Imerslund-Gräsbeck syndrome: the significance of the annular deposits or structures determined by electron microscopy

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INTRODUCTION

Imerslund-Gräsbeck syndrome (IGS) or juvenile megaloblastic anemia 1 or selective vitamin B12 (cobalamine) malabsorption with proteinuria independently described from Finland and Norway (Imerslund and Gräsbeck et al.) in 1960 is a rare autosomal recessive familial disorder characterized by selective malabsorption of intestinal cobalamine (vitamin B12) from the terminal ileum and renal tubular protein reabsorption deficiency [1, 2]. This syndrome is usually resulting in megaloblastic anemia which is responsive to parenteral vitamin B12 therapy appearing in childhood. Recessive mutations in the cubulin (CUBN) and amnionless (AMN) genes underlie IGS have been described. The protein products from these genes together constitute the intestinal and renal receptor for vitamin B12, called Cubam complex that functions as the receptor for the intrinsic factor-Cobolamin I complex in the ileal enterocytes and for proteins found in the renal proximal tubule epithelial cells [3-5]. Mild proteinuria is frequently present [6, 7]. Proteinuria may either glomerular or tubular [8, 9]. However, most accepted hypothesis is that the proteinuria is originated from defective tubular function. No structural glomerular or tubular damage. To explain the proteinuria, defective or lack cubulin, which participates in the tubular reabsorption of some...
proteins in the primary urine, may be considered [6]. Amnionless (AMN) is thought to bind to cubilin (CUBN) and is essential for apical membrane localization and endocytic functions and its conduction to the subcellular region [5, 6]. Light and electron microscopic findings of kidney biopsies demonstrated that no or slight and nonspecific changes. These morphological findings suggested that was result of the familial cobalamine deficiency rather than true kidney disease [6]. In IGS with proteinuria, urine protein electrophoresis revealed that the excretion of total protein and albumin and in the lesser amount of transferrin, immunoglobulin light chains, and α1- and β2-microglobulins was increased [6, 7].

Herein, we detailed emphasized the renal ultrastructural features in IGS with proteinuria rather than its light and immunofluorescence findings in kidney and tried to investigate the significance of annular deposits or structures detected by electron microscopy.

CASE REPORT

The patient was a 19-year-old-male who was under vitamin B 12 and folic therapy for 18 years because of megaloblastic anemia known since 1-year-old. There was no history of renal disease such as cryoglobulinemia, plasma cell dyscrasia and systemic lupus erythematosus (SLE), use of nephrotoxic drugs, renal stones, urinary tract or upper respiratory tract infections. He admitted nephrology service with flank pain. His blood pressure was 120/80 mmHg. His routine blood chemistry, total protein, albumin, creatinine, blood urea nitrogen (BUN), cryoglobulin, immunoglobulins, C3 and C4 were normal. Urinalysis showed 3 + protein, 1-2 WBCs/HPFs. No hyaline and granular casts were present. Urine specific gravity was 1.020 and pH was 6.00. A 24-hour urine collection showed a total volume of 1500 ml and total protein of 518 mg/24 h. On admission, gross or microscopic hematuria was not present. Antinuclear antibody (ANA) titer was normal and anti-double-stranded DNA antibody and antineutrophil cytoplasmic antibodies (ANCA) were negative. Hepatitis B and C markers were negative. The albumin fraction was dominant in the urine protein electrophoresis and other proteins were low level. Kidney biopsy was performed because of the persistent proteinuria.

Light microscopy revealed minimal mesangial hypercellularity and increase in the amount of mesangial matrix in some glomeruli (Fig. 1). Tubulointerstitial compartment was well preserved. No amyloid deposition was identified with Congo red stain. Vascular structures well preserved. In immunofluorescence examination was unremarkable and only detected mesangial and peripheral minimal (1+/2+) finely granular immunoglobulin (Ig) A (Dako, Denmark; dilution 1:100), Ig M (Dako, Denmark; dilution 1:100) and C3 deposition (Dako, Denmark; dilution 1:100). The other antibodies, which were Ig G (Dako, Denmark; dilution 1:100), C1q (Dako, Denmark; dilution 1:100), fibrinogen (Dako, Denmark; dilution 1:100), kappa light chain (Dako, Denmark; dilution 1:100), and lambda light chain (Dako, Denmark; dilution 1:100), were negative. Semi-thin section obtained from plastic-embedded tissue blocks was stained with toluidine blue. In semi-thin section, minimal mesangial hypercellularity and mild thickening and thinning in glomerular basement membranes were seen (Fig. 2). Electron microscopic examination was operated by Zeiss Libra 120 (Carl Zeiss microscopy GmbH, Germany). In electron microscopic examination of thin sections with stained uranyl acetate sited on the copper grids, electron dense and/or fibrillary deposits were not seen (Fig. 3). Annular deposits or structures in podocytes (Fig. 3, 4, 5 and 8), mesangial (Fig. 3, 6 and 7), endothelial (Fig. 8) and proximal tubular epithelial cells (Fig. 9) were the most striking finding, ultrastructurally. The glomerular basement membrane (GBM) thickness was ranged from 119 nm to 503 nm (Fig. 4 and 5). There were swelling in endothelial cells and podocytes (glomerular epithelial cells) with “watery” or “filamentous” appearance. Microvillus formation, minimal focal foot process fusion and pinocytotic vesicles were also seen in some podocytes (Fig. 3, 4 and 5).

Figure 1. Minimal mesangial hypercellularity (H&E, x400).
Figure 2. In semi-thin section, minimal mesangial hypercellularity and mild thickening and thinning in glomerular basement membranes (Toluidine blue, x1000).

Figure 3. Annular deposits or structures (polyribosomes) in podocytes, mesangial, endothelial. The swelling, filamentous appearance and microvillus formation have been seen in podocytes (Electron micrograph, x4000).

Figure 4. Annular deposits or structures (polyribosomes) in podocytes. The swelling, filamentous appearance and pinocytic vesicles have been seen in podocytes. Mild thickening in glomerular basement membranes are seen (Electron micrograph, x6300).

Figure 5. Annular deposits or structures (polyribosomes) in podocytes, and endothelial. The swelling and filamentous appearance has been seen in podocytes. There are also minimal and focal fusions in foot process. Mild thickening in glomerular basement membranes are seen (Electron micrograph, x6300).
Figure 6. Annular deposits or structures (polyribosomes) in cytoplasm of mesangial cell (Electron micrograph, x16000)

Figure 7. In high power field, annular deposits or structures (polyribosomes) has been seen in cytoplasm of mesangial cell (Electron micrograph, x31500)

Figure 8. Annular deposits or structures (polyribosomes) in podocytes. The swelling and filamentous appearance has been seen in podocytes. There are also minimal and focal fusions in foot process (Electron micrograph, x8000)

Figure 9. Annular deposits or structures (polyribosomes) in cytoplasm of proximal tubule epithelial cell (Electron micrograph, x8000).
DISCUSSION
Imerslund-Gräsbeck syndrome (IGS) was first described in 1960 by Imerslund and Gräsbeck et al., independently [1, 2]. Cobalamine (Cbl, vitamin B12) is a coenzyme for the several enzymes and its deficiency causes to potentially lethal disorder or disease present with megaloblastic anemia and severe neurological symptoms [5]. There are polymorphisms in a number of genes involved cobalamine metabolism. The mutations of genes involved cobalamine metabolism affect its absorption (intrinsic factor deficiency, Imerslund-Gräsbeck syndrome), transport (transcobalamin deficiency) and intracellular metabolism affecting adenosylcobalamin synthesis (cblA and cblB deficiencies), methionine synthase function (cblE and cblG deficiencies) or both (cblC, cblD and cblF deficiencies) [10]. IGS is a rare heritable disorder of intestinal cobalamin (Cbl, vitamin B12) absorption and renal tubular protein reabsorption [1-9]. IGS seems to be more common in Scandinavian countries and Israel [1, 2, 9, 10]. Typical neurological disorders reported in cobalamin deficiency are seldom pronounced in IGS [7, 9, 11]. Neurological abnormalities detected in 9 % of Turkish population [9].

Proteinuria often, but not always, accompanies IGS. Proteinuria is traditionally categorized as either glomerular or tubular. In the former, albumin and bigger molecules are excreted, in the latter less albumin and some smaller proteins such as transferrin, immunoglobulin light chains, and α1- and β2-microglobulins are found [1, 2, 8]. Wahlstedt-Fröberg et al study suggested that moderate or clearly evident proteinuria was present in 46 % (in 6 of 13 cases) of Finnish patients. They purposed that the proteinuria was neither typically glomerular nor tubular in their study [6]. Moreover, they concluded that proteinuria does not clearly correlate with CUBN or AMN mutations, varies in total urinary protein amounts (clear-cut, mild and borderline proteinuria), as well as in urinary protein types and cannot be classified as glomerular or tubular [6]. Broch et al detected proteinuria (range 13-1460 mg/h) in 100 % (in all of 14 cases) of Norwegian patients [8]. They concluded that the proteinuria is predominantly of glomerular origin, but some is also of tubular origin. Proteinuria was detected in 78 % of Turkish population [9]. These studies indicated that the proteinuria persisted over the years in properly treated patients and however, kidney function did not deteriorate and the renal lesions did not seem to be progressive. In presented case, proteinuria was mild and persistent.

Light and electron microscopy of kidney biopsies have revealed normal or slight and uncharacteristic changes such as mesangial hypercellularity [1, 8]. In presented case, light microscopic findings were unremarkable with slight mesangial hypercellularity as previous studies. However, in electron microscopy, there was different finding which was not described before. In our case, the most striking ultrastructural finding was annular deposits/structures in podocytes, mesangial, endothelial and proximal tubular epithelial cells. These deposits are usually associated with cryoglobulinemia, plasma cell dyscrasia, systemic lupus erythematosus and immunotactoid glomerulopathy [12]. These deposits usually associated with morphological changes seen in light microscopy. In presented case, there were neither clinical nor morphological findings of these diseases. Light microscopic findings in the kidney biopsy are unremarkable exclude the presence of annular deposits or structures only detected by electron microscopy. In presented case, we considered that annular deposits/structures can be polyribosome. The increases in the number of polyribosome may be associated with vitamin B12 supplement therapy [13].

In conclusion, annular structures considered as polyribosome was ultrastructurally first described in a patient with IGS. Our results also suggested that fine structural changes not seen with conventional light microscopy might be seen by electron microscopy in IGS. However, these structures have been carried out the possibility of confused with annular organized immune deposits. Indeed, these structures are not deposits. It must be kept in mind that this polyribosome may increase and be prominent in these patients with IGS, who take vitamin B12 therapy.

REFERENCES
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