	Negative	Negative
ANCA	Negative	Negative
C3	<20	<20
C4	44	53.4
Clinical diagnosis	**Renal failure, ?C3G**	**CKD stage III**

RBC: Red blood cells, ANA: Antinuclear antibody, ANCA: Antineutrophil autoantibodies, C3G: C3 glomerulopathy, CKD: Chronic kidney

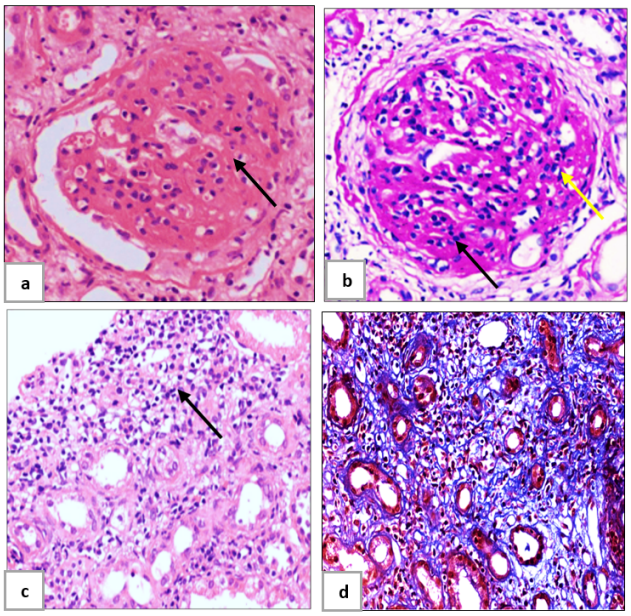

Figure 1: a): Glomerular basement membrane thickening(black arrow), double contouring and segmental sclerosis (40X, H&E); **b)**: Mesangial (black arrow) and endocapillary (yellow arrow) hypercellularity (40X, PAS); **c)**: Tubular injury with regenerative changes and interstitial inflammation (black arrow) (20X, H&E); **d)**: Interstitial fibrosisand tubular atrophy (20X, MT stain)

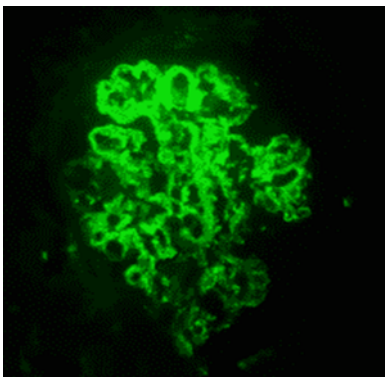

Figure 2: Immunofluorescence C3 stain showing strong capillary and focal mesangial deposits (40X, C3 stain)

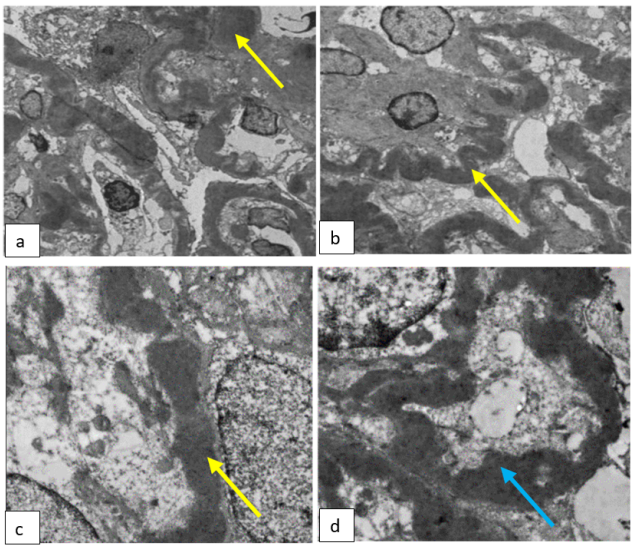

Figure 3: Corresponds to case 1 and 2 respectively. Few large confluent "sausage" like electron dense deposits are seen and highlighted in blue arrow on image d.

3. Discussion

C3G has been gaining recognition since its first description in 2007. Though the knowledge of the disease has been increased, the pathophysiology and treatment of the same remains unclear. The rarity of this condition presents a challenge for the treating physician and nephrologist.[4] C3G is a unique and rational disease classification encompassing DDD and C3GN. DDD is characterized by electron dense deposits on the glomerular basement membrane, mesangium, tubular basement membrane and Bowman's capsule secondary to dysregulated alternative complement pathway. Various studies showed that more than 50% of patients with DDD of 10 or more years progress to end stage kidney disease.[3] Studies by Jansi Prema et al. represented that incidence of DDD is increased in Indian population and highlights the significance of performing electron microscopy analysis of kidney biopsies as it is not possible to definitively diagnose DDD.[5]

The pathogenesis of C3G is based on dysregulation and excessive activation of the alternative complement pathway but the detailed pathophysiological aspects of DDD remains unknown. Dysregulation takes place at the level of C3 convertase and also includes some of the factors such as C3Nefs, genetic mutations in complement genes, and autoantibodies to complement proteins, such as complement factor H.[6] Some of the screening tests for alternate complement pathway such as hemolytic assay, alternative pathway functional assay, and levels of serum membrane attack complex may help in the diagnosis of C3G and provide us the information about physiological details of the same. It was also stated that these markers did not correlate with the outcome of the disease.[7] The characteristic age limit for DDD diagnosis is between 5 and 15 years and does not show a gender bias. The variety of clinical features include nephrotic syndrome, sub nephrotic range proteinuria, episodic synpharyngitic macroscopic or persistent microscopic hematuria and acute nephritic syndrome.[8] Non renal manifestations like ocular drusen, acquired partial lipodystrophy (PLD), Type 1 Diabetes Mellitus and Monoclonal gammopathy of undetermined significance (MGUS) are typical of DDD and hence to be considered while giving a definitive diagnosis.[9]

Histologically, intramembranous C3 accumulation can be depicted based on the thickening of glomerular basement membrane giving a homogenous and eosinophilic glassy appearance on hematoxylin and eosin stain, fuchsinophilic appearance on MT stain and non-argyrophilic appearance on silver stain. These changes can also be seen in tubular basement membrane.[10] The deposits of DDD are quite subtle and may be missed at the initial stage during examination and are picked up in the immunofluorescence and EM studies. The other features include proliferation of the mesangial cells, glomerular basement membrane thickening, endocapillary hypercellularity with lobular accentuation (classic MPGN pattern) and crescentic glomerulonephritis.[11] Under immunofluorescence, the deposits in DDD are classically highlighted as linear, band-like staining of glomerular basement membrane with fine or coarse granular to semiconfluent with peripheral accentuation and/or some tubular basement membrane staining. The coarse granular to large rounded forms of deposits are called as 'mesangial rings'.[10] The intensity of C3 immune deposits for C3G two times greater than the other conjugates.

Although light microscopy and immunofluorescence findings can be suggestive of the diagnosis, electron microscopy is essential and will show ribbon-like linear, confluent and elongated deposits in the middle layer (lamina densa) of glomerular basement membrane. The electron dense deposits are hyperosmiophilic, showing variability in thickness ranging from segmentally thin to thick, referred to as "sausage on a string" appearance. Some may show segmental breaks which may be present in the paramesangial regions.[10] Similar deposits can be seen in mesangium, Bowman's capsule and tubular basement membrane.[11]

4. Summary

The pathologist should be aware of identifying various histomorphological patterns in DDD. A combined approach with the help of light microscopy, immunofluorescence and electron microscopy analyses helps in diagnosing the disease. Clinical investigations for identifying complement dysregulation such as genetic testing, assays of complement function, measurement of complement protein levels and screening for autoantibodies are ensured for proper diagnosis. DDD is distinguished histologically from other forms of C3G by the presence of osmiophilic, intramembranous, ribbon-like deposits. Some of the non-renal manifestations are to be considered at the time of evaluation. One-fifth of DDD patients have silent symptoms who are found on routine physical examinations according to studies. Recurrence rate of DDD is high in case of post renal transplant biopsies which is considered to be a unique feature. EM is mandatory for a definite diagnosis of DDD.

5. Conclusion

There is a need to increase health-care provider awareness of DDD to optimize best patient care practices. In order to bring out the underlying pathogenesis and provide best therapy, an integrated and combined multidisciplinary approach with the help of treating clinician, pathologist and biochemical or genetic laboratory work up is very essential.

6. Source of Funding

Nil.

7. Conflicts of Interest

Nil.

8. Patient Consent

The authors certify that they have obtained all appropriate patient consent forms and that the patient has given his consent for his images and other clinical information to be reported in the journal. The patient understands that his name and initials will not be published and due efforts will be made to conceal his identity, but anonymity cannot be guaranteed.

9. Ethical Statement

Nil.

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