CELL-FREE PLASMA DNA (cfpDNA) IN PERITONITIS IS ORIGINATED FROM APOPTOTIC EVENTS

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INTRODUCTION AND AIMS

Peritonitis are a frequent complication of peritoneal dialysis (PD) and a common cause of technique failure. Cell-free plasma DNA (cfpDNA) is a circulating extracellular DNA fragment and originates from necrotic and apoptotic cells derived from inflammation and tissue damage. High levels of cfpDNA have been reported in many clinical conditions. In particular, cfpDNA is increased in PD patients plasma with a recent episode of peritonitis.

The first aim of this study was confirmed the variation in plasma levels of cfpDNA after an episode of peritonitis in chronic PD patients. The second purpose of this study was to elucidate the putative causative mechanism involved in cfpDNA formation during peritonitis.

METHODS

This cross-sectional study was conducted over a 2-month period in the Peritoneal Dialysis Center, in the Nephrology Department at St Bortolo Hospital in Vicenza, Italy. We enrolled 54 PD patients undergoing maintenance PD for a minimum of 3 months and we divided them into 3 different groups: 25 PD patients without any history of peritonitis, 21 PD patients whose last episode of peritonitis was more than 3 months prior to enrollment, and 8 patients who had an episode of peritonitis within the 3 months prior to enrollment.

Blood samples were collected from all 54 patients into EDTA-containing tubes and processed within 30 minutes after venipuncture. Samples were subsequently centrifuged for 10 minutes at 3500 rpm. Plasma was carefully removed without touching the cell pellet and re-centrifuged at 13000 rpm for 10 minutes. After centrifugation, the supernatant was placed into a clean polypropylene tube and stored at -80°C until use.

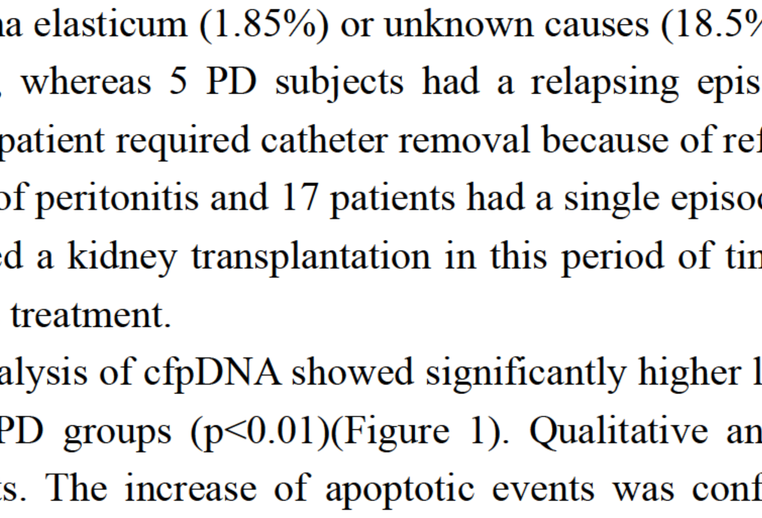
cfpDNA was extracted from plasma and was quantified by Real time PCR for the β-globin gene, in triplicate. Subsequent, qualitative analysis of apoptosis was performed by DNA Ladder kit and quantitative plasma levels of Caspase-3 was measured by Human Instant enzyme-linked immuno-sorbent assay (ELISA) kit (eBioscience). Statistical analysis was performed using the *SPSS 15* software. A *p*-value of <0.05 was considered statistically significant.

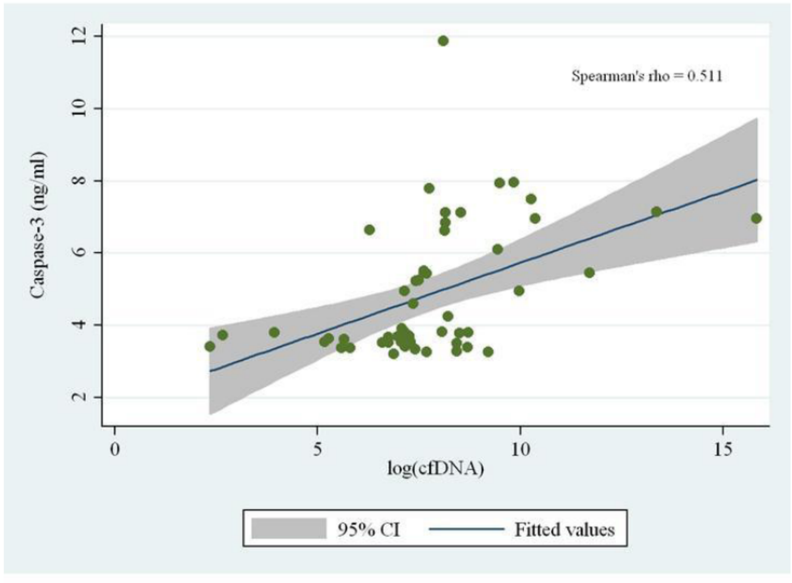
RESULTS

A total of 54 PD patients (29 male and 25 female; mean age 63.1 ± 16.3, median time on PD 25.5 months; IQR: 13.2-49.4 months) were enrolled in this study. End stage renal failure in the study population was attributed to diabetic nephropathy (25.9% patients), glomerulosclerosis (25.9% patients), nephroangiosclerosis (20.3% patients), autosomic polycystic kidney disease (5.5% patients), vesico-ureteral reflux (1.85% patient), Pseudoxanthoma elasticum (1.85%) or unknown causes (18.5% patients). 24 (82.0%) patients had a first episode of peritonitis and responded to first-line antibiotics, whereas 5 PD subjects had a relapsing episode of peritonitis, but subsequently responded to another course of intra-peritoneal antibiotics. No patient required catheter removal because of refractory peritonitis without need to switch to HD. (41%) patients had multiple episodes (maximum=5) of peritonitis and 17 patients had a single episode of peritonitis in their clinical history. No patient died within the study period and no patients received a kidney transplantation in this period of time.Normal WBC count in peritoneal effluent were observed in all patients by day 10 from peritonitis treatment.

Quantitative analysis of cfpDNA showed significantly higher levels in patients who had an episode of peritonitis within the 3 months compared with the other two PD groups (p<0.01)(Figure 1). Qualitative analysis of apoptosis showed higher DNA ladder formations, suggesting presence of apoptotic events. The increase of apoptotic events was confirmed by Caspase-3 activation (p<0.01) and a significant correlation was observed between cfpDNA and Caspase-3 levels (Figure 2). We observed lower levels of cfpDNA and Caspase-3 in patients with a longer peritonitis-free period. cfpDNA levels tend to progressively decrease in correlation with peritonitis-free time.

cfDNA GE/ml	15025 (4280-330687.9)	1433.39 (540.02-3680.24)	1564.22 (1154.2-2200.84)
Caspase-3 ng/ml	7.1 (7.0-7.9)	3.7 (3.5-5.4)	3.7 (3.5-4.9)
Days from the last peritonitis	12 (2-27)	583 (265-925)	NA





CONCLUSIONS

In conclusion, our data has demonstrated that cfpDNA is increased in the plasma of PD patients with recent peritonitis and cell apoptosis induced by Caspase-3 activation is one of the potential sources of cfpDNA.

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456--SP
M) Dialysis. M2) Peritoneal dialysis.
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DOI: 10.3252/pso.eu.51era.2014
Poster presented at: 51 ERA-EDTA CONGRESS AMSTERDAM 2014