Exfoliated Mesothelial Cell and CA-125 in Automated Peritoneal Dialysis (APD) and Continuous Ambulatory Peritoneal Dialysis (CAPD) Patients

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Objective: *Automated peritoneal dialysis (APD) becomes the first option for peritoneal dialysis, nowadays overtaking continuous ambulatory peritoneal dialysis (CAPD) in many countries. The comparison of peritoneal membrane alteration in CAPD and APD is inconclusive. The authors therefore compared the peritoneal membrane changes in patients undergoing CAPD and APD.*

Material and Method: *In naive end stage renal disease patients, the choice of PD modes (CAPD or APD) was dependent on the patient's decision. Thirty-six CAPD and 25 APD patients with a total of 287 patient-months were compared. The peritoneal mass parameter, exfoliated mesothelial cell (MTC) and dialysate CA-125, as well as modified peritoneal equilibrium test (mPET) with 4.25% dextrose solution was simultaneously evaluated at 1 and 6 month follow-up.*

Results: *Although the peritoneal function (as measured by D/P creatinine, D/D0 glucose, sodium dipping, and dialysate protein loss), adequacy, serum albumin, nutritional status, and residual renal function showed no significant differences between groups at 1 and 6 months, CA-125 but not MTC was higher in APD compared with CAPD at the first month of PD beginning. Due to the single time-point measurement limitation, the authors compared the peritoneal mass parameter differences between 1 and 6 month. During 6-month follow-up, CA-125 decreased 30 $\pm$ 5% vs. 7 $\pm$ 5% and MTC decreased 5 $\pm$ 12% vs. 40 $\pm$ 11% in APD and CAPD, respectively. The higher CA-125 reduction in APD and greater changes of MTC in CAPD suggested that there was less viable mesothelial cell in APD compared with CAPD.*

Conclusion: *The authors observed that both APD and CAPD damaged peritoneum. However, there might be higher peritoneal injury in APD patients. The proper randomization study in longer follow-up period is mandatory to confirm this observation.*

Keywords: *CAPD, APD, Mesothelial cell mass, CA-125, Exfoliated mesothelial cell*

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Utilization of automated peritoneal dialysis (APD) has rapidly grown in the last decade because APD give more flexible lifestyle while dialysis efficiency remains uncompromised. APD becomes the first mode for PD which nowadays overtaking continuous ambulatory peritoneal dialysis (CAPD) in some centers, especially in Europe and North America. In Thailand, APD is usually performed in the night-time shift, leaving the abdomen dry in the day-time; the mode is termed nightly intermittent PD (NIPD). The common prescription is 2 L/cycle and 5 cycles/night or 10 L for

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10-12 hours/day. On the other hand, CAPD patients are dialyzed in the lower amount (8 L/day) and less frequent exchanges (4 cycles/day) but around the clock. Therefore, peritoneal membrane of the APD patient inevitably contact with fresh peritoneal dialysate more frequently in the larger amount but shorter duration compared with CAPD. The fresh dialysate is composed of high dextrose concentration (10-15 times higher than plasma glucose) and various kinds of toxic components, such as acidic pH, hyperosmolarity, high lactate, high glucose degradation products, and plasticizer, that might affect peritoneal membrane function and anatomy. The longer the peritoneal dialysate retained in peritoneal cavity, the lesser osmolarity and more neutral pH of the fluid and theoretically should be less harmful. Thus, it seems that the more fresh dialysate exchange in APD (5 times/day) might cause more damage to peritoneal resident cells, particularly mesothelial cells, than the less frequent exchange in the CAPD (4 times/day). The continuous contact between dialysate and the mesothelial cell (MTC) in the CAPD mode without time for resting, unlike APD, might, on the other hand, worsen affect to peritoneum. Nevertheless, the comparative incidence of peritoneal membrane alterations in patients treated with CAPD and APD is controversial.

In peritoneal dialysis (PD), patient outcome is largely influenced by the adequate peritoneal transport[1,2]. Generally, adequate transport indirectly reflects healthy peritoneal MTC. The cells line interface between dwelled PD fluid and the peritoneum, hence it acts as a functional barrier protecting the PD membrane from various injuries and preventing the membrane adhered to a nearby structure. In addition, the cells renovate the damaged membrane by producing various growth factors. Thus, capacity of the intracorporeal dialyzer is maintained with the aids of the cells.

Currently, CA-125, a high molecular weight glycoprotein, is routinely used as an indicator reflecting peritoneal cell mass[3,4]. However, the debate regarding the association between CA-125 and peritoneal cell mass is on-going[5,7]. The direct count of exfoliated MTC might be a better representative. The authors postulated that the detection of total exfoliated MTC with flow cytometry should be more appropriate as the authors recently reported that higher apoptotic MTC was associated with worsen peritoneal membrane function and could predict future ultrafiltration loss[6]. The authors therefore compared the incidence of MTC changes in patients undergoing CAPD only using the "flush before fill" technique and APD in four different centers during 1-year period. The peritoneal parameters were monitored by direct MTC count assessed by flow cytometry, dialysate CA-125, and modified peritoneal equilibrium test (mPET).

Material and Method

Patients

Fifty-eight patients were included in the present study; 35 CAPD and 23 APD patients from 4 different PD centers, including King Chulalongkorn Memorial Hospital (representative of university hospital), Sappasit Hospital (representative of tertiary hospital in the countryside), Prommitr branch of Banphaeo Hospital-Public Organization (representative of private hospital), and Police General Hospital (representative of military or specific care hospital) with a total of 287 patient-months were followed. Inclusion criteria were all new PD patients with age more than 15 years old. The clinical courses were recorded. Peritoneal function (mPET with 4.25% dextrose solution), dialysate CA-125, and overnight exfoliated MTC in the spent effluents were simultaneously evaluated at 1 and 6 months of follow-up.

Peritoneal function and peritoneal mass

Patients provided written consent before the test procedure was initiated. Overnight dialysate fluid was collected to measure exfoliated MTC and CA-125. The peritoneal function was assessed by using mPET with 4.25% dextrose solution (Dianeal; Baxter). The standardized 4-hour dwell with the test bag was conducted. Repetitive dialysate sampling of the test bag was performed at 0, 60, 120, and 240 min while blood samples were drawn twice (at 0 and 120 min). Glucose, sodium, and creatinine were determined by the enzymatic method. D/P creatinine, D/D0 glucose, and sodium dipping were calculated. Peritoneal masses, CA-125 was measured by enzyme chemiluminescent immunoassay (Roche Diagnostic, Indianapolis, IN, US) based on the monoclonal antibody against CA-125 and overnight exfoliated MTC were stained with anti-cytokeratin AE1/AE3 (Fitzgerald Industries International, Concord, MA, US) counted, and measured by flow cytometry as described by Kanjanabuch et al[6]. In brief, overnight (8-hour) effluent from a dwell with 1.5% dextrose PDF was collected and centrifuged for 5 min at 300 g. The total fluid volume was recorded, and nucleated cells were counted. Exfoliated MTC were identified after staining of the cells with monoclonal antibodies directed against human cytokeratin then were counted in a FACSCalibur

flow cytometer (BD Bioscience, San Jose, CA, US). Events were gated on forward scatter vs. side scatter in such a way that cell debris was excluded. All tests were simultaneously evaluated at 1 and 6 months of follow-up. The percent difference of dialysate CA-125 and MTC were calculated from the following formula (the individual value at 6 month-value at 1 month) x 100/ (the value at 6 month + the value at 1 month).

Statistical analyses

Results were presented as medians or means ± standard deviation, according to the characteristics of each variable. Distribution was tested to be normal. Paired and unpaired t-tests were used for statistical analysis where appropriate. An alpha error at $p < 0.05$ was judged to be significant.

Results

There were significant differences in several baseline characteristics; more elderly and diabetic patients in the APD but higher baseline serum creatinine (Scr) in the CAPD group. However, the liver function, albumin, and lipid profiles were not significantly different between both groups (Table 1). The peritoneal function at 1 and 6 month between CAPD and APD were comparable (Table 2). Additionally, the serum albumin, dialysate protein loss, and residual renal function were not different (data not shown).

Dialysate CA-125 but not exfoliated MTC was different at early first month after beginning PD. Dialysate CA-125 was higher in APD; 36 ± 7 and 17 ± 6 U/L in APD and CAPD, respectively ($p < 0.05$) (Fig. 1). At 6 month, both dialysate CA-125 and exfoliated MTC were higher in APD (24 ± 4 and 14 ± 3 U/L for CA-125 ($p < 0.05$) and 3 ± 0.7 and 1.2 ± 0.3 cells/ bag for MTC ($p < 0.05$) in APD and CAPD, respectively) (Fig. 1 and 2). However, the difference between the parameters at 1 and 6 months follow-up might be an effect of demographic disparity (Table 1) and hence a single value was less informative[3]. Thus, the alteration of the parameters should be more appropriate in representing peritoneal mass. The authors found that both dialysate CA-125 and exfoliated MTC in either APD or CAPD decreased during 6 month follow-up. However, there were discrepancies between the degree of CA-125 and MTC reduction. The percent reduction of dialysate CA-125 in APD was higher than CAPD; 30 ± 5% vs. 7 ± 5% in APD and CAPD, respectively. On the other hand, the reduction of exfoliated MTC in APD was less; 5 ± 12 vs. 40 ± 11 x 10^5 cells/bag in APD and CAPD, respectively (Fig. 3). There was a reverse correlation between percent change of CA-125 and MTC, $r^2 = 0.062$ (data not shown).

Discussion

Adequate renal replacement therapy of PD was clearly dependent on the peritoneal membrane function as the intracorporeal dialyzer. The long term loss of peritoneal function is the major factor for technical failure of PD and finally can lead to death[8,9]. It is widely accepted that functional changes in the peritoneal membrane are strongly correlated with histomorphological alterations from uremia and chronic exposure to dialysate or peritonitis[10,11]. The dialysate

Table 1. Baseline patient characteristics

Parameters	CAPD (36)	APD (25)	p-value
Age (years)	50.5 ± 4	70.3 ± 2	< 0.001*
Male gender	20 (57.1%)	15 (65.2%)	NS
Body weight (kg)	58.7 ± 2	60.3 ± 3	NS
Height (cm)	158 ± 0.05	162 ± 0.02	NS
Underlying diseases			
Diabetes mellitus	10 (28.5%)	18 (78.2%)	< 0.001*
Hypertension	28 (80%)	21 (91.3%)	NS
ACEI/ARB usage	10 (28.5%)	8 (34.7%)	NS
Blood chemistries:			
Blood urea nitrogen (mg/dl)	53.5 ± 3	54.3 ± 4	NS
Creatinine (mg/dl)	10.6 ± 0.7	6.6 ± 0.3	< 0.001*
Cholesterol (mg/dl)	191 ± 12	183 ± 9	NS
Triglyceride (mg/dl)	155 ± 17	153 ± 14	NS
HDL (mg/dl)	46 ± 3	57 ± 6	NS
Albumin (g/dl)	3.3 ± 0.1	3.5 ± 0.05	NS

Table 2. Peritoneal functions assessed by mPET at 1 and 6 month after starting PD

Parameters	CAPD (35) 1 month	APD (23) 1 month	CAPD (35) 6 months	APD (23) 6 months	p-value
Total weekly Kt/V	1.9 ± 0.1	1.8 ± 0.1	1.7 ± 0.1	2.0 ± 0.2	NS
Weekly Ccr (L)	57 ± 2	55 ± 4	61 ± 5	69 ± 6	NS
Sodium dipping (mEq/L)	8 ± 0.6	6.6 ± 0.7	7.7 ± 0.8	7.2 ± 0.5	NS
D/P creatinine	0.7 ± 0.02	0.6 ± 0.02	0.7 ± 0.07	0.7 ± 0.02	NS
D/D0 glucose	0.3 ± 0.02	0.4 ± 0.02	0.3 ± 0.01	0.4 ± 0.02	NS

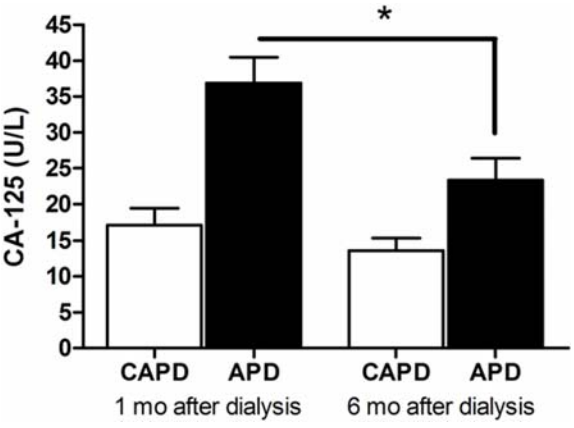

Fig. 1 Dialysate CA-125 in CAPD (white bar: n = 35) and APD (black bar; n = 25) at 1 month (A) and 6 month (B). *p < 0.05 value at 1 month vs. 6 month

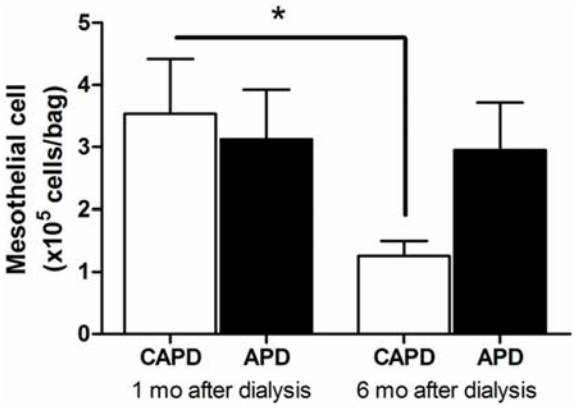

Fig. 2 Peritoneal fluid exfoliated mesothelial cell in CAPD (white bar: n = 35) and APD (black bar; n = 25) at 1 month (A) and 6 month (B). *p < 0.05 value at 1 month vs. 6 month

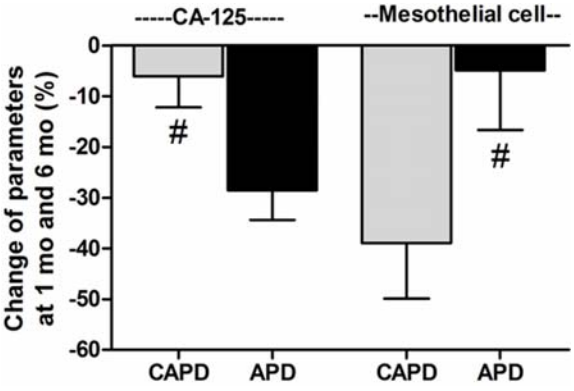

Fig. 3 The percent change of peritoneal fluid CA-125 in CAPD (light bar: n = 35) and APD (dark bar; n = 25) (A) and reciprocal peritoneal fluid exfoliated mesothelial cell in CAPD (B). # p < 0.05; CAPD vs. APD within each group

contents such as glucose, glucose degradation products (GDPs), and advanced glycation end-products (AGEs) can induce inflammation, fibrosis, and angiogenesis[12]. In APD, larger volumes of dialysate and more frequent exchanges with fresh dialysate, might have a negative impact on peritoneal membrane. In CAPD, continuous contact with dialysate might affect peritoneal cell. To compare the MTC injury of APD and CAPD, the *in vitro* system (a double chamber cell cultures system with human MTC on top of a permeable membrane and growth medium below) was used for mimicking CAPD and APD[13]. The results showed that the cell viability and CA-125 release did not differ between PD modes. Thus, the authors aimed to assess the effect of PD mode on MTC injury in clinical milieu. In the present study, no significant differences in small solute transport data between APD and CAPD were observed and this concurred with an earlier study[14]. Regarding to the peritoneal cell mass, the authors both measured the percent changes of dialysate CA-125 and exfoliated MTC in order to compensate for the baseline difference. The authors found a huge reduction in dialysate CA-125 but less MTC decrease in APD (Fig. 3). It is likely that APD caused more peritoneal injury as indicated by the CA-125 result. Then there was less viable peritoneal MTC in APD peritoneum resulting in less detected exfoliated

cells.

However, there was no change in peritoneal function as measured by mPET, the peritoneal mass detection might be an earlier marker compared to the peritoneal function change. The longer follow-up study is needed. Recently, the authors found the negative correlation between CA-125 and MTC compatible to the authors' previous report[6]. However, the positive correlation had been previously described by Sanusi et al[4] and Breborowicz et al[5], higher CA-125 represents more peritoneal cell mass then more exfoliated MTC can be detected. Unfortunately, both researchers[4,5] measured MTC by counting with immunohistostaining which was a semi-quantitative test. The discrepancy between the studies might be a result of the difference in MTC detection method. The head-to-head comparison of anti-cytokeratin flow cytometry and calretinin immunohistostaining might be required.

There were several limitations in the authors report. First, the authors could not random patients because of financial problems. Second, there were significantly differences in several baseline characteristics of the patients, more elderly and high prevalence of diabetic patients with less baseline Scr in the APD group. Third, this was just a short term follow-up, the longer term follow-up data might show more obviously different results. By determining the percent change in parameters, the effect of confounders was partly reduced. However, the higher reduction in peritoneal mass might also be a result of older age or direct effect of diabetes. In conclusion, more peritoneal membrane alterations develop in APD than CAPD. The proper randomization with the longer time of follow-up is needed to confirm the authors observation.

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Potential conflicts of interest

None.

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การศึกษาเปรียบเทียบการเปลี่ยนแปลงของเยื่อบุผนังช่องท้องในผู้ป่วยโรคไตวายเรื้อรังระยะสุดท้ายที่ได้รับการรักษาด้วยการล้างไตทางช่องท้องแบบต่อเนื่องด้วยตนเองกับการล้างไตทางช่องท้องโดยใช้เครื่องอัตโนมัติ

เถลิงศักดิ์ กาญจนบุษย์, นพดล พุฒิพิทยาธร, อัษฎาศ์ ลีฬหวนิชกุล, ทรงเกียรติ หลิวสุวรรณ์, พิสุทธิ์ กตเวทิน, นันทา มหัธนันท์, กานดา ศรีอุดม, ธนิต จิรนันทวันท์, นิศา ทองบ่อ, สมชาย เอี่ยมอ่อง

วัตถุประสงค์: *มีการเพิ่มจำนวนการใช้เครื่องอัตโนมัติช่วยการล้างไตทางช่องท้อง (เอพีดี) มากขึ้นในปัจจุบัน ในหลายประเทศพบปริมาณการใช้สูงกว่าการล้างไตทางช่องท้องด้วยตนเอง (ซีเอพีดี) อย่างไรก็ตามยังไม่มีหลักฐานทางวิชาการชัดเจนสนับสนุนว่าเอพีดีเหนือกว่าซีเอพีดีในแง่การเสื่อมของเยื่อบุผนังช่องท้องจากการล้างไตทางช่องท้องด้วยเหตุนี้จึงเป็นที่มาของการศึกษา*

วัสดุและวิธีการ: *ผู้ป่วยโรคไตวายเรื้อรังระยะสุดท้ายที่จำเป็นต้องเริ่มการบำบัดทดแทนไตจะเป็นผู้ตัดสินเลือกประเภทการล้างด้วยตนเอง (ระหว่างซีเอพีดี หรือ เอพีดี) ทำการศึกษาเปรียบเทียบในผู้ป่วย ซีเอพีดี จำนวน 35 รายและผู้ป่วยเอพีดี จำนวน 23 ราย คิดเป็น จำนวนผู้ป่วยรวม 287 ผู้ป่วย-เดือน ประเมินการเปลี่ยนแปลงของเยื่อบุผนังช่องท้อง 2 ครั้ง ณ เดือนแรกและเดือนที่ 6 ด้วยวิธีนับปริมาณเซลล์เยื่อบุผนังช่องท้องที่หลุดลอกออกมาในน้ำยาล้างไตทางช่องท้องที่ได้รับทิ้งค้างไว้ในช่องท้องตลอดคืน วัดระดับ ซีเอ-125 ในน้ำยาล้างไต และการทดสอบประสิทธิภาพของเยื่อบุผนังช่องด้วยวิธี เพ็ทเทส แบบดัดแปลงโดยใช้น้ำยาล้างไตเข้มข้น 4.25% แทนการใช้ 2.5%*

ผลการศึกษา: *แม้ว่าไม่พบความแตกต่างระหว่างประเภทของการฟอกในด้านประสิทธิภาพของเยื่อบุผนังช่องท้อง (ประเมินด้วยดัชนี อัตราส่วนของค่าครีแอทินินในพลาสมาและในน้ำยาล้างทางช่องท้อง, อัตราส่วนของน้ำตาลกลูโคส ณ เวลา 4 ชั่วโมงและที่เวลาเริ่มต้น, ปริมาณการลดต่ำลงของโซเดียมในน้ำยาล้างไต และปริมาณการสูญเสียของโปรตีนในน้ำยาล้างไตทางช่องท้อง) ความเพียงพอในการล้างไต พลาสมาแอลบูมิน ระดับโภชนาการของผู้ป่วย และปริมาณการขจัดของเสียของไตที่ยังคงเหลืออยู่ ณ เวลา 1 และ 6 เดือน อย่างไรก็ตามพบว่ามีเพียงความเข้มข้นของซีเอ-125 ในน้ำยาล้างไตของผู้ป่วยเอพีดีที่สูงกว่าของผู้ป่วยซีเอพีดี ตั้งแต่เดือนแรกของการล้าง ด้วยข้อจำกัดของการเก็บดีซีพีเพียงครั้งเดียว คณะผู้นิพนธ์ตัดสินใจประเมินการเปลี่ยนแปลงของเยื่อบุผนังช่องท้อง 2 ช่วงเวลา คือ 1 และ 6 เดือน พบว่า ณ เวลา 6 เดือน ระดับ ซีเอ-125 ลดลงร้อยละ 30 $\pm$ 5 เทียบกับ ร้อยละ 7 $\pm$ 5 และปริมาณการหลุดลอกของเซลล์เยื่อบุผนังช่องท้องลดลง ร้อยละ 5 $\pm$ 12 เทียบกับร้อยละ 40 $\pm$ 11 ในกลุ่ม เอพีดี และ ซีเอพีดี ตามลำดับ การลดลงอย่างมากของ ซีเอ-125 ในกลุ่มเอพีดี และการเปลี่ยนแปลงของปริมาณการหลุดลอกของเซลล์ในกลุ่มซีเอพีดีบ่งชี้ว่า มีเซลล์บุผนังช่องท้องเหลือในผู้ป่วยเอพีดีน้อยกว่าผู้ป่วยซีเอพีดี*

สรุป: *คณะผู้นิพนธ์พบว่าทั้ง 2 วิธีของ การล้างไตทางช่องท้อง ทำลายเยื่อบุผนังช่องท้อง โดยพบแนวโน้มว่าการทำลายเกิดมากในวิธีเอพีดีอย่างไรก็ตาม ควรใช้การศึกษาแบบสุ่ม และติดตามผู้ป่วยนานขึ้น เพื่อยืนยันผลการศึกษาอันนี้*