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Separation of Urinary Proteins with Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis in Patients with Secondary Nephropathies

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Abstract : Background: Proteinuria is an important feature of secondary nephropathies. The quantitative and qualitative analysis of proteinuria plays an important role in determining the types of proteinuria (glomerular, tubular and mixed), in the diagnosis and prognosis of secondary nephropathies. The damage of the glomerular basement membrane is responsible for a proteinuria characterized by the presence of large amounts of protein with high molecular weights such as albumin (69 kilo Daltons-kD), transferrin (78 kD) and immunoglobulin G (150 kD). An insufficiency of proximal tubular function is the cause of a proteinuria characterized by the presence of proteins with low molecular weight (LMW), such as retinol binding protein (21 kD) and α1-microglobulin (31 kD). In some renal diseases, a mixed glomerular and tubular proteinuria is frequently seen. Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) is the most widely used method of analyzing urine proteins for clinical purposes. The main aim of the study is to determine the type of proteinuria in the most common secondary nephropathies such as diabetic, hypertensive nephropathy and preeclampsia. Material and methods: In this study were included 90 subjects: subjects with diabetic nephropathy (n=30), subjects with hypertensive nephropahty (n=30) and pregnant women with preeclampsia (n=30). We divided all subjects according to UM/CR into three subgroups: macroalbuminuric (UM/CR >300 mg/g), microalbuminuric (UM/CR 30-300 mg/g) and normolabuminuric (UM/CR<30 mg/g). In all subjects, we measured microalbumin and creatinine in urine with standard biochemical methods. Separation of urinary proteins was performed by SDS-PAGE, in several stages: linear gel preparation (4-22%), treatment of urinary samples before their application on the gel, electrophoresis, gel fixation, coloring with Coomassie blue, and identification of the separated protein fractions based on standards with exactly known molecular weight. Results: According to urinary microalbumin/creatinin ratio in group of subject with diabetic nephropathy, nine patients were macroalbuminuric, while 21 subject were microalbuminuric. In group of subjects with hypertensive nephropathy, we found macroalbuminuria (n=4), microalbuminuria (n=20) and normoalbuminuria (n=6). All pregnant women with preeclampsia were macroalbuminuric. Electrophoretic separation of urinary proteins showed that in macroalbuminric patients with diabetic nephropathy 56% have mixed proteinuria, 22% have glomerular proteinuria and 22% have tubular proteinuria. In subgroup of subjects with diabetic nephropathy and microalbuminuria, 52% have glomerular proteinuria, 8% have tubular proteinuria, and 40% of subjects have normal electrophoretic findings. All patients with maroalbuminuria and hypertensive nephropathy have mixed proteinuria. In subgroup of patients with microalbuminuria and hypertensive nephropathy, we found: 32% with mixed proteinuria, 27% with normal findings, 23% with tubular, and 18% with glomerular proteinuria. In all normoalbuminruic patiens with hypertensive nephropathy, we detected normal electrophoretic findings. In group of subjects pregnant women with preeclampsia, we found: 81% with mixed proteinuria, 13% with glomerular, and 8% with tubular proteinuria. Conclusion: By SDS PAGE method, we detected that in patients with secondary nephropathies the most common type of proteinuria is mixed proteinuria, indicating both loss of glomerular permeability and tubular function. We can conclude that SDS PAGE is high sensitive method for detection of renal impairment in patients with secondary nephropathies.

Keywords : diabetic nephropathy, preeclampsia, hypertensive nephropathy, SDS PAGE
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