Endotoxin in Patients with Terminal Renal Failure Undergoing Dialysis with Re-Processing Dialyser

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ABSTRACT

**Aim:** to determine the level of endotoxin in the blood of patients with renal failure prior to and following hemodialysis using re-processing dialyser to know possibility of pyrogenic reactions in hemodialysis patients.

**Methods:** This study subjects consisted of 10 patients with terminal renal failure undergoing regular hemodialysis. The collected samples were then sent in frozen condition for endotoxin examination in Japan. The normal level of endotoxin in the blood was < 9.8 pg/ml based on standard E. Coli E.0111 endotoxin quantitatively measured using Limulus Amoebocyte lysate test (the endospecy test). Statistical analysis was performed using paired student test.

**Results:** Ten patients with terminal renal failure who were undergoing hemodialysis were obtained, consisting of 1 female and 9 males. The mean age was 55.5 years (SD 6.74), the mean hemoglobin level 7.26 g/dl (SD 2.19), mean white blood cell (WBC) count 8660/mm³ (SD 3064.2), and mean albumin level 3.59 g/l (SD 247). The etiologies of renal failure were as follows: glomerulonephritis (GN) 30%, Diabetic nephropathy (DN) 20%, hypertension (HT) 10%, interstitial nephritis (IN) 10%, obstruction/infection (OI) 10%, unknown (U) 10%. The mean duration of hemodialysis was 97.9 month (SD 54.86). The mean endotoxin level prior to hemodialysis (ET pre-hemodialysis) was 5.4 pg/dl (SD 8).

**Conclusion:** We conclude that terminal renal patients who undergoing re-processing hemodialysis did not have endotoxemia both prior to and following hemodialysis unless if they associated with infection, or other complications.

Key words: endotoxin level, terminal renal failure, dialysis.

INTRODUCTION

Hemodialysis using a re-processing dialyser could increase the risk of infection. The risk of infection could occur due to entry of endotoxin from the dialyser and dialyzing fluid. The potential for exposure of dialysis patients to greater levels of microbial and endotoxin contamination has increased dramatically with the increase in reuse of hemodialyzers, and the use of bicarbonate dialysate and high flux dialysis.

There is a concern that endotoxins or bacteria may cross or interact at the membranes of these dialyzers, triggering the release of endogenous pyrogens (cytokines) by peripheral blood mononuclear cells to cause pyrogenic reactions (PR). Pyrogenic reactions (PR) are a well-recognized complication of hemodialysis and have been associated with dialyzer reuse, high-flux dialysis, and bicarbonate dialysate. However, the roles of bacteria and endotoxin in dialysate for producing PR are not well defined. If such condition continues, it may cause chronic inflammation, increasing the long-term morbidity and mortality of patients with terminal renal failure who undergo hemodialysis.

Endotoxins are made up of the outer most component of Gram negative bacteria, which play an important role for stimulating the humoral system, the macrophage cell, and other inflammatory cells. Endotoxins are also called lipopolysaccharide (LPS).

Endotoxin or LPS layer consists of 3 structures, as follows: (1) polysaccharide, consisting of O specific chain, (2) a middle polysaccharide layer consisting of an outer and an inner layer, and (3) Lipid A layer.

The Lipid A layer is a layer that plays an important role in endotoxin toxicity. All Gram negative bacteria are similar in the structure of their middle layer, polysaccharide, and Lipid A layer, but are different in their O specific polysaccharide chain.
Endotoxins enter the blood circulation from bacterial translocation as well as from the use of a re-processing dialyser. Pasquale, defined bacterial translocation as the passage of either live or dead bacteria through the intestinal mucosal epithel into the lamina promale and mesenteric lymph nodes, unaccompanied by intestinal anatomical abnormality.

Several conditions that could cause bacterial translocation from the digestive tract are: patients with malignancy who have received chemotherapy, patients with granulocytopenia, bone marrow transplant recipients, patients with burns, as well as patients with multi-organ failure.

Other conditions that could promote bacterial translocation are: severe hypoxia that disturbs intestinal mucosa integrity, malnutrition, and intestinal ischemia.

Currently, the chromogenic test (endospecy test) is a quantitative limulus test that is perfect for the detection of plasma endotoxin. Basically, this test consists of two phases, enzyme clotting, and diazotization (color formation by chromogenic substrate). The level of endotoxin is read using an optimal density spectrophotometer with a wavelength of 545 nm.

The aim of this study is to determine the level of endotoxin in the blood of patients with renal failure prior to and following hemodialysis using re-processing dialyser to know possibility of pyrogenic reactions in hemodialysis patients.

METHODS

The study subjects consisted of 10 patients with terminal renal failure undergoing regular hemodialysis at the Renal and Hypertension Division, Department of Internal Medicine of the Faculty of Medicine of the University of Indonesia/Cipto Mangunkusumo Hospital, Jakarta. The blood was obtained from healthy veins prior to and following hemodialysis. Two millimeters of venous blood was inserted into a pyrogen-free tube (made by Terumo) filled with pyrogen-free heparin, centrifugated at 3000 rpm for 40 seconds and stored in the refrigerator at –80°C prior to examination. The collected samples were then sent in frozen condition for endotoxin examination in Japan. The normal level of endotoxin in the blood was < 9.8 pg/ml based on standard E.Coli E.0111 endotoxin quantitatively measured using Limulus Amoebocyte lysate test (the endospecy test).

Statistical analysis was performed using paired student test.
Study Results

Ten patients with terminal renal failure who were undergoing hemodialysis were obtained, consisting of 1 female and 9 males. The mean age was 55.5 years (SD 6.74), the mean hemoglobin level 7.26 g/dl (SD 2.19), mean white blood cell (WBC) count 8660/mm³ (SD 3064.2), and mean albumin level 3.59 g/l (SD 247). The etiologies of renal failure were as follows: glomerulonephritis (GN) 30%, Diabetic nephropathy (DN) 20%, hypertension (HT) 10%, interstitial nephritis (IN) 10%, obstruction/infection (OI) 10%, unknown (U) 10%. The mean duration of hemodialysis was 97.9 month (SD 54.86).

The mean endotoxin level prior to hemodialysis (ET pre-hemodialysis) was 5.4 pg/dl (SD 8), while the level of endotoxin following hemodialysis (ET post-hemodialysis) was 4.63 pg/dl (SD 8). No significant difference was found between the level of endotoxin prior to and following hemodialysis (p=0.406). The characteristics of the subjects could be found in Table 1.

DISCUSSION

From this study, we could see that the mean endotoxin level both prior to and following hemodialysis was not increased (< 9.8 pg/dl). In addition, the level of endotoxin was not increased following hemodialysis (p=0.406, paired student test). This finding differed from other studies who suspected an increased endotoxin level in terminal renal failure patients undergoing re-processing hemodialysis. This may have been due to a different method of examination. Using the conventional (toxicolor) examination, the β-D-glucan factor is not inhibited, resulting in false positives. One of the reasons β-D-glucan factor can be found in the blood is due to the use of a cellulose dialyzer in terminal renal failure patients undergoing re-processing hemodialysis.12,13

False positives due to the use of non-specific endotoxin tests could also be induced by the use of polysaccharide anti-cancer treatment, which has a similar structure to glucan.14,15

In this study, we found 1 (one) case with an endotoxin of 26 pg/dl (>9.8 pg/dl), which may be due to Gram negative infection. This suspicion was supported by leukocytosis and obstruction/infection as the etiology of terminal renal failure. In urinary tract infection, the most commonly found microorganism is E. coli (80-90% of cases) which is a negative Gram bacteria.16 Nevertheless, further examination must be performed using blood as well as urine culture, to determine the cause of infection in this case.

In this study, we also found 2 cases (cases no:8 and 9) with leukocytosis unaccompanied by endotoxemia, which may be due to infection by something other than Gram negative bacteria or non-infection inflammatory process, resulting in an endotoxin level that is not increased. Studies of urinary tract infection demonstrate that endotoxin levels are only increased if caused by Gram negative infection.17 Another study found a level of endotoxin of 4.9±4.5 pg/dl (n=48) in bacteremia due to Staphylococci, which was not significant (p=0.43) when compared to normal subject.

A study of patients with non-infective systemic inflammatory response syndrome (SIRS), such as due to burns or trauma, demonstrated an endotoxin level of 5.6±4.5 pg/dl, which was not significant when compared to normal subjects (p=0.79). However, if SIRS was caused by infection (sepsis and septic shock) the
endotoxin level was increased up to 66.7±155.0 pg/ml (p<0.0001, unpaired Wilcoxon test), which was significant when compared to normal subjects (n=62).18

CONCLUSION

We conclude that terminal renal patients who undergo re-processing hemodialysis did not have endotoxemia both prior to and following hemodialysis unless they associated with infection, or other complications.

REFERENCES