Proteomic Profile of Retained Proteins from Hemodiafiltration with on-line Endogenous Reinfusion (Supra-HFR) Cartridge

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BACKGROUND

Hemodiafiltration with on-line endogenous reinfusion (Supra-HFR) is a dialytic method, which combines the processes of diffusion, convection and adsorption. The performance of this system is linked to the optimal combination of the membrane permeability and cartridge resin bed [Wratten 2007].

Lupus nephritis (LN) is one of the most severe manifestation of systemic lupus erythematous (SLE), associated with considerable morbidity and mortality. Cytokine plays a key role in disease initiation and progression, in fact in the kidney immunocomplexes (ICs) deposition activate mesangial cells. Once activated, by ICs and/or autoantibodies, renal resident cell secret the cytokines which may further amplify inflammatory processes [Borchers 2012].

In this preliminary study, ESI-QTOF-MS (Electrospray Ionization with Quadrupole Time-of-flight Mass Spectrometer) was used for protein identification of ultrafiltrate (UF) and for the protein captured by resin bed, obtained from one dialysed patient with LN.

METHODS

Plasma, UF (pre and post cartdrige) of one patient with LN treated with Supra-HFR, were collected at the 15 min and at 235 min of the dyalitic session. The cartridge utilized during treatment, containing styrenic resin, was opened and the proteins kept by the resin were eluted by incubation O/N with 60% ACN and 1%TFA. Samples were desalted and separated by SDS-page, interesting band were picked and "in-gel" triptic digested before ESI –QTOF-MS analysis.

RESULTS

ESI-QTOF analysis of eluted proteins resulted in the identification of several biomarker of kidney injury in LN, such as Retinol binding protein 4, Neutrophil gelatinase-associated lipocain, and Cystatin-C (and Serotransferrin, Alpha-1-acid glycoprotein, Prostaglandin-H2 D-isomerase, Transthyretin). Moreover we identified several fragments of Immunoglobulin, that are implicated in the etiopathogenesis of LES.
Another important protein in phatophisiology of LES is beta-2-glycoprotein 1, protein involved in antiphospholipid syndrome, associated to arterial and venous thrombosis, characterized by up to 30 different autoantibodies [Shoenfeld 2008]. In fact McNeil et al. identified beta-2-glycoprotein 1 as a cofactor required for antiphospholipid antibodies (APA) to bind to cardiolipin [McNeil 1990].

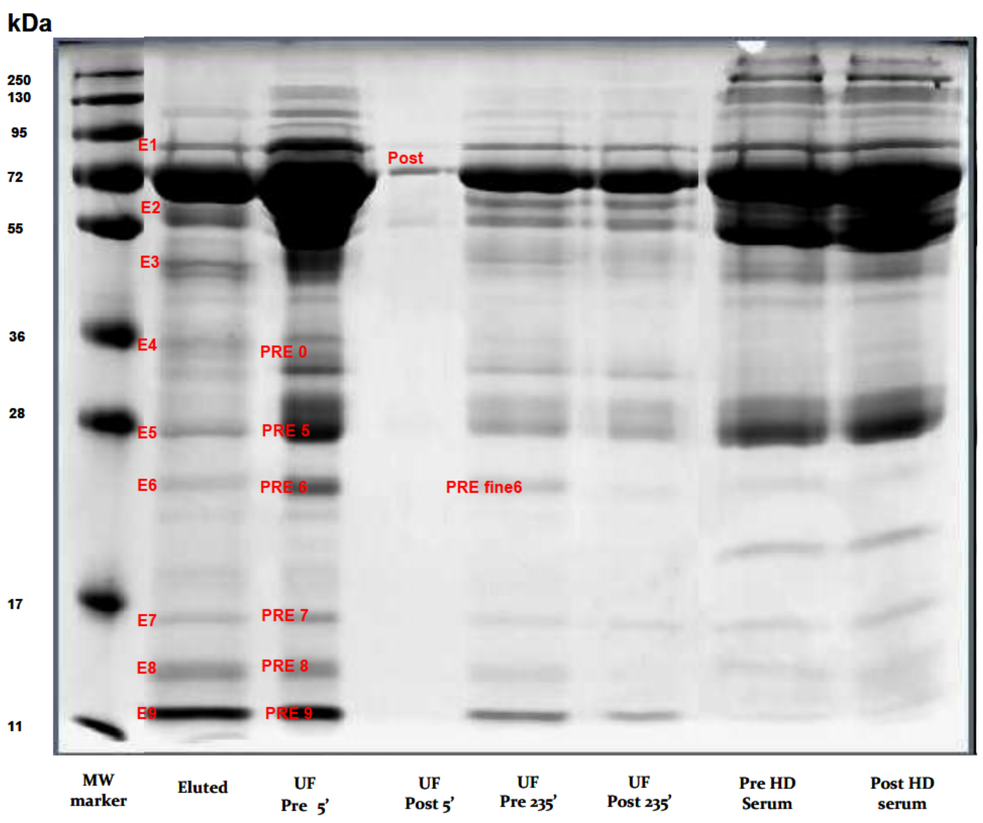

Fig . 1D gel of Plasma, UF (pre and post cartdrige) and eluted of cartdrige samples and ESI-QTOF identification result of protein gel band .

Eluted	UF Pre 5'	UF Pre 235'
	PRE 0 Protein AMBP Ig gamma-1 chain C region	
E1 Serotransferrin Serum albumin		
E2 Serum albumin Vitamin D-binding protein Alpha-1-antitrypsin Beta-2-glycoprotein 1 Ig gamma-1 chain C region Alpha-2-HS-glycoprotein Ig gamma-2 chain C region Monocyte differentiation antigen CD14		
E3 Apolipoprotein A-IV Zinc-alpha-2-glycoprotein Alpha-1-acid glycoprotein 1 Alpha-1-acid glycoprotein 2 Complement factor H-related protein 1 Leucine-rich alpha-2-glycoprotein Protein AMBP		
E4 Insulin-like growth factor-binding protein 2 Protein AMBP Ig gamma-1 chain C region		
E5 Complement factor D Apolipoprotein A-I Prostaglandin-H2 D-isomerase Immunoglobulin lambda-like polypeptide 5	PRE 5 Immunoglobulin lambda-like polypeptide 5 Complement factor D Apolipoprotein A-I Prostaglandin-H2 D-isomerase Ganglioside GM2 activator	
E6 Retinol-binding protein 4 Tetranectin Neutrophil gelatinase-associated lipocalin	PRE 6 Retinol-binding protein 4 Neutrophil gelatinase-associated lipocalin Tetranectin	PRE fine 6 Retinol-binding protein 4 Neutrophil gelatinase-associated lipocalin Tetranectin
E7 Transthyretin Ribonuclease 4	PRE 7 Transthyretin	
E8 Lysozyme C Cystatin-C Neutrophil defensin 1 Angiogenin Guanylin	PRE 8 Cystatin-C Lysozyme C Ig kappa chain C region Ig lambda-2 chain C regions Neutrophil defensin 1 Angiogenin Profilin-1	
E9 Beta-2-microglobulin Neutrophil defensin 1	PRE 9 Beta-2-microglobulin Neutrophil defensin 1	

CONCLUSIONS

The results of this study demonstrate that, styrenic resin retain several proteins implicated in the Lupus nephritis pathogenesis because the corresponding protein bands disappear in UF samples confirming the remotion of these proteins by the cartridge. This means that Supra-HFR is a dialytic method that reduce inflammatory status, uremic toxin level and antiphospholipid syndrome in LN patient.

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H) Clinical Nephrology, CKD. H1) Lab methods, progression & risk factors for CKD 1-5, nutrition in CKD, renal diseases (except GNs and cystic diseases).
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