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Proteomics in Peritoneal Dialysis

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1. Introduction

Relatively little is known about proteins in peritoneal effluent, that are lost or changed during peritoneal dialysis (PD) and in different diseases, leaving various unclear questions. Biomarkers that can indicate damages caused by peritoneal dialysis, like cancer antigen 125 and interleukin-6 are some examples. Therefore, tools such as proteomic approaches that can globally identify, characterize, and quantify a set of proteins and their changes in peritoneal dialysate, could shed light to the mechanisms of peritonitis and membrane damage. The availability of fluid from dialysis for study and the potential importance of specific protein change during peritoneal dialysis making this a potentially fruitful area for further observation. Since the renal community is embracing proteomic technologies at an increasing rate, growing numbers of studies that would be carried out through this process can be envisaged. In this chapter we intend to introduce basic proteomic tools and highlight important advances in peritoneal dialysis using proteomic approaches as well as the future perspective that proteomic tools can contribute in the field of peritoneal dialysis

2. Proteomic tools

In recent years, proteomic analyses of particular biological samples or clinical samples have drawn much interest and provided much information. Proteomic tools such as two dimensional gel electrophoresis (2DE) and mass spectrometry analysis have been widely applied in the study of body fluids, e.g. cerebrospinal fluid, pleural and pericardial effusions (Liu et al. 2008; Tyan et al. 2005a; Tyan et al. 2005b), and urine (Bennett et al. 2008; Tan et al. 2008). For peritoneal dialysis, several issues have been addressed as described in the following sections. The advantages and disadvantages of the various techniques have been reviewed previously (Fliser et al. 2007; Mischak et al. 2007).

2.1 Two dimensional gel electrophoresis (2DE)

Proteins are separated by isoelectric point and size. The protein spot can be visualized by gel staining. It is widely available and the posttranslational modification of the protein can be

revealed by separation of charge forms. However, low-abundance, large, and hydrophobic proteins are difficult to be detected. 2DE is technically demanding and time-consuming. The low number of independent datasets and the high variability of the gel make the definition of biomarkers difficult or even impossible. 2DE with fluorescent labeling of proteins before separation in gel (DIGE) has been proposed to improve reproducibility. Additional expense for fluorescent dyes and three color imaging system is required.

2.2 Liquid chromatography-tandem mass spectrometry (LC-MS/MS)

Proteins are digested before separation by liquid chromatography coupled to MS instruments. MS detection is more sensitive than 2DE. It is easily automated, allowing analyzing a series of samples. Drawback in comparison to 2DE is that information on the molecular mass of the actual biomarker as well as on any posttranslational modifications (PTM) is generally lost. This requires additional tools.

2.3 Surface-enhanced laser desorption ionization (SELDI)

Proteins are bound to affinity surface on a MALDI chip. Samples can be enriched for specific low-abundance proteins. Bound proteins are detected in a mass spectrometer. The SELDI technology draws a lot of interest because of its ease of use and its high throughput for biomarker discovery. However, the low-resolution of the mass spectrometer, the large amount of variability between labs, and its lack of reproducibility, hamper its potential clinical application (McLerran et al. 2008).

2.4 Capillary electrophoresis coupled to mass spectrometry (CE-MS)

Proteins were separated by elution time in CE and by size in MS. High reproducibility, robustness, high resolution and sensitivity make it a potential technique for biomarker discovery. Its limitation is that proteins can't be identified without additional steps and only proteins/peptides <20 kDa can be analyzed.

3. Analyses of peritoneal dialysis by proteomics tools

3.1 Preliminary proteomic studies on peritoneal dialysis effluent

A descriptive study was performed on the dialysate of nine paediatric PD children patients to obtain a representative overview of the proteome of peritoneal fluid (Raaijmakers et al. 2008). None of the patients suffered from peritonitis in the 3 months before the collection. Proteins were resolved on the SDS-PAGE and the protein spots identification was achieved by nanoLC-MS/MS. A total of 189 proteins were identified, with 88 proteins shared by all the patients. The function of these shared proteins were classified into 8 classes. As listed in Table 1, acute phase proteins, complement factors, hormones, coagulation factors, and apolipoproteins were found.

These factors were related to the number of frequently occurring proteins in the dialysate (Pecoits-Filho et al. 2004; Reddingius et al. 1995; Saku et al. 1989; van der Kamp et al. 1999). The proteome of PD fluid also reveals some interesting proteins, Gelsolin, intelectin, and paraxonase, which could possible involve in protecting the mesothelial cell damage and against infection, against parasites, and anti-atherogenic capacities, respectively. The proteome of PD may help understand the functional mechanism of the peritoneum.

Classification	Different proteins	Mean emPAI	SD
1. Acute phase proteins	24	1435	706
(a) Antiproteases	13	84.4	18.8
(b) Transport proteins	3	1333	713
(c) Other acute phase proteins	8	18.4	4.26
2. Complement factors	18	31.9	7.9
3. Apolipoproteins	7	40.4	22.8
4. Coagulation proteins	8	6.46	1.73
5. Extracellular matrix proteins	8	6.74	2.23
6. Hormone and vitamin binding proteins	5	19.8	7.98
7. Enzymes	4	7.02	1.38
8. Others	14	25.2	9.8

Table 1. Protein classification according to function of the proteins present in all the patients, relative abundances are given with mean emPAI values [Adapted from (Raaijmakers et al. 2008)]

3.2 Dialysis-related peritonitis

Peritonitis caused by CAPD may lead to peritoneal abnormalities. To search for potential biomarkers for peritonitis, Lin et al. have compared the proteome of peritoneal dialysate from 16 patients with and without peritonitis (Lin et al. 2008). Proteins were separated on 2DE, indicating several differential expressed spots (Figure 1).

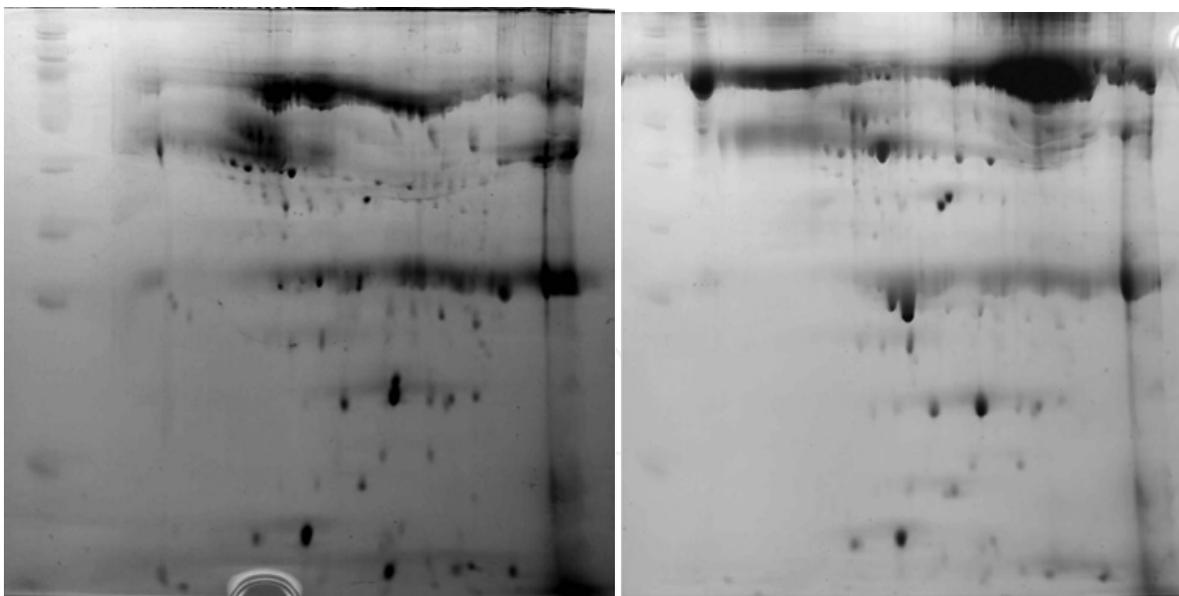


Fig. 1. Protein map, obtained by 2DE, of protein lysates prepared from CAPD dialysis effluent without (left) and with (right) peritonitis. Proteins are first separated according to their pI using iso-electric focusing and then separated according to their respective molecular weight using (10%) SDS-PAGE. [Adapted from (Lin et al. 2008)]

Samples were also analyzed by SELDI-TOF MS, revealing that signal peak at m/z of 11117.4 only appeared in the peritonitis sample. This signal was identified as β 2-microglobulin by

using MALDI-TOF/TOF MS. Protein β 2-microglobulin has been linked to CAPD peritonitis in previous studies. In CAPD dialysate from patients with bacterial peritonitis, β 2-microglobulin showed higher levels than in those without peritonitis (Carozzi et al. 1990). Minami et al. the level of β 2-microglobulin in the peritoneal dialysate was correlated with peritoneal injural (Minami et al. 2007). Using the protein profile approach, this study confirmed β 2-microglobulin as a biomarker for CAPD peritonitis.

3.3 Different types of peritoneal membranes

The efficacy and clinical outcome of CAPD depend on peritoneal membrane function. Peritoneal membranes can be classified as high (H), high average (HA), low average (LA), and low (L) transporters by using peritoneal equilibration test (PET). Whether there is a difference in proteins removed by different types of peritoneal membranes has been discussed in a study conducted by Sritippayawan et al. (Sritippayawan et al. 2007). They performed a proteomic analysis of peritoneal dialysate in CAPD patients with H, HA, LA, and L transport rates. Five patients were included for each group, makes up to 20 patient samples. Proteins from each sample were resolved in each 2D-gel (total 20 gels). Representative gels are shown in Figure 2. After gel visualization by staining and spot quantitation by image analysis software, the mean values of individual parameters were compared among the four different groups. Five proteins were found to show differed levels among groups. They were identified as serum albumin in a complex with myristic acid and triiodobenzoic acid, α 1-antitrypsin, complement component C4A, immunoglobulin κ light chain, and apolipoprotein A-I by MALDI-Q-TOF MS and MS/MS analyses. ELISA was used to confirm the difference expression of C4A and immunoglobulin κ in a set of other 24 patients. Functional significance of differential levels of these proteins may associate with dialysis adequacy, residual renal function, risk of peritonitis, and nutritional status. The level of serum albumin in a complex with myristic acid and triiodobenzoic acid was higher in the L LA groups, implying that the modified or complexed form of albumin may be associated with peritoneal membrane transport. The level of C4A was higher in H and HA group. In peritoneal dialysate, C4A originates from vascular leakage, resulting in the lower C4A level in L group. The immunoglobulin κ light chain VLJ region, whose level was higher in H and HA groups also tended to be higher in patients with peritonitis. The higher level of immunoglobulin κ light chain VLJ region might be related to poorer function of neutrophils. Patients in H and HA group also had higher apolipoprotein A-I in peritoneal dialysate compared to L and LA groups, which may explain that high solute transporters are prone to develop atherosclerosis.

3.4 The role of glucose in peritoneal dialysis

The abdominal cavity is covered by the mesothelial cell (MC) layer. Peritoneal dialysis fluid may remove solutes and fluid from the patients due to its hypertonicity. Long period and frequent peritoneal dialysis could lead to structural and functional alterations of the MC layer, leading to a final failure of peritoneal dialysis (Davies et al. 2001; Heimburger et al. 1990; Ho-dac-Pannekeet et al. 1997; Imholz et al. 1993; Williams et al. 2002). Hperosmolarity in peritoneal dialysis effluents is generated by high concentrations of glucose which could be degraded into carbonyl compounds as various glucose degradation products (GDPs) after heat sterilization (Linden et al. 1998; Nilsson-Thorell et al. 1993; Pischetsrieder 2000; Witowski and Jorres 2000; Witowski et al. 2003). These GDPs are reported as being

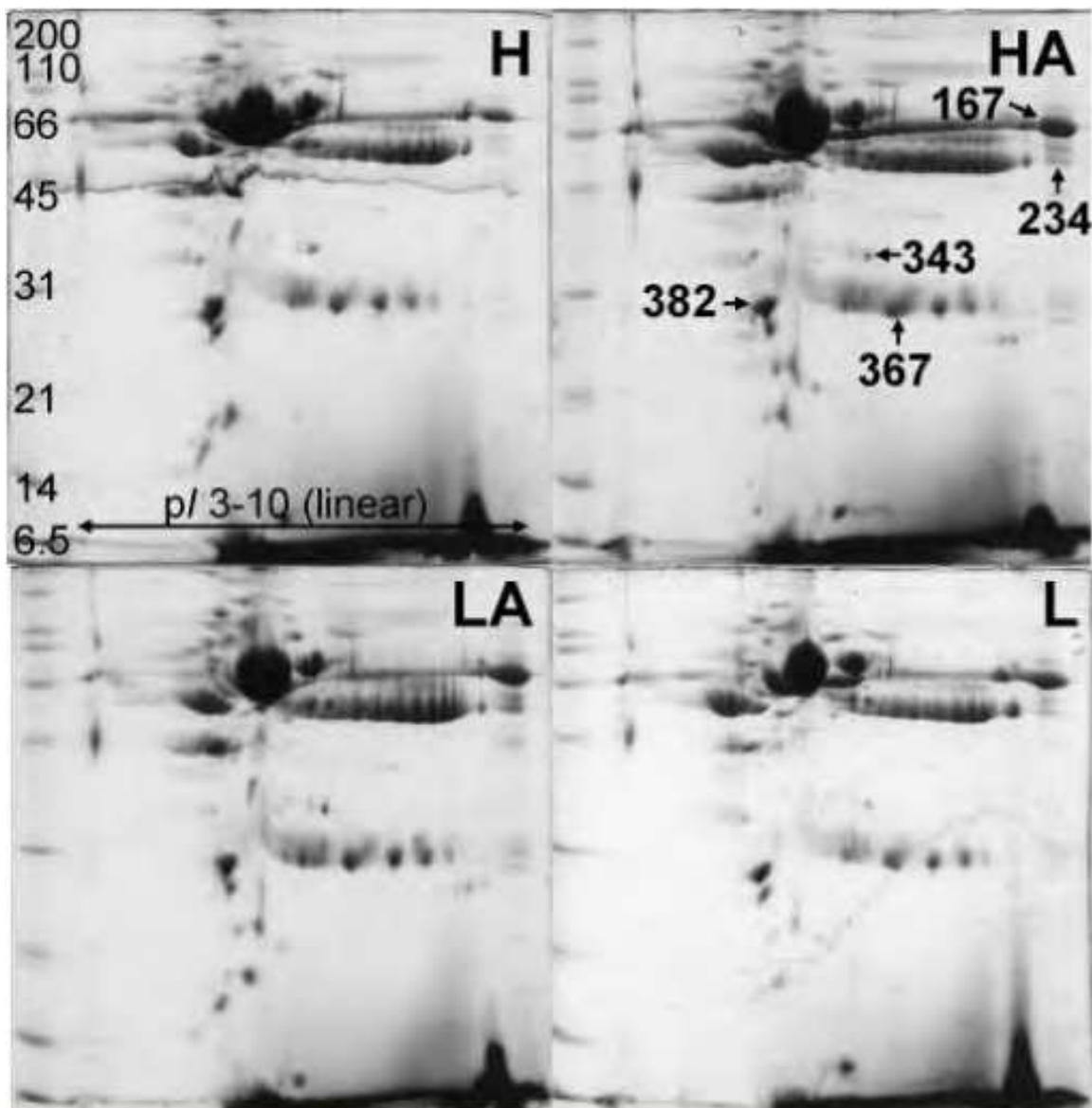


Fig. 2. Representative 2-D gel images of the PDE proteins derived from different types of peritoneal membranes. Proteins were precipitated with 75% ethanol, and an equal amount of total protein (200 μ g) obtained from each patient was resolved in each 2-D gel (n = 5 gels for each group; total n = 20). The resolved protein spots were visualized by CBB-R250 stain. Quantitative intensity analysis and ANOVA with Tukey's posthoc multiple comparisons revealed five protein spots whose intensity levels significantly differed among groups (see Table 2). These protein spots (labeled with numbers) were subsequently identified by MALDI-Q-TOF MS and MS/MS analyses. [Adapted from (Sritippayawan et al. 2007)]

mitogenic and cytotoxic. Thus, peritoneal dialysis effluent is considered as a significant stressor for the MC layer (Brebrowicz et al. 1995; Wieslander et al. 1991). To address this issue, a cell line derived from the MC layers was used as a model to study the glucose-related pathways induced by high concentration of glucose (Lechner et al. 2010). Using two-dimensional fluorescence-difference gel electrophoresis (DIGE), altered proteins upon glucose stress in Met-5A cell were revealed. A total of 947 spots were present in 32 gels (16 controls, 16 glucose stress). A representative gel is shown in Figure 3. Among them, 140

spots were of differential expression under full-peritoneal dialysis fluid stress consisting of high glucose concentration, pH 5.8, and the presence of GDPs, when compared to untreated cells, of which 100 proteins can be identified by MALDI-MS and MS/MS techniques. Further studies on these factors suggested that glucose exposure alone was not sufficient to explain the differential abundance of these proteins, supporting the hypothesis that stressors, pH, lactate, and GDPs, might have essential impact for activation of the glucose-related pathways. By comparing peritoneal dialysis effluent with different glucose concentrations, four proteins were found to be under-expressed in the highest osmolar solution. All of them were considered to be involved in the inflammatory processes.



Fig. 3. CBB stained 2-DE gel of a MeT-5A cell lysate. Proteins, differently abundant after full-PDF exposure and assigned to significantly enriched glucose associated pathways, are indicated with a circle and labeled with their Swiss-Prot entry names. Protein isoforms are distinguished by numbers in brackets. [Adapted from (Lechner et al. 2010)]

Another laboratory works on analyzing the protein composition of peritoneal fluid from patients receiving peritoneal dialysis with different concentration of glucose (Cuccurullo et al. 2010). Peritoneal dialysis effluent with different glucose concentrations were revealed by 2DE. The representative gels for each group are shown in Figure 4.

Combining the data from 2DE and shotgun proteomics analysis, 151 non-redundant identifications were reached. Through the cellular component analysis, proteins related to extracellular region were over-expressed. As for the molecular function and biological process, proteins associated with protein binding and inflammatory processes were over-represented. Four proteins, Alpha-1-antitrypsin (1603), fibrinogen beta chain (4308- 5303), transthyretin (4303 and 2101), and apolipoprotein A-IV (4208) were found to be under-

expressed in the highest osmolar solution. The result provides potential targets for future therapeutic implementation in preventing inflammatory processes induced by the exposure to dialysis solutions.

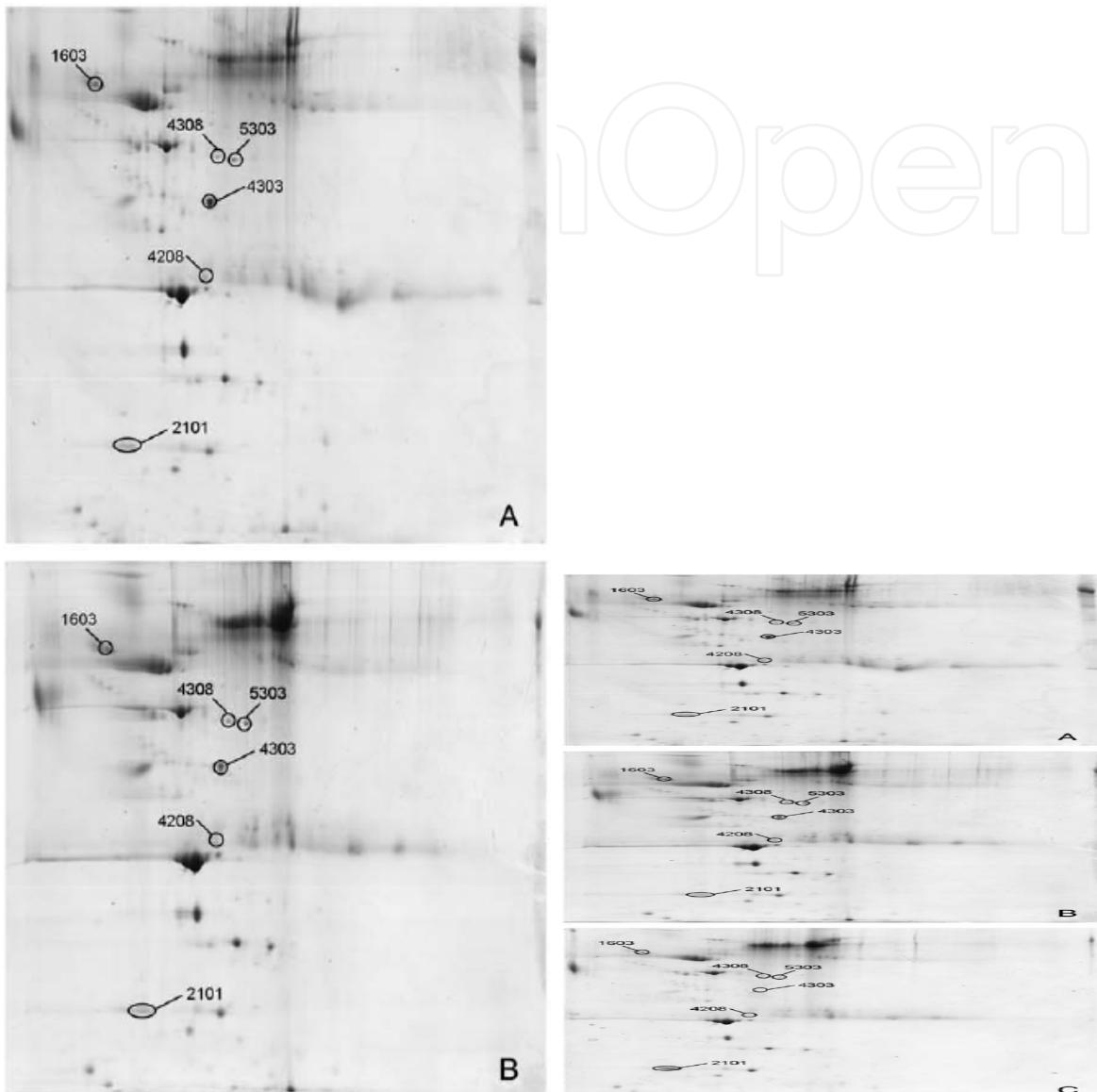


Fig. 4. Representative 2D gel images of PDE protein profiles from one (out of five) adult patient treated with peritoneal dialysis solutions differing for glucose percentages (A = glucose percentage 1.5%, B = glucose percentage 2.5%, C = glucose percentage 4.25%). Circled spots correspond to proteins whose expression undergoes quantitative changes. Alpha-1-antitrypsin (1603), fibrinogen beta chain (4308- 5303), transthyretin (4303 and 2101), apolipoprotein A-IV (4208). [Adapted from (Cuccurullo et al. 2010)]

3.5 Peritoneal dialysate from diabetic patients

Fluid overload related cardiovascular disease is one important factor to mortality in patients receiving CAPD (Brown et al. 2003). Other proposed mechanism is the glucotoxicity cause by the high concentration of glucose contained in PD fluid (Sitter and Sauter 2005).

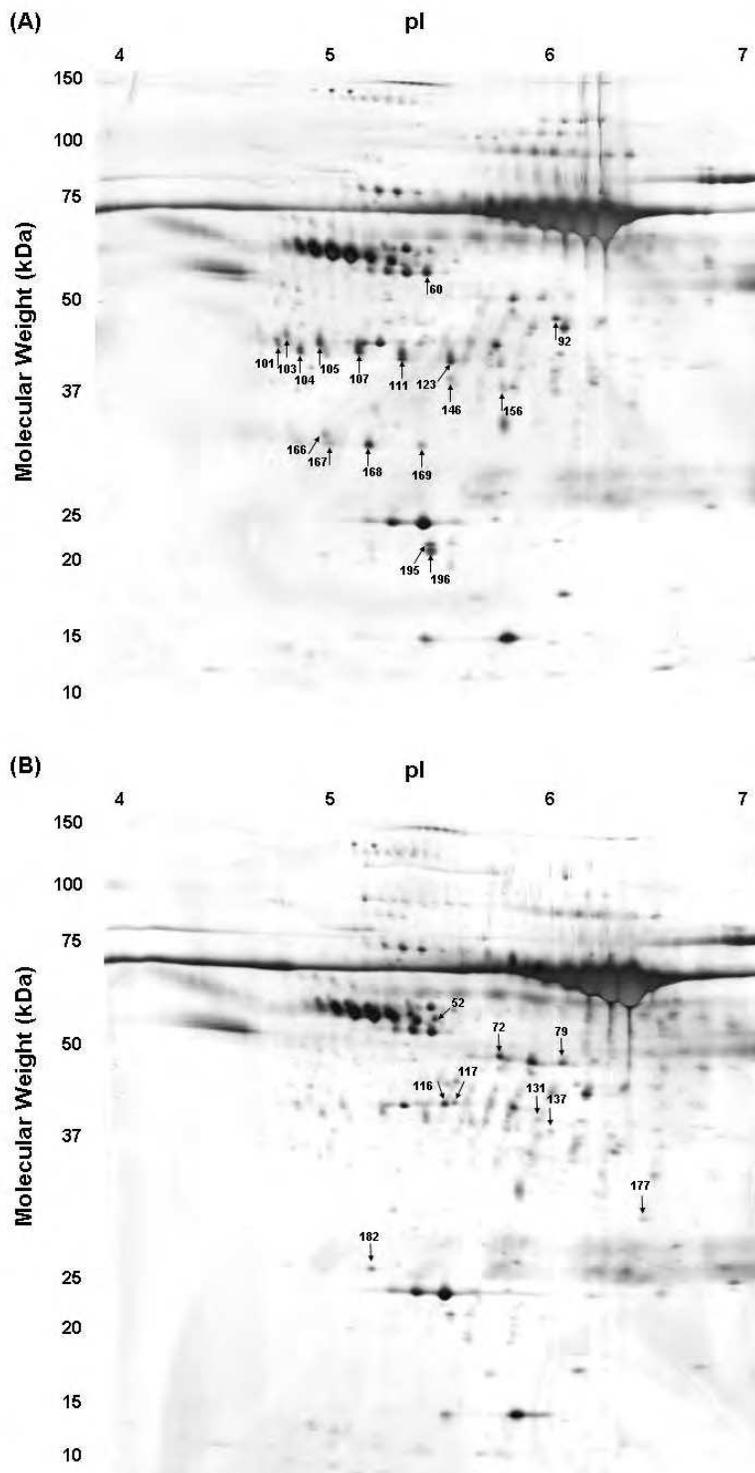


Fig. 6. Representative 2DE gels for the DM samples (A) and the non-DM (B) samples. DM samples and non-DM samples were pooled separately for 2DE analysis (pH 4-7). A total of 120 μg was used. The analysis of each group was repeated six times and two representative gels are shown. An average of 200 protein spots were detected in both gels. Among these, 17 spots were found with higher levels in the peritoneal dialysate (indicated in A by arrows) and 9 spots were found with higher levels in the control samples (indicated in B by arrows) [Adapted from (Wang et al. 2010)]

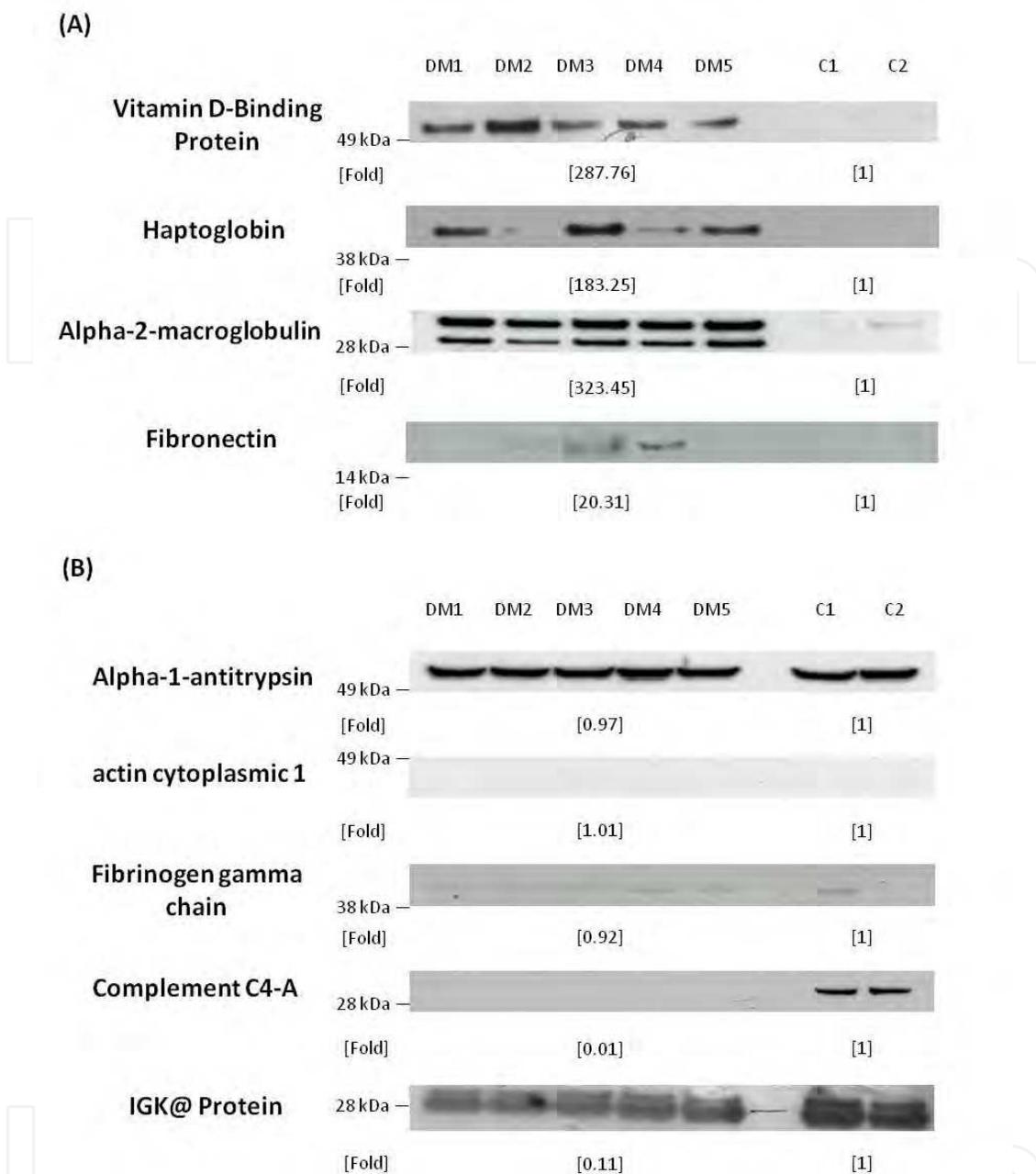


Fig. 7. Western blotting of the identified proteins in five individual DM samples and two individual control samples: the mean band density of five DM samples and two control samples were calculated and fold change between the two groups was calculated by dividing the mean band density of the DM samples by that of the control samples; the mean density of the control group was adjusted as 1, and the fold change was put in the bracket below each set of bands [(A) proteins, vitamin D-binding protein, haptoglobin and α -2-microglobulin show higher expression levels in the DM samples than in the control samples; (B) proteins, complement C4-A and IGK@ protein show higher expression levels in the two control samples than in the five DM samples]. [Adapted from (Wang et al. 2010)]

However, the details of the pathogenic mechanism remain unclear. Protein changes between peritoneal dialysate from specific disease and normal peritoneal fluids may shed light to better understanding of the mechanism involved in peritoneal damage resulting from

peritoneal dialysis. For clinical application, altered proteins in the peritoneum may function as biomarkers for monitoring which functions as a non-invasive way of detecting peritoneal damage. Wang et al. have compared the diabetic peritoneal dialysate versus normal peritoneal fluid (Wang et al. 2010). From the 2DE (as shown in Figure 6), 26 protein spots were considered altered between two sample groups.

According to the western confirmation results (Figure 7), vitamin D-binding protein, haptoglobin and α -2-microglobulin showed higher levels in the DM samples, while complement C4-A and IGK@ protein were of lower levels compared to the control samples. The work concluded that the loss of some specific proteins may be due to a change in the permeability of the peritoneal membrane to middle-sized proteins or leakage from peritoneal inflammation. It has been reported that PD leads to loss of DBP, and this causes loss of vitamin D. Vitamin D deficiency results in reduced insulin secretion in rats and humans, and its replacement improves B cell function and glucose tolerance (Boucher et al. 1995; Kumar et al. 1994; Norman et al. 1980; Tanaka et al. 1984). Thus the level of Vitamin D binding protein should be monitored after long-term PD. It has been suggested that haptoglobin may play a role in defending against haemoglobin toxicity, mainly renal toxicity (Lim et al. 2000). The observation of α -2-microglobulin in dialysate may be indicative of high levels of α -2-microglobulin in serum and a potential for amyloidosis. Complement activation happens in the peritoneal cavity in patients on chronic PD (Reddingius et al. 1995; Young et al. 1993), suggesting that local production of complement may be a possible inflammatory injury effectors in the initiation of chronic peritoneal damage. Lower levels of complement C4-A in dialysis effluent may indicate the beginning of peritoneal scleroses. The limited studies of the role of IGK@ protein in the peritoneum posted this protein a novel target for further investigation of its defect in dialysate, which may provide a new insight for peritoneal change or damage during PD.

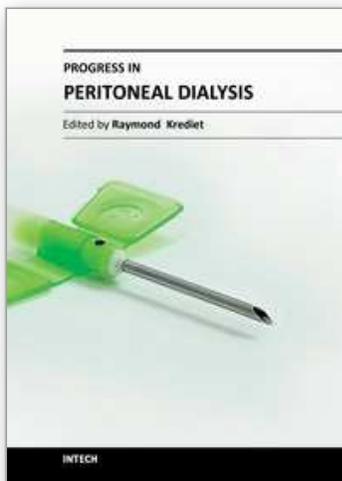
4. Concluding remarks and outlook

Recent publication of potential biomarkers is only based on limited datasets in the absence of any validation. Future implementation of proteomics to the peritoneal dialysis will depend largely on establishment of generally accepted validity of the identified biomarkers. Development of standardization procedures for clinical proteomic studies is also required, including the sample collection procedures. However, these primary works suggested that proteome analysis may be a helpful tool to evaluate therapeutic effects of drugs on a molecular level. These studies may serve as a basis for the future identification of toxins and biomarkers for monitoring and improving the PD. Although it is evident that significant efforts, including larger studies are required to reach these goals, those studies also provide a possible platform for future diagnostic and therapeutic applications in the field of peritoneal dialysis and allowed the identification of potential targets to be used in preventing inflammatory processes induced by the exposure to dialysis solutions. Some reviews on this field claimed that recent findings underscore proteomic tools in defining molecules removed by the treatment modalities. Most of the studies were conducted by using 2DE which is widely used but also lacking in sensitivity and reproducibility. Thus, we can envisage that in the future, using rapidly evolved proteomic tools such as LC-MS/MS and accurate quantitative proteomic approaches such as iTRAQ and label free analyses will bring more information and novel insight into this field.

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