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ORIGINAL ARTICLE

# Ultrastructural Study of Electron Dense Deposits in Renal Tubular Basement Membrane: Prevalence and Relationship to Epithelial Atrophy

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## ABSTRACT

This study reports the prevalence of immune deposits associated with the proximal and distal tubules in a series of routine renal biopsies received in our department during a single calendar year. From 87 cases, 65 (74%) were found to have glomerular immune deposits by immunofluorescence. Tubular immune deposits were found in 12 cases (18%), 3 of which had no glomerular deposits. By transmission electron microscopy (EM), 58 cases (66%) were found to have deposits of granular or vesicular material associated with the tubular basement membranes (TBM). Finely granular electron dense deposits appeared to correspond to the immune deposits seen by immunofluorescence microscopy (IF) and may be a sensitive marker of immune deposition.

**Keywords:** Immune deposit, renal biopsy, renal tubule, transmission electron microscopy

## INTRODUCTION

In renal biopsy diagnosis, the main emphasis in histological examination is usually directed at finding glomerular abnormalities. Tubular lesions are also frequently encountered and although they also reflect the renal function, they are not as well studied or understood in the overall assessment of renal pathology [1]. Those who specialise in renal biopsy diagnosis will appreciate that in a significant number of renal biopsies from patients with renal failure, there are no or minimal glomerular abnormalities and, in these patients, tubular lesions can appear subtle by light, immunofluorescence, and electron microscopy (EM). Tubulointerstitial nephritis may be secondary to a spectrum of primary glomerulonephritis or occurs as a primary disease which may be due to immune complex deposition of antibodies directed against a structural component of the tubule, due to direct toxic effects of poisons or drugs or infections. Tubular necrosis with or without deposits of various classes of immunoglobulins and/or complements is often observed in renal biopsies but the significance and exact mechanism of how tubular lesions are formed are not well understood despite the vast amount of experimental studies and collective renal biopsy experience. The proximal tubular epithelium carries out specialized functions of protein, vitamin, and trace element reabsorption [2] and active sodium transport. Tubular lesions may impair these functions.

We have studied tubular lesions in 87 routine renal biopsies received in our department during a single calendar year to assess their prevalence. Using EM, we have examined the nature and distribution of various deposits associated with the tubular basement membrane (TBM) and correlated them with positive immunostaining for

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immunoglobulin, light chain, and complement seen by IF. Recognition of the ultrastructural features of immune tubular deposits would enhance our general understanding of the pathogenesis of tubulointerstitial nephritis.

## MATERIALS AND METHODS

In our laboratory, all renal biopsies are examined by brightfield light microscopy (LM), immunofluorenence microscopy (IF), and transmission EM when adequate tissue is available. In calendar year 2006, 101 renal biopsies were received. Of these, 87 biopsies from 87 patients were included in this study and 14 were excluded due to poor fixation, insufficient tissue for EM or no renal tissue present. All biopsies were processed using routine methods [3]. They were received fresh in the laboratory in phosphate buffered saline (PBS) for examination under the dissecting microscope and each was then sampled for IF and EM with the remainder processed for LM.

For routine histology, the biopsy was fixed with B5 fixative, processed and cut at 2 and 4 um, stained for hematoxylin and eosin, periodic acid–Schiff, Masson trichrome, methenamine silver, and Congo red.

For IF, 4 μm cryostat sections were air dried, stained with a standard panel including antibodies to immunoglobulins IgA, IgG, IgM, kappa light chains (KLC), lambda light chains (LLC), complement C1q, C3c, and fibrinogen.

Tissue samples for transmission EM were fixed in 2% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.4) at 4 °C. They were then routinely processed and embedded in Spurr low-viscosity resin. *En bloc* staining included 2% osmium tetroxide and 1% uranyl acetate. Ultrathin sections were stained with uranyl acetate and lead citrate and sections were examined and imaged in a Morgagni 268D transmission electron microscope (FEI, Eindhoven, The Netherlands) at 80 kV.

Tissue examined in this study was obtained routinely with full patient consent and all images and analysis was acquired as a part of routine laboratory assessment. This work was carried out in due regard for the provisions of the Declaration of Helsinki.

## RESULTS

A survey of renal biopsies received during a single calendar year has been carried out to assess the prevalence of immune complex deposition associated with the proximal and distal tubules. This cohort comprised 87 native renal biopsy cases diagnosed by routine histology, IF, and EM. The patients ages ranged from 23 to 85 years comprising 48% male and 52% female cases. At the time of examination, detail of any pathology affecting tubules of the kidney cortex was noted. Deposition of immune complex in proximal tubules by IF and abnormality of the TBM by EM were the main assessment criteria.

Screening of all 87 cases by immunofluorescence was based on a standard antibody panel to identify IgA, IgG, IgM, KLC, LLC, C1q, C3c, and fibrinogen. Positive glomerular staining for immunoglobulin or light chain was found in 65 cases (74%) and positive staining for complement was found in 51 cases (58%). The tubules demonstrated less positive staining for both immunoglobulin/light chain and complement with only 12 cases (14%) and 11 cases (12%) respectively (Table 1). This staining was found either in a granular pattern with positivity randomly distributed in the cytoplasm and TBM or linear surrounding the tubule perimeter and corresponding to the TBM (Figure 1).

Examination by EM revealed 58 cases to have some changes evident in the TBM including deposition of vesicular, vacuolar or granular material, and/or thickening of the lamina densa region (Figure 2). Of these, only 12 cases had corresponding positive tubular staining for immunoglobulin, light chains, or complement by IF. Electron dense granular TBM deposits were found in 27 cases mainly with immune complex disease, SLE, and tubulointerstitial nephritis. Vesicular material was found in 31 cases spread across most groups and vacuolar changes were found in 14 cases likewise dispersed through many groups including cases considered to have no specific pathology and only normal ageing (Table 1). Twenty-nine (29) cases showed no specific ultrastructural abnormality of the TBM. In addition to TBM deposits, EM was also able to demonstrate crystalline material in lysosomes of the epithelial cytoplasm and in luminal cast deposits (Figures 2 and 3). This material is considered to represent altered light chain material and corresponded to linear circumferential positive staining by IF.

Of the 27 cases found to contain granular deposits by EM, 12 exhibited corresponding positive staining for immunoglobulin or light chain by IF. The ultrastructural features of these deposits were high electron density and fine granularity (Figure 2). This observation was supported by ultrastructural examination of unequivocal immune deposits seen in glomeruli of lupus nephritis cases and the characteristic tubular deposits seen in light chain disease.

Deposits considered to be non-immune were located mainly within or external to the TBM and appeared to be of more moderate electron density. They contained mainly membranous and vesicular material located within the lamina densa (Figure 4).

A single case we considered to be primary immune complex TBM disease with no glomerular abnormality showed degeneration of the tubular epithelium

TABLE 1. Numbers of cases with positive immunostaining with corresponding EM findings.

| Primary diagnosis | Cases | Glomeruli IF Immunoglobulin or light chain | Glomeruli IF Complement | Tubules IF Immunoglobulin or light chain | Tubules IF Complement | Tubules EM Granular deposits in TBM | Tubules EM Vesicular deposits in TBM | Tubules EM Vacuolar changes in TBM |
|---|---|---|---|---|---|---|---|---|
| Normal aged (within normal limits) | 5 | 2 | 2 | 1 | 0 | 0 | 5 | 1 |
| Amyloid | 1 | 1 | 1 | 0 | 0 | 0 | 1 | 0 |
| Diabetes | 7 | 6 | 4 | 2 | 2 | 2 | 4 | 1 |
| Focal and segmental glomerulosclerosis, necrotising glomeruolonephritis | 11 | 5 | 5 | 1 | 1 | 3 | 6 | 2 |
| Immunoglobulin deposition (including IgA, IgG, and IgM), light chain disease, immune complex tubular basement membrane disease | 41 | 40 | 29 | 6 | 6 | 8 | 9 | 5 |
| Lupus nephritis (SLE) | 8 | 8 | 8 | 1 | 1 | 8 | 4 | 3 |
| Minimal change nephropathy | 5 | 0 | 0 | 0 | 0 | 0 | 1 | 2 |
| Scleroderma | 1 | 1 | 0 | 0 | 0 | 0 | 1 | 0 |
| Tubulointerstitial nephritis, tubular atrophy, and transplant glomerulopathy | 6 | 2 | 2 | 1 | 1 | 6 | 0 | 0 |
| Drug toxicity | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| TOTAL | 87 | 65 | 51 | 12 | 11 | 27 | 31 | 14 |

Renal biopsy cases grouped into main disease entities based on the primary diagnosis. Types of deposits seen in the glomerulus and tubules by immunofluorescence (IF) with corresponding deposits associated with tubular basement membrane (TBM) identified by transmission electron microscopy (EM).

in association with electron dense granular deposits internal to the TBM (Figure 5). The patient was a 68-year-old female who presented with proteinuria (1 g/24 h), microscopic hematuria, serum creatinine 150 μmol/L, and a creatinine clearance rate of 54 mL/min. She also had hypercholesterolemia and clinically did not have features of lupus nephritis. Laboratory tests for SLE were also negative for lupus nephritis. This case also showed positive IF staining of tubules for IgA, IgG, IgM, and C1q and C3c. EM showed electron dense granular deposits lying internal to the lamina densa indenting the basal plasma membrane of the epithelial cells (Figure 5). Epithelial cells in various stages of degeneration from cytoplasmic vacuolation to cytoplasmic fragmentation were associated with this material and, in nearby regions, tubular epithelial cell atrophy was observed.

## DISCUSSION

In immune complex tubulointerstitial nephritis, deposits of immunoglobulin and complement may be found in the proximal tubule lumen, tubular epithelial cell cytoplasm, TBM, and the interstitium. These locations are likely sites of hold-up or blockage in the normal pathway of protein metabolism in the proximal tubule [4], although the factors that determine where the deposition of proteins occurs are not well understood. Under normal physiological conditions, protein is taken up from the urine by endocytosis into the tubular epithelial cells where it is metabolized in lysosomes. Amino acids are returned to the blood after traversing the basement membrane and interstitial space [4].

In diseased states, there may be alteration of the nature of the protein or excessive accumulation of protein in any of the tubular or interstitial compartments. This may lead to complement activation, endocytic activity, or upregulation of genes leading to the production of mediators that promote inflammation, cause tubular degeneration, and fibrosis [5]. It is, therefore, important to establish ultrastructural morphological indicators of chronic injury and impending tubular degeneration. In addition, it is considered that apoptosis of tubular epithelial cells might be caused by stimuli other than protein overload [5] so it is important to look for mechanisms responsible for tubular cell death.

Our study has demonstrated that deposits in the TBM and ultrastructural alterations of the TBM in various forms are almost always present in many routine renal biopsies. The tubular deposits were associated with both the proximal and distal tubules as identified by their specific ultrastructural appearance; however, collecting tubules were rarely affected.

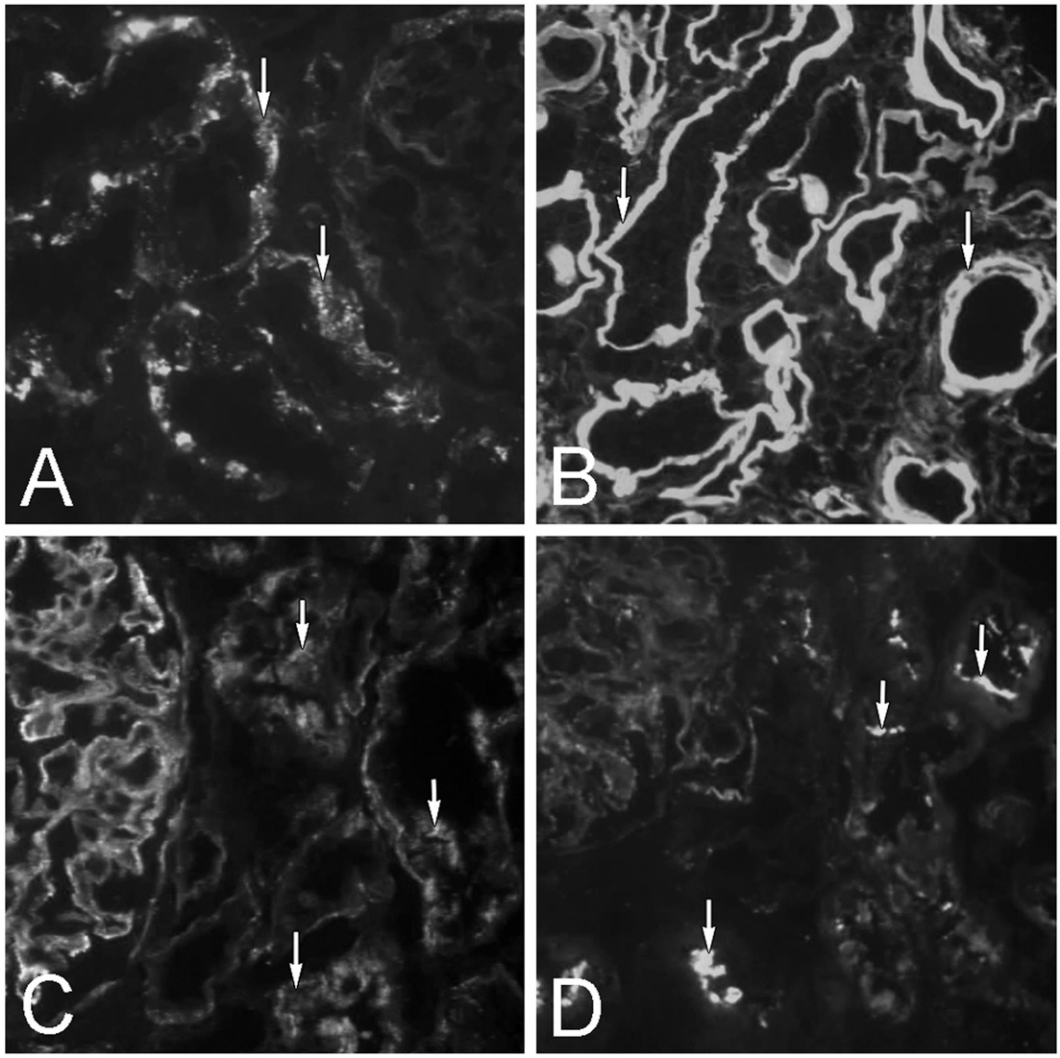


FIGURE 1. Immunofluorescence microscopy showing examples of staining patterns for immune deposits associated with proximal and distal tubules. (A) Granular staining of the TBM with anti-C3c (arrows) from a case of immune complex tubular basement membrane disease. (B) Linear staining of the TBM with anti-KLC (arrows) from a case of KLC disease. (C) Diffuse cytoplasmic staining of the epithelium with anti-LLC (arrows) from a case of lupus. (D) Focal staining of the apical cytoplasm and desquamated debris with anti-C3c (arrows) from a case of lupus.

We have found that there was no direct correlation between the tubular deposits and the severity of glomerulonephritis, severity of renal failure, proteinuria, or hematuria. We drew this conclusion because in some cases with severe renal failure, little, or no tubulointerstitial deposits were present whereas, in contrast, large amounts of deposits were found in cases with minor impairment to renal function. There were also cases where electron dense deposits were found when no tubular deposits were detected by light microscopy or immunofluorescence (IF). In these patients, there was no significant proteinuria or hematuria.

On the basis of our observations, the cases can be broadly divided into two categories for comparison. Included in one category are those cases with positive IF changes with clinical history of renal impairment and electron microscopic TBM abnormalities. In the other category are those cases which were negative by IF examination and with no clinical history of renal failure but in which TBM EM-detected deposits were found. In both groups, there was no direct correlation with the severity of proteinuria.

Immune deposition was found to occur in both intracellular and extracellular locations in cases with light chain disease and myeloma. Crystalline deposits were found in tubules as intracellular elongated crystals in lysosomes, intracellular rhomboid masses in phagolysosomes, extracellular crystals in the tubular lumen, and as linear deposits external to the TBMs. Crystalline cytoplasmic inclusions showing rhomboid and oval shapes have been described previously in myeloma by us and others [6,7] and the content of light chain fragments confirmed by immunoelectron microscopy [1].

Immunoglogulin deposits identified by IF appeared to correspond to finely granular electron dense deposits by EM. There was good correlation in

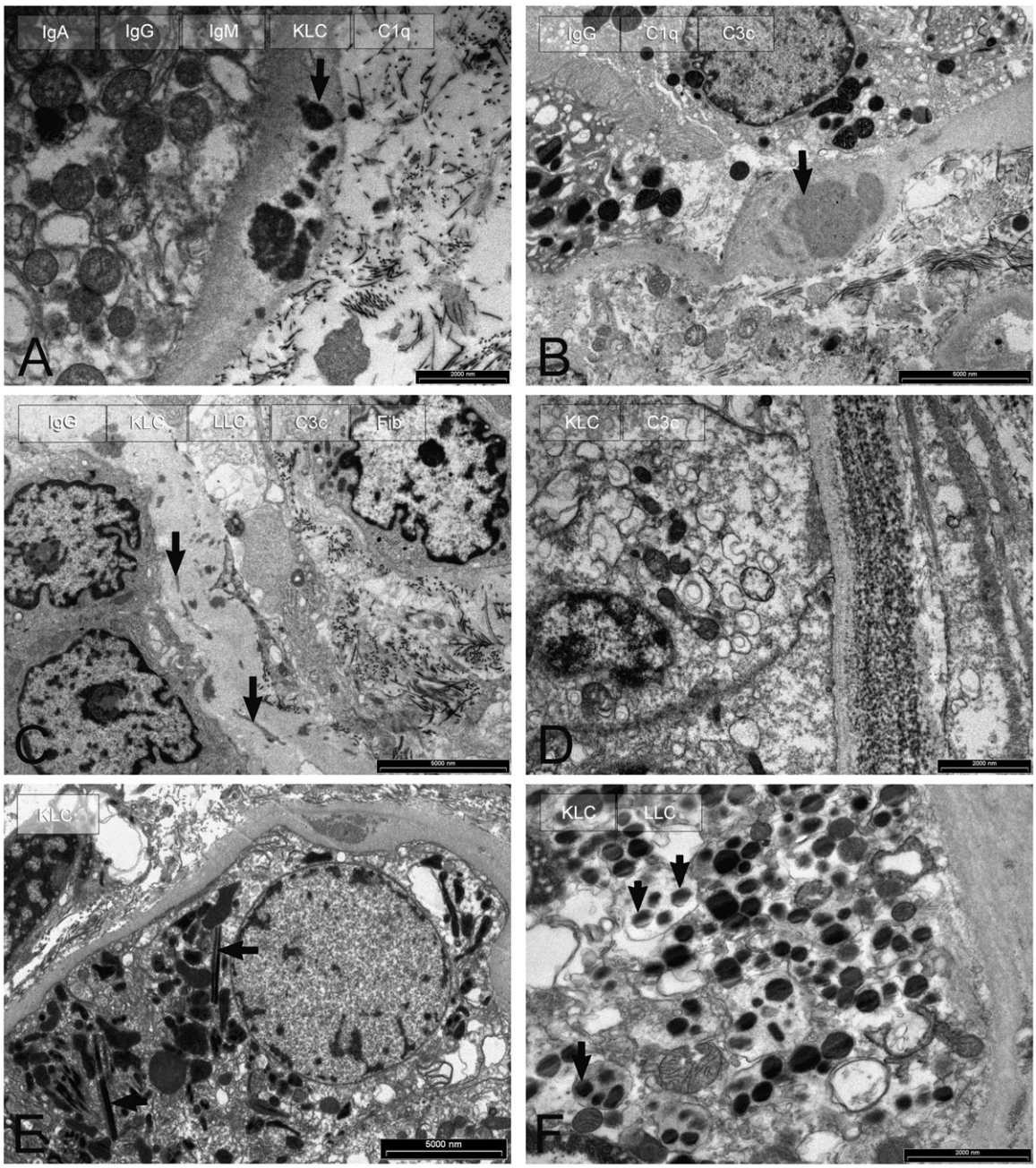


FIGURE 2. Examples of immune deposition associated with tubular epithelium with positive immunofluorescence staining indicated in boxes. (A) Electron dense granular material embedded in the basement membrane (arrow). (B) Large focal deposit of electron dense granular material corresponding to Figure 1(A) (arrow). (C) Patchy electron dense granular material and fibrinogen fibers (arrows) embedded in the thickened basement membrane. (D) Fine granular immune deposit forming a linear pattern external to the tubular basement membrane corresponding to Figure 1(B). (E) Granular deposits in the basement membrane and elongated crystalloids (arrows) within the epithelial cytoplasm from a case of myeloma. (F) Rhomboid crystalloids in lysosomes (arrows) within the epithelial cytoplasm from a case of myeloma.

lupus nephritis cases where IF positive immune deposits in the glomerulus corresponded with electron dense granular deposits. This correlation supported the interpretation that immune deposits appear electron dense by EM. Electron dense deposits were rarely seen in patients with no history of immune positive disease although other TBM abnormalities such as vesicular material and vacuolar spaces were present.

There was some overlap in the appearance of immune and non-immune granular deposits associated with the TBM. There were some immune-like deposits which were detected by EM but not found by IF examination emphasizing the importance of electron microscopic examination of the TBM, in particular, in those cases which were IF negative. Our data suggest that EM may be a more sensitive method of detecting immune deposits as the electron

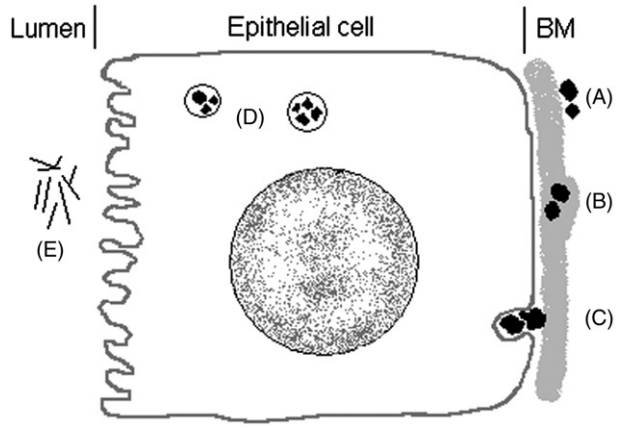


FIGURE 3. Diagram depicting sites of immune deposition associated with the tubular epithelium. (A) Linear and granular deposits external to the TBM. (B) Granular deposits within the TBM lamina densa. (C) Granular deposits internal to the TBM. (D) Cytoplasmic accumulation of crystalloids in lysosomes. (E) Luminal deposition of crystalloids.

dense granular material was found in the TBM of 27 cases overall compared with 12 cases by IF. We speculate that the presence of EM detected deposits may explain renal insufficiency in patients which were otherwise normal by light and IF microscopy. In this study, we were able to distinguish the non-immune granular deposits which were generally electron pale, located external to the TBM, and may be larger in size than immune deposits. Non-immune granular deposits also were found early in life so may represent another disease process affecting the tubules. They remain of uncertain pathological significance and require further immunocytochemical characterization.

In addition to granular deposits, other changes in the TBM including splitting of the lamina densa and incorporation of membranous and vesicular material were also seen. In none of these cases was there clinical evidence of tubulointerstitial nephritis. At worse, some cases had non-specific patchy tubular atrophy and minimal interstitial changes not considered to be significant. It is most likely that these changes are degenerative or non-specific resulting from non-immune related injury or possibly transient biochemical disturbance. Similar changes have been noted in nephropathia epidemica and were thought to result from viral injury [8]. Membranous structures and lucent areas in the TBM have also been found in a variety of glomerular diseases and have been significantly correlated with increased membrane attack complex (MAC) or terminal complement complex deposits [9]. In cases which displayed linear immune deposition by IF, such as in cases of KLC disease, the corresponding EM findings showed electron dense material in linear deposits, sometimes in multiple layers extending into the surrounding stroma. Once again our

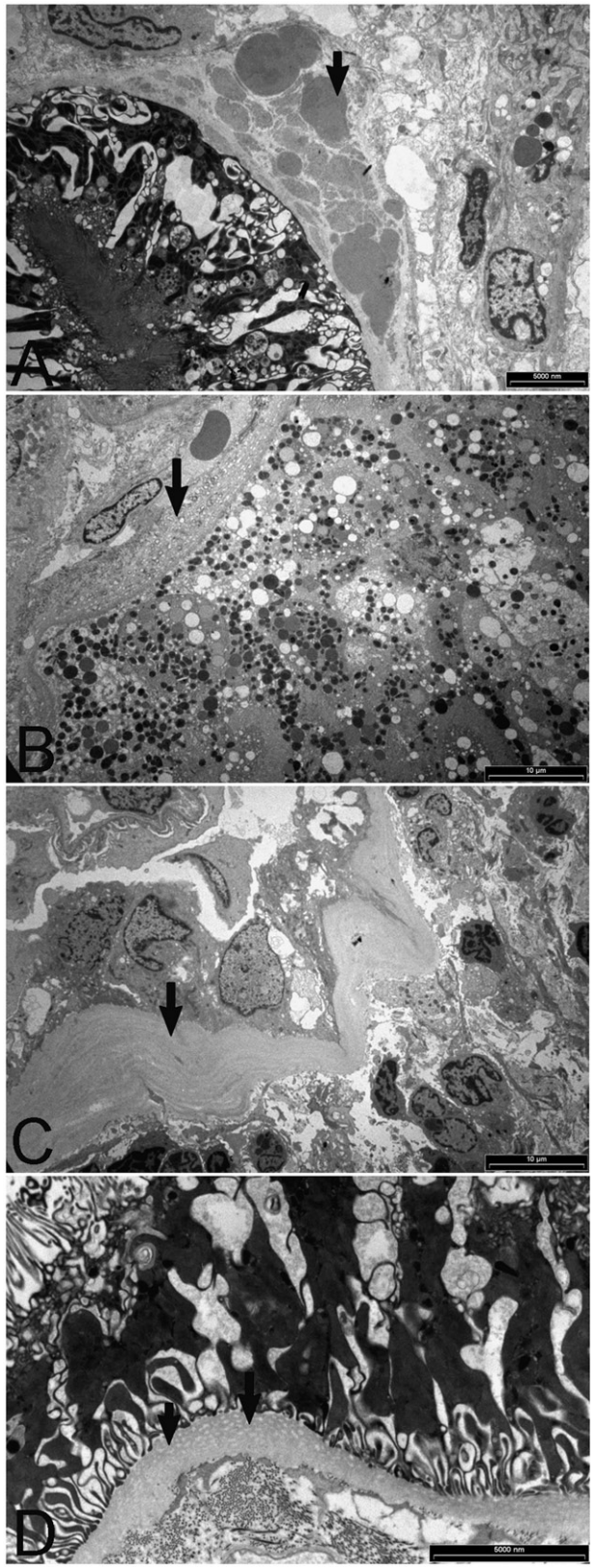


FIGURE 4. Non-immune deposits of the TBM. (A) Massive deposits of vesicular material within the TBM (arrow). (B) Vesicular material (arrow). (C) Thickened basement membrane (arrow). (D) Vacuolation of the basement membrane (arrows).

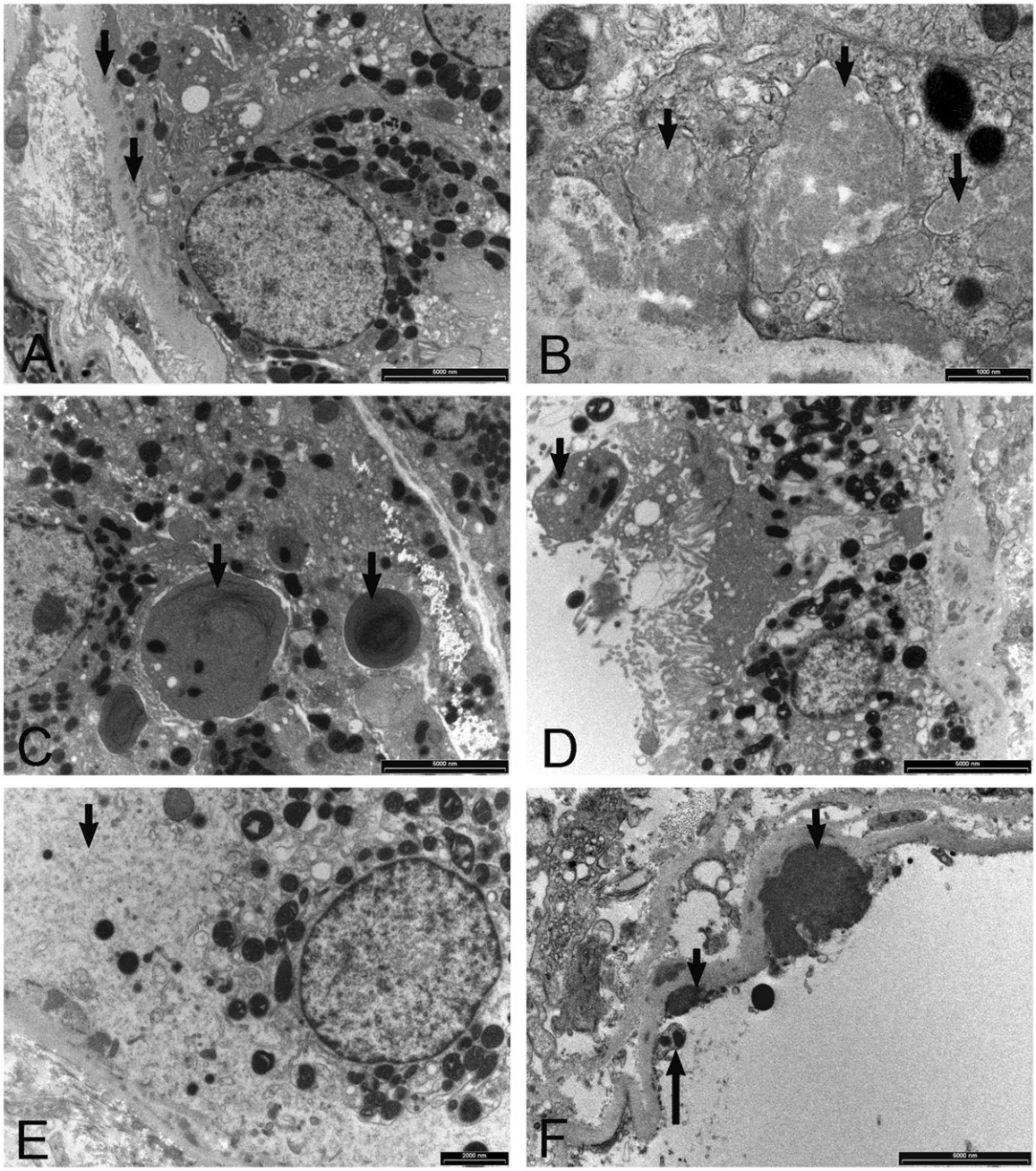


FIGURE 5. Build up of immune complex deposits leading to epithelial cell apoptosis and atrophy in a case of ICTBM disease. (A) Patchy granular deposits (arrows) within the TBM. (B) Extensive granular deposits (arrows) building up internal to the TBM. (C) Autophagic vacuoles or apoptotic fragments (arrows) of degenerating epithelial cytoplasm. (D) Jettisoned apoptotic fragments of epithelial cytoplasm (arrows). (E) Epithelial cell with ruptured plasma membrane in the region of arrow. (F) Complete atrophy of epithelium associated with large granular deposits (arrows) lying internal to TBM, remnant epithelial cytoplasm (long arrow).

findings in this study highlight the need to examine TBM changes carefully by EM.

In the single case we considered to be primary immune complex TBM disease, there was heavy immune complex deposition with atrophy of tubular epithelium. EM showed the deposits to be lying internal to the TBM and extending inwards displacing the tubular epithelial cells and disrupting cell junctions. The affected cells displayed large lysosome or autophagosome-like inclusions which occasionally were ejected into the tubule lumen, suggesting these cells were under stress or undergoing a protection mechanism against impending apoptotic cell death [10]. These tubules also displayed regions of deleted epithelial cells suggesting that epithelial cell loss had occurred. We could not be sure whether this cell loss had resulted from cell death or epithelial transdifferentiation and migration into the stroma. Epithelial mesenchymal transformation [11] or histiocytic transdifferentiation [12] are processes

thought to arise after tubular injury and can result in loss of epithelial cells and the generation of an interstitial fibrotic phenotype [13,14]. The results of our study suggest that apoptosis preceded epithelial cell atrophy in agreement with similar findings from experimental studies of accelerated interstitial fibrosis [15], microembolism-induced chronic ischaemic injury in rats [16], and human diabetic renal disease [17].

In conclusion, this study is not intended to be a comprehensive study of ultrastructural changes in tubules related to severity of proteinuria but to highlight that in renal biopsies ultrastructural tubular changes are frequently present and may contribute to the pathology. Also, to point out that careful EM examination for tubular deposits must be carried out in all renal biopsies, especially in those cases where no glomerular lesions are seen. The deposition of immune material in and adjacent the TBM is a part of a spectrum of ultrastructural changes that can indicate changes in function of the tubular epithelium. In most cases, immune deposition indicates worsening renal function from preexisting glomerular injury and may indicate the onset of chronicity. However, in some cases, such as ICTBM disease immune deposition associated with the TBM may represent the primary pathology and indicate impending tubular atrophy.

## DECLARATION OF INTEREST

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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