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Cholesterol crystals tubulointerstitial injury during nephrotic syndrome; can be classified as tubular crystallopathy?

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ABSTRACT

Introduction: Cholesterol crystals and granulomas in tubular lumen and interstitium of the kidney are infrequent findings during nephrotic syndrome (NS) and are poorly described. We attempt to discuss cholesterol crystals in NS as a form of crystallopathy.

Case Presentation: Three cases of 207 (1.5%) performed kidney biopsies, between 2001 and 2019, in patients with NS, showed cholesterol crystals deposition in tubules, interstitium and even cholesterol granulomas with some degree of interstitial mononuclear inflammation with giant cells, interstitial fibrosis and variable tubular atrophy. Oil Red O staining revealed lipid laden macrophages in interstitium and lipid droplets in tubular epithelium. Two patients had membranous glomerulonephritis (MGN) and one membranoproliferative glomerulonephritis (MPGN). The proteinuria ranged from 6.08 to 12.57 g/24 hours, lasting from 1 to 22 months. All had hypertension, high values of serum cholesterol and triglycerides.

Conclusion: Clinically significant deposition of cholesterol crystals in kidney is mainly in atheroembolic renal disease, however deposition of cholesterol crystals during NS is rare and it is not considered a form of crystallopathy. Cholesterol crystals do not seem to be correlated with degree of proteinuria, persistence of NS or type of glomerulonephritis, but it is correlated with amount of serum cholesterol which is strongly associated with the severity of NS. Therefore we propose it as tubular crystallopathy, in the setting of NS associated hypercholesterolemia which may cause chronic kidney disease (CKD).

Implication for health policy/practice/research/medical education:

Cholesterol crystals deposition in the tubulo-interstitium, forming granuloma, in patients with nephrotic syndrome are infrequent and can be classified as tubular crystallopathy.

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Introduction

Crystalline nephropathy is a kind of intratubular precipitation of different exogenous or endogenous crystals. Cholesterol crystals are described in vascular crystalline nephropathies (1-3). They arise from the rupture of the atherosclerotic plaque developing cholesterol crystal embolism (4,5). The presence of cholesterol crystals in the kidney tubular lumen and interstitium, with possible development of granulomas during nephrotic syndrome (NS), is an unusual histopathological finding and poorly

described in the literature (6,7). This prompted us to review our database of kidney biopsies performed between 2001 and 2019 in patients with NS. We found three cases with cholesterol crystals in tubules or interstitial granulomas. In this paper, we attempt to classify more exactly this form of crystallopathy in NS.

Cases Presentation

We reviewed 207 samples of performed kidney biopsies in patients with NS in our hospital between 2001 and 2019.

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Only 3 cases (1.5%), with histologic reports of the presence of cholesterol crystals, were re-evaluated. Hematoxylin and eosin (H&E), periodic acid Schiff (PAS), Masson's trichrome, Jones' methenamine silver (JMS), Oil Red O, CK7 (clone RN7, Leica) and CD 68 (clone PGM-1, Falini) were performed for light microscopy (LM). Accordingly, fluorescein isothiocyanate-conjugated antibodies to IgG, IgM, IgA, C3, C1q, C4, kappa and lambda chains, fibrinogen (DAKO, Carpinteria, CA, USA) were used for immunofluorescence (IF). For transmission electron microscopy (TEM), which performed in one case, the

specimen was processed according to the usual procedures for electron microscopy.

The main clinicopathological features of 207 patients with NS are given in Table 1. NS was defined according to KDIGO guidelines (8) as proteinuria >3.5 g/24 hours, hypoalbuminemia and edema. Clinical features of selected patients are summarized in Table 2. One of these 3 patients underwent a kidney biopsy one year after a first kidney biopsy because of worsening of renal function.

The main histopathological features of three cases are shown in Table 3. Histological glomerular lesions and IF data were consistent with membranous glomerulonephritis (MGN) in two cases and in the other one case with lupus like membranoproliferative glomerulonephritis (MPGN), where TEM excluded fibrillary/immunotactoid glomerulopathy.

The amount of crystals in three cases, as shown in Table 3 was variable.

In all cases cholesterol crystals were present both in the lumen of the tubules and in the interstitium (Figure 1A). The crystals were present mainly in distal tubules, as showed by immunostaining for CK 7 (Figure 1B). They appeared often deformed, damaged with rupture of the basement membrane and surrounded by macrophages (Figures 1B-C-D).

In case 1 cholesterol crystals were present in the interstitium surrounded and englobed by multinucleated foreign-body giant cells forming granuloma.

In all cases the cholesterol crystals appeared on H&E sections, as elongated, empty, narrow cleft with a

Table 1. Clinico-pathological features of 207 patients with nephrotic syndrome

Clinical features	No. (%)
Female	85 (42)
Male	122 (58)
Median age	53 y
Age range	13-83
Urinary protein range (g/24 h)	3.5-26
Renal function (eGFR > 60 mL/min)	119 (57.5)
Renal function (eGFR < 60 mL/min)	88 (42.5)
Histological diagnosis	
Membranous GN	106 (51)
Focal segmental glomerulosclerosis	28 (14)
Minimal change disease	9 (4)
Diabetic renal disease	12 (6)
Amyloidosis	14 (7)
IgA nephropathy	8 (4)
Membranoproliferative GN	5 (2)
Other forms	25 (12)

GN: Glomerulonephritis; eGFR: estimated glomerular filtration rate.

Table 2. Clinical features of selected cases

Case	Year	Sex/ Age	Urinary protein (g/24h)	Renal function sCr (mg/dL)/ CrCl (mL/min)	Total cholesterol/ HDL (mg/dL)	TG (mg/dl)	Urinalysis	Clinical history	Overt nephrotic syndrome -kidney biopsy interval (months)
1	2008	F/73	8	11/51.1	340/40	-	-Glomerular hematuria -Hyaline-granular Casts	Systemic hypertension, renal colic	1
2	2014	M/45	6.08	109/77.6	359/44	269	-Glomerular hematuria	Systemic hypertension	5
3	2017	M/46	12.57	178/60	331/40	192	- Glomerular hematuria (intense) - Hyaline-granular, erythrocytic, lipidic casts - Oval fat bodies	Rheumatoid arthritis treated with MP, MTX, ETC, systemic hypertension	4
	2018	M/46	8.06	328/30	194/43	138	- Glomerular hematuria (intense), - Leukocytes - Hyaline-granular, erythrocytic casts - Tubular epithelial cells	Rheumatoid arthritis, systemic hypertension	22

F: female; M: male; sCr: sieri creatinine normal values 74-106; CrCl: clearance creatinine (sec MDRD) normal values >60; Total cholesterol normal values 0-200, HDL normal values >35; Triglycerides normal values 0-150; MP: methylprednisolone; MTX: methotrexate; ETC: etanercept; TG, triglycerides.

Table 3. Histopathological features of selected cases

Case	Year	Glomerulus			Interstitial/tubules		Vessels	Immunofluorescence	Diagnosis
		Hypercellularity	Basement membrane	Sclerosis segmental	Fibrosis and tubular atrophy	Cholesterol crystals (amount)			
1	2008	Mesangial	Thickened	Absent	5%	Intratubular Interstitium (few) +granuloma	Hyalinosis Arterio-nephrosclerosis	+++IgG, κ and λ chains, diffuse, global, granular along the capillary wall +C3, diffuse, global, granular along the capillary wall +C1q, C4, fibrinogen focal along the capillary wall	MGN
2	2014	Absent	Thickened, Spike-like protrusions	Present (50%)	15%	Intratubular Interstitium (several)	Hyalinosis	+++IgG, κ and λ chains diffuse, global, granular along the capillary wall ++ IgM, diffuse, segmental, granular along the capillary wall	MGN
3	2017	Mesangial, endocapillary and extracapillary	Thickened Duplication	Present (>50%)	<5%	Absent	Normal structure	+++IgG, IgA, C3, fibrinogen, κ chains diffuse, segmental in mesangium and along the capillary wall +C1q focal and segmental in mesangium and along the capillary wall	MPGN lupus like
	2018	Mesangial, endocapillary and extracapillary	Duplication	Present (>70%)	30%	Intratubular Interstitium (many)	Normal structure	+++ C3, diffuse, segmental in mesangium and along the capillary wall ++ IgA, diffuse, global in mesangium and along the capillary wall IgG, fibrinogen, κ chains several deposits along the capillary wall	MPGN lupus like worsening

MGN: Membranous glomerulonephritis; MPGN: Membranoproliferative glomerulonephritis

rhomboid shape and pointed end as shown also by TEM (Figure 1E). There was variable degree of interstitial mononuclear inflammation (Figure 1A) associated with interstitial fibrosis and tubular atrophy. In all cases, cytoplasmic vacuolization of tubules and interstitial foamy macrophages were evident. These cells, on Oil Red O staining, resulted filled with lipids (Figure 1F).

Discussion

Cholesterol crystals in kidney parenchyma are usually known to have an atherosclerotic origin (1-5), by cholesterol embolism, while their presence in renal tissue of patients with NS is not well reported. In the literature few papers described the intra-tubular cholesterol crystals, particularly focusing on the cholesterol granuloma (6,7,9-12). The authors suggested that cholesterol crystals and granulomas might be related to lipid metabolism abnormalities, increased permeability of the glomerular capillary wall and persistence of NS (9). Renal cholesterol granulomas, by analogy with other sites in the body, may be a rare manifestation of chronic inflammatory damage of renal tissue (9).

In our series, we observed cholesterol crystals in 3 of 207 (1.5%) patients with NS. One case (0.5%) showed granulomas. The literature reports a frequency of crystals between 2.7% and 10.5 % and granulomas between 0.6% and 1.2%.

We found crystals and granulomas regardless of degree of proteinuria (Tables 1 and 2) as opposed to available reports (2,5). In fact, we did not observe any cholesterol crystals in patients with more proteinuria than these 3 cases. In support of our data, cholesterol granulomas were identified in a renal biopsy of a diabetic patient without proteinuria and their formation was related to the changes in lipid metabolism occurring in diabetes mellitus (9).

Persistence of NS as shown in Table 2, does not seem to be correlated with crystal formation.

It seems that crystal formation is not correlated with the type of glomerulonephritis, but more cases are necessary to clarify this concept.

The morphological findings of cholesterol crystals and granulomas, in renal biopsies from patients with NS, were first studied in the 80s. Nast et al (7) described the lesions by LM and TEM. Initially, small cholesterol crystals form in tubular epithelium also exfoliated in the lumen, often lodging against tubular cells. Intracellular lipids are also present. Then, the enlarged crystals are released into tubular lumen where they elongate, or pass into the urine. The largest crystals destroy tubular cells, and they pierce basement membranes with subsequent invasion to interstitium where they are ingested by monocytes and finally are transformed to multinucleated giant cells

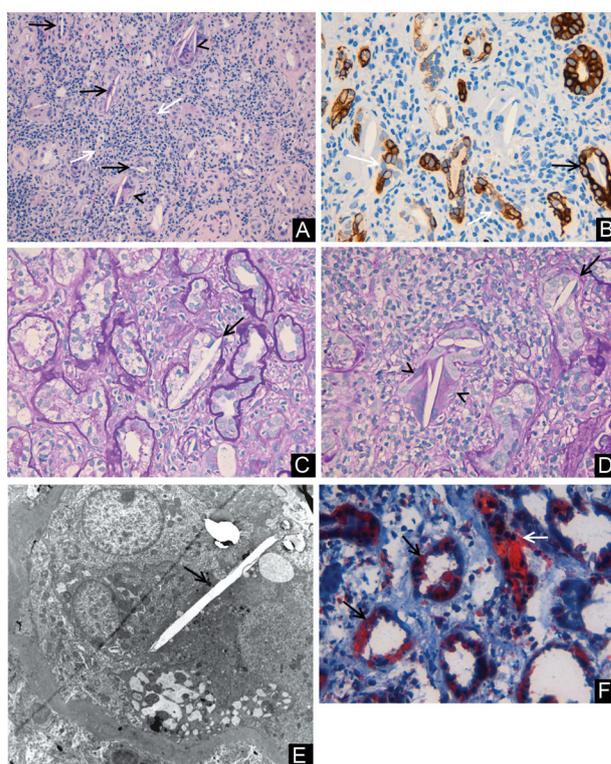


Figure 1. (A) Cholesterol crystals in the tubular lumen (black arrows) and in the interstitium engulfed by multinucleated foreign-body giant cells (arrowheads). Diffuse interstitial mononuclear inflammation (white arrows) (H&E $\times 200$). (B) Cholesterol crystals in distal tubules often damaged (black arrows) (CK 7×400). (C-D) Tubules deformed by crystals (black arrows) with rupture of the basement membrane and engulfed by multinucleated foreign-body giant cells (arrowheads) (PAS $\times 400$). (E) Cholesterol crystals with rhomboid shape (black arrow) (TEM $\times 14000$). (F) Lipid droplets in tubular cells (black arrows) and in interstitial macrophages (white arrow) (Oil Red Red O $\times 400$)

with formation of granulomas, fibroblasts activation and resulting interstitial fibrosis.

Kidney inflammation and injury related to cholesterol crystals during NS could be classified as “crystalline nephropathy” (1-3). Depending on the dynamics of crystal formation, acute kidney injury (AKI) or chronic kidney disease (CKD) can develop. According to the localization of crystal deposits, the crystalline nephropathies are classified as renovascular (type 1), tubular (type 2), urinary tract (type 3). The crystals of type 1 crystallopathy are made up of cholesterol or apatite. Under different conditions, cholesterol crystals present in atherosclerotic plaque are sent which may obstruct arcuate and interlobar arteries causing cortical and medullary infarcts. Type 2 crystallopathy is characterized by different kinds of crystals and according to their composition it can be divided into 4 categories (13): in the setting of dysproteinemia, drug induced, related to calcium deposition and a genetic and metabolic form. Type 3 crystallopathy is characterized by

occurrence of stones in the urinary system.

The intra-tubular cholesterol crystals that can be formed during NS are not reported among crystals induced tubular crystallopathy. We would like to include, for the first time, cholesterol crystals in NS among crystals induced tubular crystallopathy.

The growth of intracellular cholesterol crystals is linked to continued precipitation of cholesterol or cholesterol esters in patients with severe proteinuria and NS, determining increased saturation. Free cholesterol is known to be tissue irritant and granuloma –inducing (7). As for other crystals the main pathogenetic mechanisms induced by cholesterol crystals, not all known yet, consist of activation of the NLRP3 inflammasome and necroptosis, crystal adhesion, in absence of crystallization inhibitors, extra-tubulation, and granuloma formation (1-3,14,15).

Cholesterol crystals could damage tubular cells either by indirect mechanisms, involving inflammation or directly inducing tubular cell death. Indirectly, phagocytosis activates a signal pathway implying NLRP3 inflammasome, a multiprotein oligomer formed by three cytosolic proteins, which specifically triggers IL-1 β -dependent inflammation, establishing the inflammation. The increased expression of cytokine and chemokines leads to infiltration of inflammatory cells.

Conclusion

Formation of cholesterol crystals during NS is rare and does not seem to be related to degree of proteinuria, persistence of NS or type of glomerulonephritis. Cholesterol crystals during NS could be considered a form of crystallopathy and classified as tubular crystallopathy (type 2) in the setting of dyslipidemia. This crystallopathy may eventually lead to CKD and fibrosis. Further studies are needed to understand relation between cholesterol crystals and NS.

Authors' contribution

RDS, CC and RB designed the study, collected clinical data, and analysed data. RS, GB and MM prepared critical revisions. AS was involved with supervision of the study. All authors revised the manuscript and approved the final version.

Conflicts of interest

The authors declare no conflicts of interest.

Ethical considerations

Ethical issues including plagiarism, double publication, and redundancy have been completely observed by the authors.

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