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Original Article

ORIGINAL ARTICLE

Dense deposit disease: a greatly increased biopsy incidence in India versus the USA

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ABSTRACT

Background. We present the largest clinicopathologic case series to date of dense deposit disease (DDD) in an Indian population and compare the renal biopsy incidence rate to that seen in a large renal laboratory in USA.

Methods. Cases of DDD were identified and evaluated from native kidney biopsies reported at Renopath, India and at Arkana Laboratories, in the USA. Renopath receives biopsies from four states, located in the South and Eastern part of India. Arkana Laboratories' biopsies came from 37 states across the USA.

Results. During the study period, there were a total of 25 patients diagnosed with DDD among the 7335 native kidney biopsies at Renopath. Thus, the biopsy incidence rate (cases of DDD/total renal biopsies/year) is 0.0034. By comparison, there were 10 cases of DDD diagnosed among 26 319 native kidney biopsies at Arkana Laboratories during the same time period, with a renal biopsy incidence rate of 0.00038.

Conclusions. DDD in this Indian subpopulation has similar clinical and pathologic characteristics when compared to previously reported studies. However, the biopsy incidence rate is about 890% or 8.9 times more common in this subset of the Indian population when compared with a broad cross-section of the US population. In addition to potential genetic factors, environmental conditions and chronic infections likely contribute to the markedly higher biopsy incidence rate. Given the much greater number of patients with DDD in this population, further retrospective and prospective studies would allow more rapid progress in understanding the pathogenesis of DDD and thus potential treatment of patients with DDD.

Keywords: dense deposit disease, incidence, India, pathology, renal biopsy

INTRODUCTION

Dense deposit disease (DDD) was first described in 1962 by Galle [1]. It was classified as a subset of membranoproliferative glomerulonephritis in 1975 [2]. The understanding of the pathogenesis has progressed, and it is now considered the prototypic glomerular disease caused by abnormalities of the alternative pathway of complement, known collectively as C3 glomerulopathy [3–6]. It is characterized by the ultrastructural finding of dense osmiophilic transformation of the glomerular basement membranes (GBMs) and mesangium. Immunofluorescence (IF) characteristically shows dominant C3 staining with up to 50% showing some degree of immunoglobulin staining [7–9]. Though historically DDD was associated with a membranoproliferative

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pattern, it is now known that there are multiple glomerular patterns seen in patients with DDD including mesangial proliferative, membranoproliferative, acute proliferative and exudative and crescentic forms of glomerulopathy [8, 9].

The aim of this study was to determine the clinicopathological features of DDD in children and adults in South and Eastern India and to compare the data with previously reported studies. We have also compared the renal biopsy incidence in this population with that seen in a large renal biopsy laboratory in the USA. To our knowledge, this is the largest study addressing the clinical features, pathological characteristics and outcome of DDD in an Indian population.

MATERIALS AND METHODS

We retrospectively reviewed the native kidney biopsies evaluated at Renopath, from August 2013 to November 2016. During that time, we received 7335 native kidney biopsies almost entirely from four states: Tamil Nadu, West Bengal, Andhra Pradesh and Karnataka. In all, 25 patients with DDD (0.34%) were identified. During the same time period, 10 cases of DDD were diagnosed among 26 319 native kidney biopsies (0.038%) at Arkana Laboratories, which receives biopsies from 37 of the 50 states in the USA. The study protocol was approved by Schulman Institutional Review Board and conformed to the principles outlined in the Declaration of Helsinki.

Table 1. Morphologic patterns of DDD

Membranoproliferative pattern	Endocapillary hypercellularity and GBM duplication
Mesangial proliferative pattern	Focal segmental mesangial hypercellularity (>3 cells/mesangial region)
Acute proliferative and exudative pattern	Endocapillary hypercellularity with neutrophilic infiltration
Crescentic pattern	Crescents in >50% of glomeruli

Clinical review

Clinical data at the time of biopsy were recorded. The series was divided into children (<16 years) and adults (16 years and older) as in the study of Nasr *et al.* [9]. In each case, the following details were compared: gender, age at onset, proteinuria, haematuria, hypertension and renal dysfunction.

Renal biopsy

Renal tissue was processed for light, IF and electron microscopy as in our previous studies and as described below [10].

Light microscopy. Renal biopsy samples were fixed in buffered formalin, dehydrated in graded alcohols and embedded in paraffin. Serial sections were cut at 3 μm and treated with haematoxylin and eosin, periodic acid–Schiff (PAS), Jones methenamine silver and Masson trichrome stain.

IF microscopy. Samples were transported in Michel media, washed in buffer and frozen in a cryostat. Sections were cut at 5 μm, rinsed in buffer and reacted with fluorescein-labelled rabbit polyclonal anti-human antibodies to immunoglobulin G (IgG), immunoglobulin A (IgA), immunoglobulin M (IgM), C3, C1q, kappa and lambda light chains (Dako Corporation, Carpinteria, CA, USA) for 1 hour, rinsed in buffer and coverslipped with an aqueous mounting media.

Table 2. Demographics

	Indian patients			American patients		
	All	Adult	Paediatric	All	Adult	Paediatric
N	25	16	9	10	5	5
Male/female	14/11	9/7	5/4	4/6	2/3	2/3
Age, mean (range), years	20.7 (5–41)	25.8 (16–41)	11.7 (5–15)	19.8 (7–44)	29.6 (18–44)	10 (7–15)
Race						
Caucasian				7	4	3
Hispanic				1	1	0
Native American				1	0	1
Middle Eastern				1	0	1

Table 3. Clinical laboratory features

	Indian patients				American patients			
	All	Adults	Paediatric	P-value	All	Adults	Paediatric	P-value
Creatinine (mg/dL)	1.4	1.1	0.54	<0.001	1.1	0.6	0.2	NS
Urine protein (U P/C)	7.12	3.4	9.04	<0.01	6.79	2.9	10.68	<0.04
Low C3 (%)	92	87.5	100	NS	90	0	100	ND
Low C4 (%)	4	0	11.1	ND	0	0	0	ND
Haematuria (%)	32	25	44.4	NS	0.5	0.5	0.5	NS

ND, not done; U P/C, urine protein: creatinine ratio; NS, not significant, $P > 0.05$ (insufficient data for the adult population).

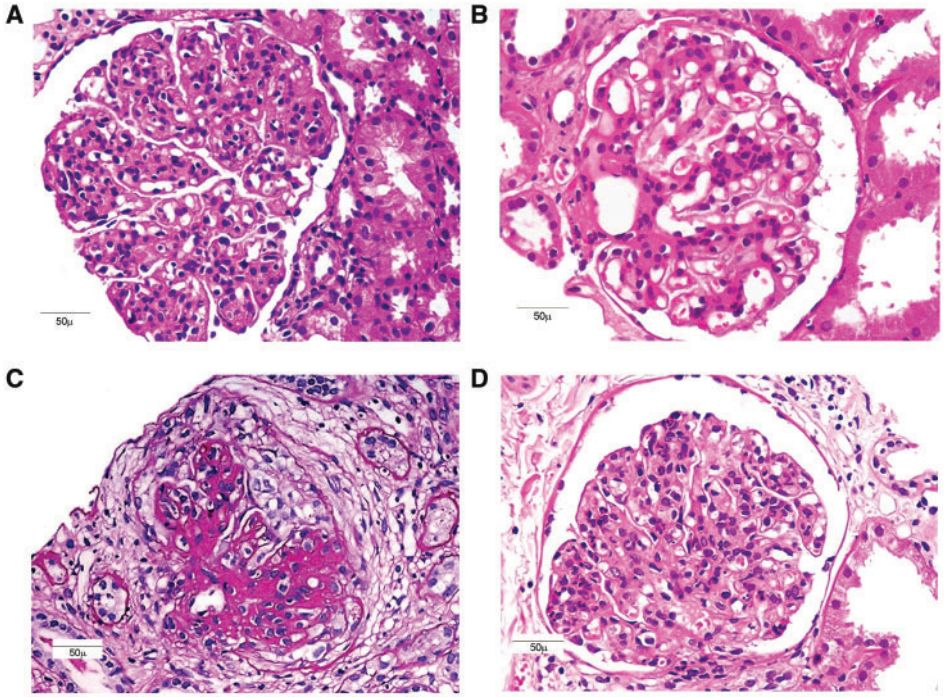

FIGURE 1: Light microscopic patterns. (**A**) Membranoproliferative pattern showing mesangial hypercellularity and capillary wall double contours (haemotoxylin and eosin, original magnification ×400); (**B**) mesangial hypercellularity (haemotoxylin and eosin, original magnification ×400); (**C**) crescentic pattern (PAS, original magnification ×400); (**D**) endocapillary proliferative pattern with many neutrophils (haemotoxylin and eosin, original magnification ×400).

Table 4. Light microscopic pattern

	India				USA			
	All	Adult	Paedriatics	P-value	All	Adult	Paedriatics	P-value
Glomerular pattern								
All (*n*)	25	16	9		10	5	5	
Mesangial proliferative	5	5	0	<0.02	3	1	2	NS
Membranoproliferative	13	9	4	NS	4	1	3	NS
Crescentic	4	1	3	NS	3	3	0	ND
Exudative	3	1	2	NS	0	0	0	ND
Tubular atrophy/interstitial fibrosis								
None	16	8	8	NS	3	1	2	NS
Mild	7	6	1	<0.001	5	2	3	NS
Moderate	1	1	0	ND	1	1	0	ND
Severe	1	1	0	ND	1	1	0	ND
Arteriosclerosis								
None	21	12	9	0	7	2	5	<0.04
Mild	2	2	0	ND	2	2	0	ND
Moderate	2	2	0	ND	1	1	0	ND
Severe	0	0	0	ND	0	0	0	ND

ND, not done; NS, not significant (insufficient data for the adult population).

Electron microscopy. Cubes of 1 mm size were removed from the ends of the biopsy sample, dehydrated with graded alcohols and embedded in Epon/Araldite resin. Sections of 1 μm size were cut with an ultramicrotome and stained with toluidine blue and examined with a light microscope. Thin sections were examined in a Jeol JEM 1011 electron microscope (Jeol, Tokyo, Japan) and photomicrographs taken at ×4000, ×12 000 and ×20 000.

Morphology

The diagnosis of DDD was based on the presence of extremely electron dense transformation of the GBMs with or without similar changes in the mesangial areas, the basement membranes of Bowman's capsule or tubular basement membranes (TBMs).

Light microscopy patterns. DDD was classified into four histological subtypes: membranoproliferative pattern, mesangial

proliferative pattern, acute proliferative and exudative pattern and crescentic pattern (Table 1) [8].

Interstitial fibrosis and tubular atrophy (IFTA), inflammation and arteriosclerosis were graded. IFTA ≤25% was graded as mild, 26–50% as moderate and >50% as severe. Interstitial inflammation was graded as none, focal (≤50%) and diffuse (>50%). Arteriosclerosis was graded based on the percentage of luminal narrowing as mild (≤25%), moderate (26–50%) and severe (>50% luminal narrowing).

IF microscopy. The intensity was graded on a scale of 0–3+ and the location of the deposits was noted.

Electron microscopy. Electron photomicrographs were examined for electron dense transformation of the GBMs, mesangium, TBMs and the basement membranes of Bowman's capsule. The presence of mesangial hypercellularity, endocapillary proliferation and new GBM formation (double contours) was also determined. The presence of subepithelial 'humps' composed of extremely electron dense material was noted.

Statistical analyses

Statistical analyses were performed using Prism statistical software for the Macintosh computer, version 7.0d (GraphPad Software, La Jolla, CA, USA). Statistical analyses used were the Kruskal–Wallis test or the Fisher Exact *t*-test as appropriate. Results were considered significant if $P < 0.05$.

RESULTS

Clinical features

During the study period, there were a total of 25 patients diagnosed with DDD among the 7335 native kidney biopsies (0.34%) at Renopath. By comparison, there were 10 cases of DDD diagnosed among 26 319 native kidney biopsies (0.038%) at Arkana Laboratories during the same time period. That is, there were about 890% more cases of DDD in biopsied patients from India than in the US series.

Among the 25 patients seen at Renopath, 16 were from Tamil Nadu, 7 from West Bengal, 1 from Andhra Pradesh and 1 from Karnataka. There were 16 (64%) adults and 9 (36%) children. The youngest patient in our study was 5 years and the oldest 41 years. Among the nine patients in the paediatric age group, three (33%) were <10 years of age. The male to female

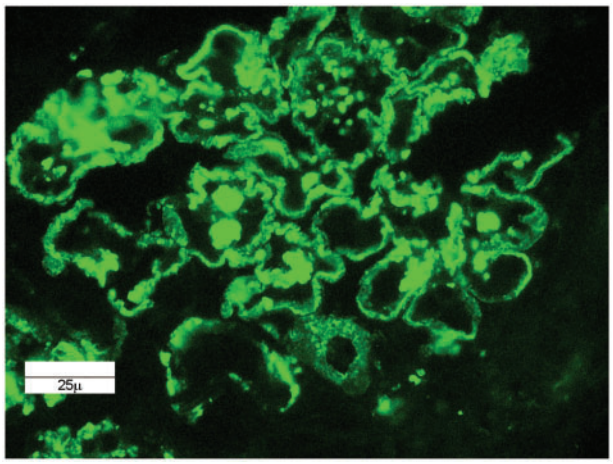

FIGURE 2: IF pattern: intense staining for C3 along the glomerular capillary loops in a semi-linear ribbon-like pattern and as 'ring' forms in the mesangium (fluorescein-conjugated anti-human C3, original magnification ×600).

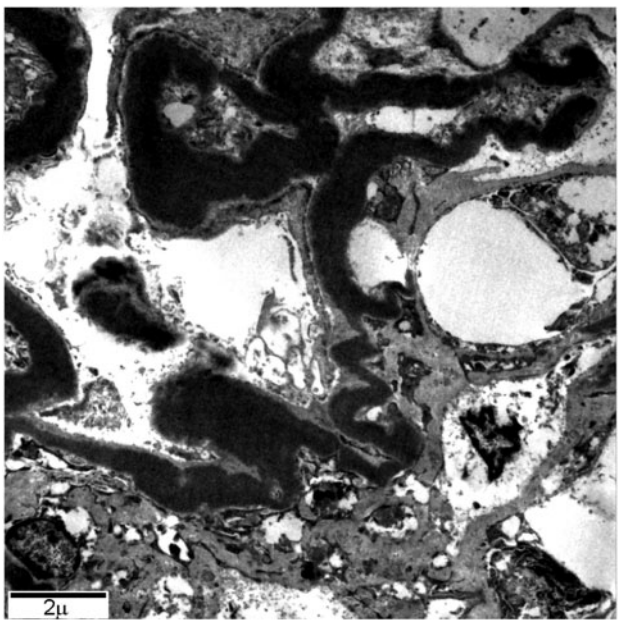

FIGURE 3: Ultrastructural pattern: interrupted, extremely electron dense transformation of the GBMs (osmicated sections, original magnification ×4000).

Table 5. IF microscopic features

	India				USA			
	All	Adult	Paedriatics	P-value	All	Adult	Paedriatics	P-value
C3	25/25 (100)	16/16 (100)	9/9 (100)	NS	9/10 (90)	4/5 (90)	5/5 (100)	NS
C4	0/25 (0)	0/16 (0)	0/9 (0)	ND	1/10 (10)	1/5 (20)	0/5 (0)	ND
C1q	1/25 (4)	0/16 (0)	1/9 (11)	ND	0/10 (0)	0/5 (0)	0/5 (0)	ND
Ig, kappa and lambda negative	22/25 (88)	14/16 (88)	8/9 (89)	NS	9/10 (90)	4/5 (80)	5/5 (100)	NS
IgG, IgM, C1q positive	1/25 (4)	0/16 (0)	1/9 (11)	ND	0/10 (0)	0/5 (0)	0/5 (0)	ND
IgG, IgM, IgA positive	1/25 (4)	1/16 (6)	0/9 (0)	ND	1/10 (90)	1/5 (20)	0/5 (0)	ND
IgM positive	1/25 (4)	1/16 (6)	0/9 (0)	ND	0/10 (0)	0/5 (0)	0/5 (0)	ND

Data are presented as *n*/*N* (%). ND, not done; NS, not significant (insufficient data for the adult population).

Table 6. Electron microscopic features

	India				USA			
Electron dense transformation, *n*/*N* (%)	All	Adult	Paedriatics	P-value	All	Adult	Paedriatics	P-value
GBMs	25/25 (100)	16/16 (100)	9/9 (100)	NS	10 (100)	5 (100)	5 (100)	NS
Mesangial	25/25 (100)	16/16 (100)	9/9 (100)	NS	9/10 (90)	5 (100)	4/5 (80)	NS
Subepithelial	2/25 (8)	2/16 (12)	0/9 (0)	NS	3/10 (30)	3/5 (60)	0/5 (0)	NS
Subendothelial	1/25 (4)	1/16 (6)	0/9 (0)	NS	3/10 (30)	3/5 (60)	0/5 (0)	NS
TBMs	6/25 (24)	3/16 (19)	3/9 (33)	NS	3/10 (30)	1/5 (20)	2/5 (40)	NS
Severe FPE	23/25 (92)	15/16 (94)	8/9 (89)	NS	10 (100%)	5/5 (100)	5/5 (100)	NS

FPE, foot process affacement; ND, not done; NS, not significant, $P > 0.05$ (insufficient data for the adult population).

Table 7. Comparative study

	Our study *n* = 25 (%)	Medjeral-Thomas *et al.* [13] *n* = 21 (%)	Nasr *et al.* [9] *n* = 32 (%)	Walker *et al.* [8] *n* = 67 (%)	Viswanathan *et al.* [12] *n* = 13 (%)
Mean age (years)	20.72	12	33	14	25.08
Patients <16 years	9 (36)	14 (68)	14 (43.8)		
Male:female	1.27:1	0.75	0.53	1.19:1	1.6:1
Membranoproliferative pattern	13 (52)	13 (62)	14 (43.8)	17 (25)	7 (58.33)
Mesangial proliferative pattern	5 (20)	3 (14)	9 (28.1)	30 (45)	3 (25)
Crescentic pattern	4 (16)	4 (19)	3 (9.4)	12 (18)	None
Exudative pattern	3 (12)	1 (5)	6 (18.7)	8 (12)	2 (16.67)
Crescents (%)					
None	18 (72)	9 (43)			
<50	3 (12)	8 (38)			4 (30.77)
>50	4 (16)	4 (19)	3 (9.4)	12 (18)	None
IF (%)					
IgA	4		13.3	12.5	
IgG	8		26.7	20.3	
IgM	12		36.7	34.4	
C3	100		100	100	
C1q	4		10	30	
Electron microscopy findings					
Intramembranous electron dense deposits	25 (100)	21(100)	32 (100)		
Mesangial deposits	25 (100)	8 (38)	32 (100)		
Subepithelial humps	None	4 (19)	10 (31.3)		
Membranous component	1 (4)	4 (19)			
Subendothelial deposits	2 (8)	3 (14)	16 (50)		

ratio was 1.27:1. In the paediatric age group, the male to female ratio was 1.25:1 and in adults it was 2.29:1 (Table 2).

Renal insufficiency was present in 33% of children and in 50% of adults. Proteinuria was universally present. Serum C3 was decreased in all patients. Serum C4 was decreased in one patient. One patient had renal tuberculosis. History of urinary tract infection was present in one. A history of an antecedent acute febrile illness was present in one child. Among the 25 patients, 2 (8%) had partial lipodystrophy and 1 child had drusen. There was no statistically significant difference in clinical features between children and adults (Table 3).

Pathological features

Light microscopy. The most characteristic feature was GBM thickening by eosinophilic, PAS-positive and silver-negative intramembranous deposits, so-called PAS-positive ribbons. The most common histological pattern seen by light microscopy was the membranoproliferative pattern, seen in 52% of patients. The mesangial proliferative pattern was present in 20% of patients, crescentic pattern in 16%, and the proliferative and exudative pattern was present in 12% (Figure 1).

Comparison between children and adults. The crescentic pattern was seen only in patients <18 years of age. The mesangial proliferative pattern was seen only in adults. Adult patients had a significantly greater degree of IFTA than children (Table 4).

IF microscopy. All patients had intense, dominant C3 staining along the peripheral glomerular capillary loops in a ribbon-like pattern (Figure 2). In the mesangium, C3 staining was seen as spherules or coarse granules, so-called mesangial 'rings'. Staining for immunoglobulins either singly or in combination was seen only in biopsies with a membranoproliferative pattern (Figure 2). Although IF on paraffin sections following pronase digestion has been shown to reveal 'masked' antigens, this

technique was not performed in this retrospective study [11]. C1q staining was seen in one patient with membranoproliferative pattern (Table 5).

Electron microscopy. Electron microscopic analysis was performed in all patients and showed highly electron dense transformation of the GBMs (Figure 3). The results are shown in Table 6.

GBM and mesangial electron dense transformation were present in all patients. Subepithelial deposits were seen in only one patient with membranoproliferative pattern. Electron dense transformation of the TBMs was seen in patients with membranoproliferative and mesangial proliferative patterns. All patients had foot process effacement. There were no differences in ultrastructural features between children and adults.

DISCUSSION

DDD was diagnosed 890% more often in this Indian renal biopsy population compared with a large renal biopsy cohort from the USA. The true incidence rate, that is, the number of new cases per population at risk in a given time period, would be difficult, if not impossible, to determine given the extreme rarity of DDD. Thus, we used the biopsy incidence rate as the best available reflection of the incidence of DDD. Using the biopsy incidence rate would, if anything, underestimate the actual incidence of DDD. This is particularly so in the Indian cohort since access to renal biopsy is much more limited. As a result, the actual incidence of DDD is likely even higher in the four states sampled in India compared with the population sampled from across the USA. Interestingly, there is an earlier study from the Post Graduate Institute of Medical Education and Research, Chandigarh in Northern India focused on the clinicopathological features of 13 patients with DDD [12]. Upon review of their data, we discovered a biopsy incidence of DDD in this Indian population of 0.006 (13 cases of DDD out of 4565 biopsies over 5 years). The geographical origin of these biopsies is not described, and, though not as common as in our cohort, this study lends support to the concept of a much higher incidence of DDD in the Indian subcontinent.

The clinicopathological findings of DDD patients in our study are similar to those that have been previously described (Table 7).

The disease most commonly affects children and young adults in India with a mean age of 20.7 in this study and 25.1 years in the previously published series of 13 Indian patients with DDD [12]. Other previously published studies have shown a mean age of 14 years in the study of Lu *et al.* [14], 40 years in the study by Bomback *et al.* [15], the median age in the study by Medjeral-Thomas *et al.* was 12 years [13], in Walker *et al.* was 14 years [8] and in the study of Nasr *et al.* [9] was 33 years. Sixty-four percent of patients in our study and 69% of patients in the study by Viswanathan *et al.* [12] were <25 years of age. The youngest patient in our study was 5 years old, and the maximum age at the time of diagnosis in our study was 41 years. C3 was the predominant immune reactant in all patients. However, only 12% also showed immunoglobulin deposition. This is in contrast to the study by Nasr *et al.* where immunoglobulin staining was seen in 47% of patients [9]. Again similar to earlier studies, only 52% of patients with DDD in our study had a membranoproliferative pattern of glomerular injury, indicating that it is pathologically heterogeneous.

The increased incidence of DDD in India emphasizes the importance of performing electron microscopy analysis of medical kidney biopsies as it is not possible to definitively diagnose DDD without ultrastructural evaluation. The renal pathologist should be vigilant to identify this disease in various histological patterns, especially in the adult population.

The reason for the 8.9-fold increased biopsy incidence of DDD in this Indian subpopulation compared with the USA is unknown. It could be the result of disease-causing genetic variants being more frequent in this subset of the Indian population as genetic aetiologies are well described in a fraction of DDD patients. Environmental factors and possibly chronic infections may contribute to the high biopsy incidence rate in this Indian population as well. However, these factors would also likely be associated with significant but more subtle genetic variants in one or more complement-related proteins [16].

Given the much greater number of patients with DDD in this population, further retrospective and prospective studies would potentially allow more rapid progress in understanding the pathogenesis of DDD and thus potential treatment for patients with DDD.

CONFLICT OF INTEREST STATEMENT

None declared.

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