Dense Deposit Disease Involving C3 and C4d Deposits

Abstract

Dense deposit disease (DDD), earlier called Type II membranoproliferative glomerulonephritis is distinct disease having frequent relapses reaching end-stage kidney disease by 10-year in up to 50%–60% of cases and high recurrence rate in the allograft. The term DDD is derived from its distinctive ribbon-like osmiophilic deposits in the lamina densa of glomerular basement membrane by electron microscopy. Pathogenetically, alternate pathway dysfunction leads to this disease, which is diagnosed by ultrastructure. Herein, we describe our observation of C4d positivity in an adolescent boy with DDD.

Keywords: *Complement C3/C4d, dense deposit disease, electron microscope, membranoproliferative glomerulonephritis*

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Introduction

Dense deposit disease (DDD) is an ultrastructural morphologic disease entity, first described by Berger and Galle in 1962.[1] Later Nasr *et al*. defined these cases as membranoproliferative glomerulonephritis (GN), Type II variant. Clinically, disease is seen in children presenting with nephritic-nephrotic illness, low C3, normal C4, and preserved renal function.[2] Relapse of symptoms and progression of disease is well known. Histologic and ultrastructural diagnosis is pivotal in management. Eculizumab is an inhibitor of terminal complement pathway and has shown promising positive results in DDD.[3] Recurrence in allograft is very high, with more than half ending up in graft loss at 5 years.

Until recently, all cases of DDD were described dominantly positive for C3. Sethi *et al*. have described C4d+ dominant DDD wherein C3 was conspicuously absent.[4] Herein, we describe an observation of C4d positivity in an adolescent boy with DDD.

Case Report

A 20-year-old male presented to nephrology services in March 2013, with facial puffiness and pedal edema. On examination, blood pressure was mildly elevated (150/90 mmHg). Urine analysis showed 3+ albuminuria and 10–15 red blood cells/high power field. Spot urine protein creatinine ratio (U-PCR) was 3.6 g/g. Complete hemogram was within normal range. Serum albumin and creatinine were 1.7 g/dl and 1.0 mg/dl, respectively. Autoimmune serologies (antinuclear antibody/antineutrophil cytoplasmic antibody) and viral markers (HIV, hepatitis C virus, hepatitis B surface antigen) were negative. Serum C3 was 0.3 mg/dl. C4 was normal. Total cholesterol was 181 mg/dl. Ultrasonogram showed normal sized kidneys with the distinct corticomedullary junction and normal echoes. History was significant for nephrotic illness 5 years ago, for which empirical steroid therapy was given outside without a renal biopsy, followed by complete remission until the current presentation. Renal biopsy showed 12 viable glomeruli revealing diffuse endocapillary proliferative changes with markedly thickened capillary basement membranes (periodic acid–Schiff [PAS]-positive and silver-weak positive) [Figure 1a]. Polymorphonuclear cells were sparsely seen. Necrotizing lesion/crescent was absent. Tubulointerstitial and vascular compartments were unremarkable. Immunofluorescence showed granular positivity with C3 (3+) along the capillary walls and mesangial region [Figure 1b]. Other panels (IgG, IgA, IgM, C1q, kappa, and lambda) were negative. Electron microscopy (EM) was not performed.

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Unfortunately, his nephrotic illness failed to respond to multiple modes of treatment-steroid (1 mg/kg × 4 months), later mycophenolate mofetil (1.5 g/day × 18 months), and subsequently to oral cyclophosphamide (2 mg/kg/day × 3 months). A repeat biopsy was performed in April 2016, for persisting nephrotic range proteinuria (U-PCR 3.7 g/g) and slowly creeping serum creatinine to 1.6 mg/dl. Light microscopy and immunofluorescence were similar to prior biopsy, except for the presence of focal global and segmental sclerotic lesions in 2 and 1 glomeurli [Figure 2a-c], respectively of total 10 glomerulil with 5 glomeruli being obsolescent; along with mild tubulointerstitial chronicity. Immunofluorescence was performed on pronase-digested paraffin material of both biopsies. Results were similar to earlier findings described. C4d was done by immunohistochemistry. Surprisingly, positive granular staining reaction was seen that mirror-imaged C3 staining [Figure 2d]. EM performed on the second biopsy showed ribbon-like osmiophilic transformation of capillary basement membranes; rarely along Bowman's capsule and tubular basement membranes [Figure 3]. A diagnosis of DDD was made. Post-biopsy he was treated with two doses of rituximab (500 mg/dose) and angiotensin-converting enzyme inhibitor therapy. Despite this renal functions have not shown significant recovery and his serum creatinine on the last follow-up was 2.6 mg/dl.

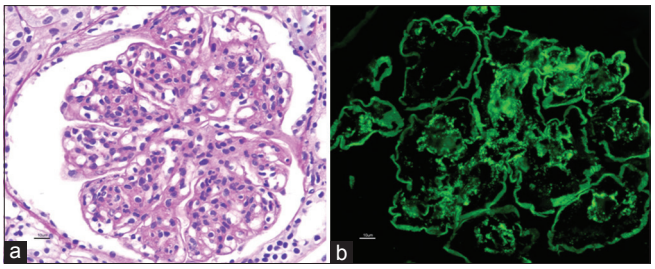

Figure 1: (a) Glomerulus revealing lobular accentuation with global proliferative tufts (PAS, ×400). (b) Thick linear accentuation of capillary walls, along with mesangial granular deposits forming mesangial rings with C3 of 3+ intensity (×400)

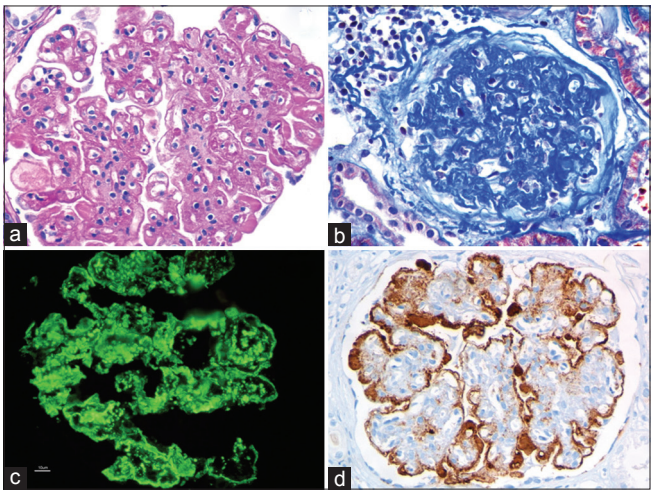

Figure 2: (a) Thickened glassy capillary basement membranes with double contour appearance and proliferation of endothelial cells/mesangial cells (PAS, ×400). (b) Globally sclerotic glomerulus with relatively preserved broad basophilic capillary walls (Mason trichrome, ×400). (c) Similar C3 positivity with mesangial rings as seen in the first biopsy (×400). (d) Band of granular positivity for C4d along the peripheral capillary walls (IHC, ×400)

Discussion

DDD is the result of alternate pathway (AP) dysfunction, most commonly due to C3 nephritic factor; an autoantibody stabilizing C3 convertase eventually causing consumption of C3. Deficiency or mutations of factor H can also result in AP dysfunction causing DDD in animals and humans.[5]

Diagnosis of DDD is rendered by a renal biopsy that demonstrates markedly thickened ribbon-like basement membranes (PAS-positive, silver-negative). Light microscopic glomerular variations (membranoproliferative GN, mesangial proliferative GN, crescentic GN, diffuse proliferative, and exudative GN) are known. Immunofluorescence findings are bright C3 staining along capillary walls and mesangium. Sparse immunoglobulins can be seen in some cases. Consensus report on diagnosing C3 glomerulopathy states C3 stain should be dominant and at least two orders in magnitude more intense that other reactants.[6] EM findings of ribbon-like osmiophilic transformation of lamina densa of basement membranes are quite characteristic of DDD. The similar osmiophilic material can also be seen in mesangium, Bowman's capsule, and tubular basement membranes. Lack of osmophilic transformation in EM renders a diagnosis of C3 GN.[3] Liquid chromatography mass spectrometry (LCMS) from the microdissected glomeruli of DDD comprised precursors of C3, C4, C8α, FHR1, vitronectin, and apolipoprotein E.[7]

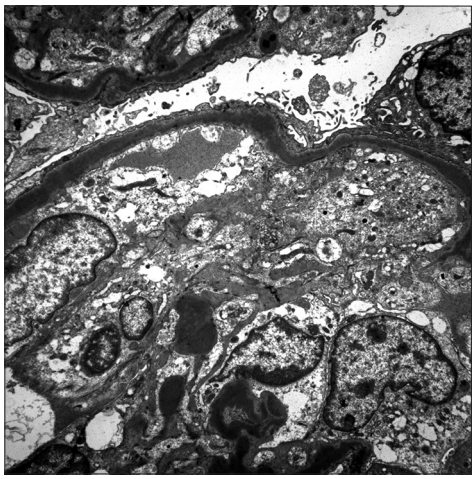

Figure 3: Ultrastructure study showing ribbon like osmiophilic deposits replacing the lamina densa of capillary basement membranes; with similar mesangial deposits and proliferative tufts (×10,000)

The utility of C4d in transplant setting is well known for more than two decades ever since Feucht *et al*. reported capillary deposition of C4d in 51 of 93 allograft biopsies with early graft dysfunction.[8] Antibodies activate classical pathway of complement through cleavage of C4, and

subsequently, C4d binds covalently to endothelial surface remaining for a longer period. C4 cleavage also occurs in lectin pathway of complement activation through interaction between bacterial carbohydrate moieties and mannose-binding lectins.[9] Studies have also shown that glomerular-C4d plays a role of activity in lupus nephritis, and is also a prognostic marker in IgA nephropathy.[10,11] It is possible that activation of C4 leads to the formation of C4 convertase and thus activation of C3.

Recently, Sethi *et al*. have described a new entity "C4d glomerulopathy" including C4d GN and C4d DDD following their observation of three cases.[4] The diagnostic approach is similar to C3 glomerulopathy.[6] Criteria for the C4d DDD is dependent on granular positivity for C4d (immunofluorescence or immunohistochemistry), and demonstration of osmiophilic deposits along glomerular basement membranes in EM. On the contrary, the absence of such characteristic EM findings and merely displaying mesangial/subendothelial electron dense deposits are labeled as C4 GN. Further, proteomic profile of all three cases corresponded to C4 by laser microdissection/LCMS. Anecdotal cases of C4d DDD associated with thrombotic microangiopathy and monoclonal gammopathy has been described by Sethi *et al*.[12,13]

We performed C4d in this case of DDD, taking the lead from the recently published utility of C4d in proliferative GN.[14] Our case is unique in demonstrating both C3 and C4d in the absence of Immunoglobulins, which has never been described before. Clinical presentation is almost similar to C3-DDD[2] and Case 1 of C4d DDD.[4] The patient had recurrent episodes of nephrotic-nephritic illness, active urine sediments, and hypocomplementinemia. C4 was normal in all cases (although C4 was borderline low in Case 3 of ref).[4] In the study by Sethi *et al*., C4d was negative in 24 of 30 biopsies of C3 glomerulopathy; and weakly positive (trace to 1+) in rest 6 cases.[14] All five cases of DDD (four native, one recurrent) in their study were found negative with C4d stain. On the contrary, our case of DDD showed 3+ bright staining along capillary walls. Messias *et al*. have noted masked immunoglobulins in fresh frozen tissue immunofluorescence that was picked up by pronase digestion in 14 of 20 patients studied.[15] These salvage techniques of looking at masked immunoglobulin's preventing misdiagnosis of C3 glomerulopathy, will probably soon become inevitable in the setting of membranoproliferative GN with significant positive reaction to C3 and C4d. This is especially for those in developing countries lacking robust EM services. Hence, we pronase-digested paraffin embedded material and repeated Immunofluorescence to look for masked immunoglobulins.

Demonstration of C3 and C4d together, along with osmiophilic character of basement membranes is thought provoking and raises question-does a third entity "C3/C4d DDD" caused by combined alternate/lectin pathway dysfunction exist under the umbrella of DDD? Answer to this is partly drawn by the presence of C4d and negative reaction to immunoglobulins/C1q together implying lectin pathway involvement.[14] It is evident in our case that there is persistent AP complement dysfunction reflected by low serum C3 and tissue C3 deposits. The analysis of renal tissue by LCMS for mannose binding proteins would throw more light on proteomic details of lectin pathway. However, it was not done in the index case due to the lack of availability.

Our patient was steroid responsive initially and maintained remission till his current presentation with relapse. His glomerular disease failed to achieve complete remission with steroids, and he maintained partial remission with mycophenolate mofetil and oral cyclophosphamide until further progression of renal azotemia at which point he was offered a rebiopsy.

He had eventual histologic progression of disease in terms of glomerular sclerotic lesions and tubulointerstitial chronicity. To summarize, DDD can be viewed as - (a) typical well known C3+ DDD, (b) C4d+ DDD, and possibly (c) C3/C4d+ DDD. It will be interesting to see in future studies if these newer entities have any similarities with C3+ DDD cases in respect to therapeutic response and rate of recurrence in the allograft.

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Conflicts of interest

There are no conflicts of interest.

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