(A) TITLE

The Efficacy of Prolonged Antibiotic Therapy for the Prevention of Relapsing Peritonitis in Peritoneal Dialysis Patients with High Dialysis Effluent Bacterial DNA Fragment Levels

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ABSTRACT

Peritoneal dialysis (PD) is the first-line treatment of end stage renal disease (ESRD) in Hong Kong. Despite the advances in antibiotic therapy and connecting system, recurrent peritonitis remains the major cause of peritoneal failure. A recent study showed that an elevated bacterial DNA fragment levels in PD effluent 5 days prior to the completion of antibiotics predicts the development of relapsing or recurrent peritonitis episodes. We hypothesize that prolonged antibiotic therapy in PD patients with peritonitis and high PD effluent bacterial DNA fragment levels could prevent the development of relapsing and recurrent peritonitis. We plan to conduct a randomized control study of 360 patients with PD peritonitis. After inform consent, they will be randomized to receive one additional week of the effective antibiotic treatment (the Preemptive Treatment Group) or no additional treatment (the Control Group). Specimens of PD effluent will be collected 5 days prior to the completion of antibiotics for the measurement of bacterial DNA fragments. All patients will be followed for 6 months after completion of antibiotic therapy for the development of relapsing, recurrent, or repeat peritonitis episodes. Our study will determine the efficacy of a test-before-treat algorithm that could reduce the incidence of relapsing and recurrent peritonitis and, at the same time, minimize the unnecessary use of prolonged antibiotic treatment.
(B) INTRODUCTION

Peritoneal dialysis (PD) is the first-line treatment of end stage renal disease (ESRD) in Hong Kong [1]. Despite the advances in antibiotic therapy and connecting system, peritonitis remains a major complication of PD. Although less than 4% of the peritonitis episodes resulted in death, peritonitis is a “contributing factor” to death in 16% of deaths on PD [2].

In addition to causing patient death directly, peritonitis is the most common cause of treatment failure in PD [1,3,4]. It is important to realize that the success of PD depends on the sustained ability of the peritoneum to act as a semi-permeable membrane [5], and recurrent peritonitis episodes is the major cause of peritoneal failure, which compels PD patients to switch to the more expensive long-term hemodialysis. Our recent study shows that around 15% of all PD-related peritonitis episodes are followed by relapsing or recurrent peritonitis, often resulting in prolonged hospitalization, expensive treatment, need of catheter removal and conversion to hemodialysis [6].

The source of the relapse is commonly from the catheter, either through biofilm or tunnel infection. Alternatively, antimicrobial resistance may develop during the treatment, resulting in an early relapse with an identical species but with different susceptibility pattern after completion of the treatment. Timely implementation of a change in therapy for high risk patients would, at least in theory, is valuable for the prevention of peritonitis recurrence. However, until recently, there is no accurate laboratory test to predict relapsing or recurrent peritonitis episodes after completion of antibiotic treatment.

Previous studies showed that bacterial-derived DNA fragments are present in clinically used fluids such as dialysis fluid [7]. DNA fragments are thought to be derived from microorganisms inhabiting body fluid [8]. Most of these microorganisms that include potential pathogens might subsist in a “viable but not culturable” state or may need specific culture media [9]. Since bacterial-derived DNA fragments could be detected by polymerase chain reaction (PCR) with excellent sensitivity, and universal primers could be used to detect most of the pathogenic bacteria without a priori knowledge of the bacteriological etiology, it represents an attractive means to test for the presence of viable bacteria in biological specimens and probably indicates incomplete treatment of the infection.

In a recent prospective observational study, we found that bacterial DNA fragment levels in PD effluent are significantly higher, both 5 days prior to and on the date of completion of antibiotics, amongst patients who subsequently develop relapsing or recurrent peritonitis than those cured by antibiotics [10]. Specifically, when bacterial DNA fragment detectable by 34 PCR cycles 5 days before the completion of antibiotics is used as the cut off, it has a sensitivity of 88.9% and specificity of 60.5% for the prediction of relapsing or recurrent peritonitis [10]. Our recent pilot study indicates that the cut-off level of 34 PCR cycles corresponds to 2 DNA copies per ml of PD effluent when measured by digital PCR. However, it remains unknown whether extending the duration of antibiotic therapy according to the bacterial DNA fragment levels in PD effluent could prevent relapsing or recurrent peritonitis.
(C) AIMS

We hypothesize that extending the duration of antibiotic therapy in PD patients with peritonitis and high PD effluent bacterial DNA fragment levels could prevent the development of relapsing and recurrent peritonitis.

The aim of this randomized control study is to determine the efficacy of prolonged antibiotic therapy for the prevention of relapsing and recurrent peritonitis in PD patients with peritonitis and an elevated PD effluent bacterial DNA fragment levels.

(D) PLAN OF INVESTIGATION

Case selection

This is a prospective randomized control study. All studies procedures are in compliance with the Declaration of Helsinki and ICH-GCP. We plan to recruit 360 patients with PD peritonitis. The diagnosis of peritonitis will be based on at least two of the followings [11]: (a) abdominal pain or cloudy PD effluent; (b) leukocytosis in PD effluent (WBC > 100/ml); and (c) positive Gram-stain or culture from PD effluent. Patients with fungal peritonitis or obvious surgical problems and require laparotomy will be excluded. All patients will be treated according to the guideline of the International Society for Peritoneal Dialysis (ISPD) [11].

Study procedures

According to the current treatment guideline, most of the peritonitis episodes would be treated with a two-week course of appropriate antibiotics, while episodes caused by Staphylococcus aureus or Pseudomonas species requires treatment for three weeks.

Informed consent will be obtained by the principal investigator or a co-investigator. In this study, after inform consent, patients will be randomized to receive one additional week of the original effective antibiotic treatment (the Preemptive Treatment Group) or no additional treatment (the Control Group). In addition, a 20-ml specimen of PD effluent will be collected 5 days before the planned completion of antibiotic treatment for the measurement of bacterial DNA fragment levels. A second 20-ml specimen of PD effluent will be collected on the day of completing antibiotic treatment for the measurement of bacterial DNA fragment levels. All patients will be followed for 6 months for the development of relapsing, recurrent, or repeat peritonitis episodes. The overall plan of study arrangement is summarized in Figure 1.

Quantification of PD effluent bacterial DNA level

DNA from 20 ml PD effluent will be extracted using the EZ1 DNA tissue kit and BioRobot EZ1 with the EZ1 bacteria card (Qiagen), according to the manufacturer’s instructions. Purified DNA was eluted in 50 μl of elution buffer before amplification. Bacterial DNA fragment level in PD effluent will be measured by QuantStudio 3D Digital PCR System (Life Technologies, Carlsbad, CA). Briefly, PCR mixture will be prepared and load into the chip according to the manufacturer’s protocol. PCR amplification will be performed by the ProFlex™ PCR system (Life Technologies). The result is captured by the QuantStudio 3D Digital PCR Instrument and analyzed by the QuantStudio AnalysisSuite Software (all from Life Technologies).

PPDNA protocol version 2.4, as of 27 October 2017
**Outcome Measures**

All patients will be followed for 6 months after completion of antibiotic therapy. Since the effect of antibiotic therapy is short-lasting, severe adverse event will be defined as death or hospitalization for any reason during antibiotic therapy.

The primary end point of this study is relapsing, recurrent, or repeat peritonitis episodes. Relapsing peritonitis is defined as an episode that occurs within 4 weeks of completion of therapy of a prior episode with the same organism (or culture negative in the second episode) [11]. Recurrent peritonitis is defined as an episode that occurs within 4 weeks of completion of therapy of a prior episode but with a different organism [11]. Repeat peritonitis is defined as an episode that occurs more than 4 weeks after completion of therapy of a prior episode with the same organism [11]. Secondary outcomes include peritonitis that requires hospitalization, catheter removal, conversion to long-term hemodialysis, death due to peritonitis, and all-cause mortality.

**Statistical analysis**

Statistical analysis will be performed by SPSS for Windows software version 18.0 (SPSS Inc., Chicago). Descriptive data will be represented as mean ± SD. Baseline demographic and clinical data will be compared between the Preemptive Treatment and the Control Groups by Student’s t test or Chi square test as appropriate. The incidence of relapsing or recurrent peritonitis will be compared by Chi square test. Data will be analyzed by both the intention-to-treat as well as per protocol approach. P values of less than 0.05 are considered significant. All probabilities are two-tailed.

**Sample size estimation**

The sample size is estimated by the Power Analysis and Sample Size for Windows software (PASS 2000, NCSS, Kaysville, Utah). All calculations use a two-sided α of 0.05. Based on our previous studies [6,10], around 15% of the patients would have relapsing or recurrent peritonitis predicted by a high PD effluent bacterial DNA level. We assume prolonged antibiotic treatment reduces the incidence of relapsing or recurrent peritonitis by 50% (i.e. absolute risk from 15% to 7.5%), which is believed to be clinically relevant. A sample size of 300 would have 80% power to detect such a difference. Allowing for 20% of drop out [10], a total sample size of 360 needs to be recruited.

**E) TIMETABLE OF WORK**

| Time Period                     | Activity                                      |
|--------------------------------|-----------------------------------------------|
| February 2016 – December 2018  | Recruitment and collection of specimen        |
| January 2019 – June 2019        | Follow up of the patients                     |
| July 2019 – December 2019      | Data analysis and preparation of manuscript    |

**F) EXISTING FACILITIES**

The renal unit of our hospital is taking care of around 400 PD patients and 150 episodes of peritonitis each year. The procedures of PD effluent collection and handling, as well as DNA quantification are familiar to our clinical and laboratory staff.
Our laboratory is equipped with the followings:
- Perkin Elmer ABI PRISM™ 7700 Sequence Detector for quantitative PCR.
- QuantStudio 3D Digital PCR System (Life Technologies)
- Pentium PC computer, SPSS for Windows version 11.0, Sigma Plot version 8.0, CAGED (Cluster Analysis of Gene Expression Dynamics) and Microsoft Office XP software.
- Freezers, refrigerators, and adequate storage space.

(G) PURPOSE AND POTENTIAL FOR IMPLEMENTATION OF RESULTS

The result of the present study will be presented in local and international conference, as well as published in peer-reviewed professional journals. The primary objective of the proposed study is to determine the efficacy of prolonged antibiotic therapy for the prevention of relapsing and recurrent peritonitis in PD patients with peritonitis and an elevated PD effluent bacterial DNA fragment levels.

An effective treatment that reduces the incidence of relapsing and recurrent peritonitis would preserve the longevity of peritoneal membrane for dialysis and reduce the need of conversion to long-term hemodialysis, which is a more expensive modality of treatment. The detection of bacteria-derived DNA fragment in PD effluent is a simple and non-invasive test, with an excellent prospect of clinical application. Our study will determine the efficacy of a test-before-treat algorithm that could reduce the incidence of relapsing and recurrent peritonitis and, at the same time, minimize the unnecessary use of prolonged antibiotic treatment.

(H) REFERENCE

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