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The Effect of Folic Acid as Anti Fibrosis in Peritoneum of Rat

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ABSTRACT

Introduction: One limitations of Peritoneal Dialysis (PD)'s is peritoneal fibrosis, which is a functional and structural change in the peritoneal membrane induced by peritoneal dialysate fluid containing high sugar levels. This may cause ultrafiltration (UF) failure leading to progressive loss of dialysis efficacy. Expression of vascular endothelial growth factor (VEGF) increases angiogenesis and vascular permeability, followed by increased macromolecular leakage and ultimately contributes to UF failure. Expression of Transforming Growth Factor-β (TGF-β) induces mesothelial thickening and peritoneal fibrosis. Folic acid inhibits progressivity of Chronic Kidney Disease (CKD) by 21% and improve Glomerular Filtration Rate (GFR) by 10% in hypertensive patients. Folic acid can reduce inflammation as cause of endothelial dysfunction that impact to progressive atherosclerosis. The aim of this study was to evaluate the effect folic acid to reduce the expression of TGF-β, VEGF and peritoneal fibrosis in a male rat treated with PD.

Methods: This was an experimental study, with post-test only control group design. Twenty-four Sprague dawley rats are divided into four groups: CKD rat group (Group 1), PD liquid 4,25% (Group 2), CKD PD and folic acid 120 µg/day oral (Group 3), and CKD PD & lisinopril 1,5 mg/day oral (Group 4). After 4 weeks, rat sacrificed expression of TGF-β, VEGF and peritoneal fibrosis are conducted by histopathology with masson's trichrome and immunology with anti-human-TGF-β and anti VEGF. Statistical analys is using Kruskal Willis test. p-value <0.05 was considered statistically significant.

Results: Compared with CKD PD group, folic acid group shown decrease of TGF-β expression (p = 0.002), VEGF expression (p = 0.01), and peritoneal fibrosis (p = 0.001). Lisinopril group also showed decrease of TGF-β expression (p = 0.06), VEGF expression (p = 0.09), but not for peritoneal fibrosis. Folic acid decreased TGF-β expression more than lisinopril. The decrease in VEGF expression in the lisinopril group was greater than folic acid group.

Conclusion: Folic acid shown lower of TGF-β and VEGF expression and reduced and peritoneum fibrosis better than control or lisinopril and statistically significant. Folic acid is potential as therapy agent to reduce peritoneal fibrosis in PD's patients

Keywords: Folic acid; ace-inhibitor; tgf-β; vegf; peritoneal fibrosis

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INTRODUCTION

End Stage Renal Disease (ESRD)is mostly treated by kidney replacement therapy including dialysis and kidney transplantation. Peritoneal Dialysis (PD) is a dialysis method using the peritoneum as a semipermeable membrane where diffusion and ultrafiltration occured.[1,2,3]Over the past 12 years, the number of peritoneal dialysis patients increase in both developing and developed countries, which increased 24.9 per million population and 21.8 per million population respectively.[4]

One study conducted by Strippoli *et al*, shown that the signs of peritoneal fibrosis was founded in 50-80 % from patients who underwent PD in the first 2 years. Some reports from the United States were also shownthat peritoneal fibrosisoccured in 4-12% of patients who undergone PD for 6 years. It alsoaffected more than 30% of all PD patients.[5,6] Formation of advanced glycosylated end products (AGE) during the PD process, followed by binding to thatAGE receptors in the peritoneal membrane causedactivations of secondary messenger system, leading to of increase production of several growth factors such as Vascular Endothelial Growth Factor (VEGF) and Transforming Growth Factor β (TGF-β). An increase of VEGF expression triggers angiogenesis, vascular permeability, followed by macromolecular leakage which later ultimately leading to ultrafiltration failure. TGF-β is a profibrotic cytokine which considered as the main molecule that responsible to peritoneal fibrosis.[7,8]

Several studies have been conducted to prevent peritoneal fibrosis. Duman *et al*, shown that administration of ACE-inhibitors (ACEI) to albino wistar rats that have been treated3.86 % intraperitoneal dextrose fluid, The TGF-β levels and morphology of peritoneal were examined afterwards. The result shown significant decreased of TGF-β levels, but peritoneal dialysis fibrosis was not completely inhibited.[9]

Xu Xin conducted a study using moderate dose of folic acid 0.8 mg to inhibit the progression of Chronic Kidney

Disease (CKD). This study shownthat enalapril and folic acid can inhibit CKD progression by21 % and delayed glomerular filtration rate (GFR) by 10 % in hypertensive patients.[10]

Folic acid can reduce homocysteine levels. However, its potential to specifically reduced peritoneal fibrosis in PD patients have not been demonstrated. So far, no data shows about the role of folic acid as an antifibrosis drug for peritoneal fibrosis. Therefore, this study aimed to evaluate effect of folic acid to reduce TGF-βexpression, VEGF and peritoneal fibrosis in a male rat treated with PD models induced by aristolochic acid.

MATERIALS AND METHOD

This study using an experimental research with post-test only controls group design. Subject were using 24 of male spraque dawley, aged 3-4 months old, and weighted 200 - 300 gram. Chronic Kidney Disease rat models were performed by using a single dose injections of 2.5 mg aristolohic acid (AA) to muse intraperitoneal. Two weeks after AA injection, the ratwere divided into 4 treatment groups. Control group (Group 1), PD liquid 4,25% (Group 2), folic acid 120 µg/day oral and PD (Group 3), and lisinopril 1,5 mg/day oral and PD (Group 4) After 4 weeks, rat was sacrificed peritoneal fibrosis were conducted using histopathology with masson's trichrome stained. TGF-β and VEGF expression were examined imunohistochemistry using anti-human-TGF-β and anti VEGF.

The interpretation of immunohistochemistry data for TGF-β and VEGF presented in semi quantitative scoring with numbers were assigned a score of 0 (no staining / negative), 1 (weak, <10% of cells staining), 2 (moderate, 10%–50% of cells staining), or 3 (high, >50% of cells staining).[11,12]Peritoneal fibrosis presented in with numbers were assigned a score of 0 (smell scellered), 1 (thin hard, <10% of cells staining), 2 (thicker, 10%–50% of cells staining), or 3 (thick and dense, >50% of cells staining).[11,12]

Statistical analysisis performed using Kruskal Willis test. A p value of < 0.05 was considered as statistically significant. SPSS 22.0 software was used for the data analysis. Ethical approval was taken prior the study.

RESULT

As shown in Figure 1, expression of TGF-β in PD group higher than control group (mean rank score = 20,00). TGF-β expression were lower in group that received folic acid therapy comparedthan PD group (mean rank score = 9,67) and lisinopril grouprespectively (mean rank score = 12.25; p = 0.002).

Figure 2 shown that VEGF expression in PD group were higher than control group (mean rank score = 17.25). VEGF expression in group that received folic acid therapy were lower compared toPD group (mean rank score 13,75) and higher than lisinopril group (mean rank score = 12,00; p = 0.01).

Figure 3 shownthat peritoneal fibrosis expression in PD group were higher than control group (score 16.00). Mean rank score of peritoneal fibrosis in group that received folic acid therapy were lower than group PD (15,42) and group lisinopril therapy respectively (score = 14.08; p = 0.001).

The results shownincreased significant expression of VEGF between control and PD groups (p = 0.01). SignificantTGF-β expression were also foundedbetweencontrol and PD group (p = 0,001). Moreover, significant differences in the expression of peritoneal fibrosis between control group and PD group were founded (p = 0,002).

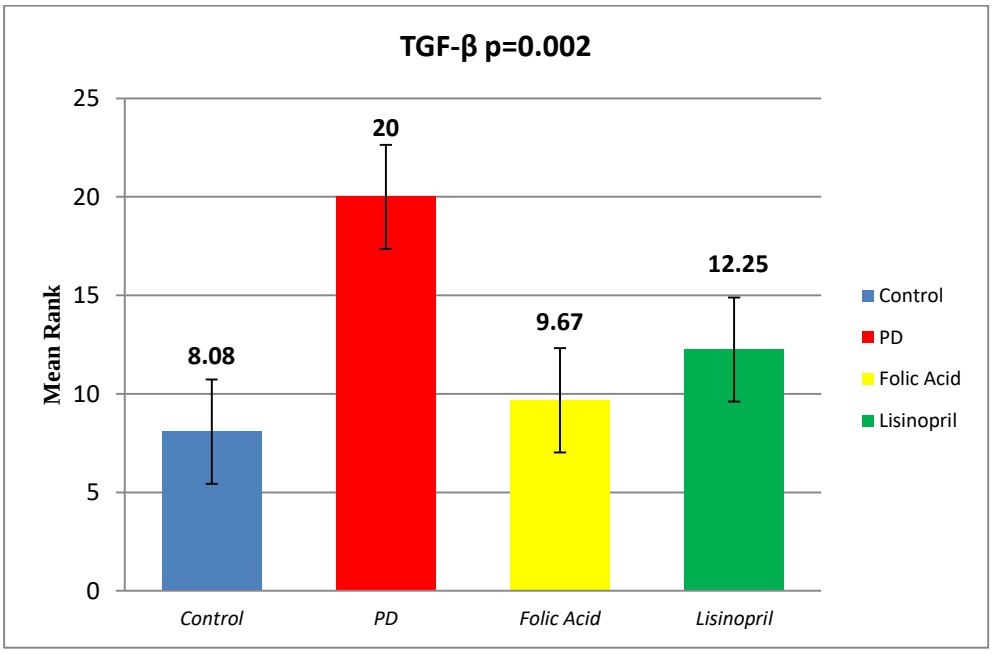

Figure 1. Expression of TGF-β peritoneum of rat peritoneal in the control group, the PD group, the group that received folic acid therapy, and the group that received lisinopril therapy after 4 weeks of getting action.

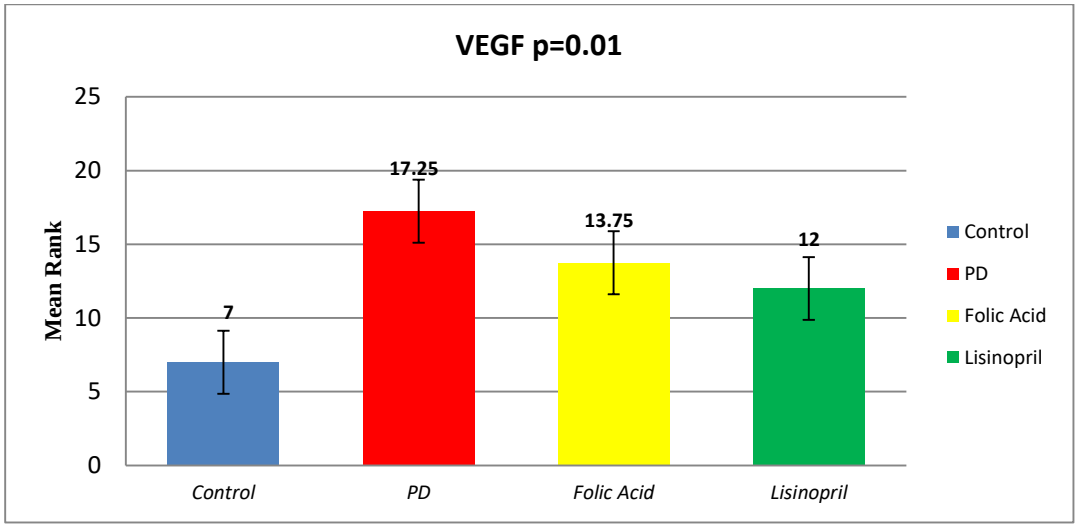

Figure 2. VEGF expression of rat peritoneal in the control group, PD group, group that received folic acid therapy, and group that received lisinopril therapy after 4 weeks of getting action.

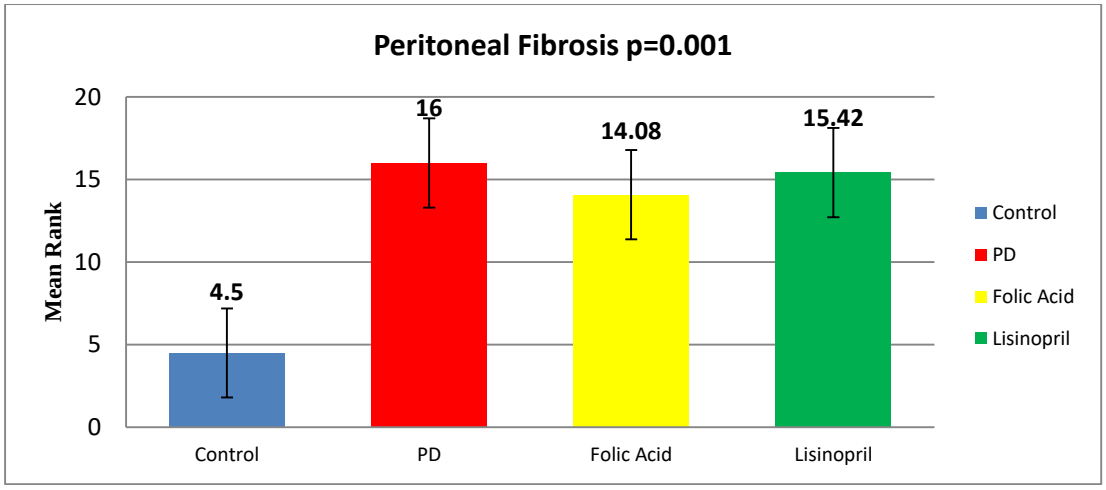

Figure 3. Peritoneal fibrosis score shownby Masson's trichrome staining in the control group, PD group, the group receiving folic acid therapy, and the group receiving lisinopril therapy after 4 weeks of getting action.

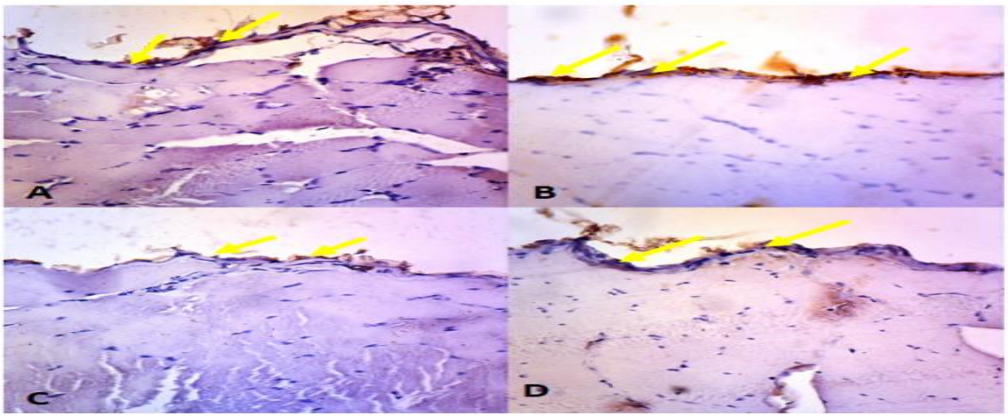

Figure 4. Expression of TGF β on peritoneum. Score 1 in group I (A); score 2 in group II (B); score 2 in group III (C); and score 2 in group IV (D). Magnification of 100x, arrows indicate TGF βexpression in the cytoplasm of mesothel and intercellular cells.0: negative; 1: light; 2: medium; 3: strong.

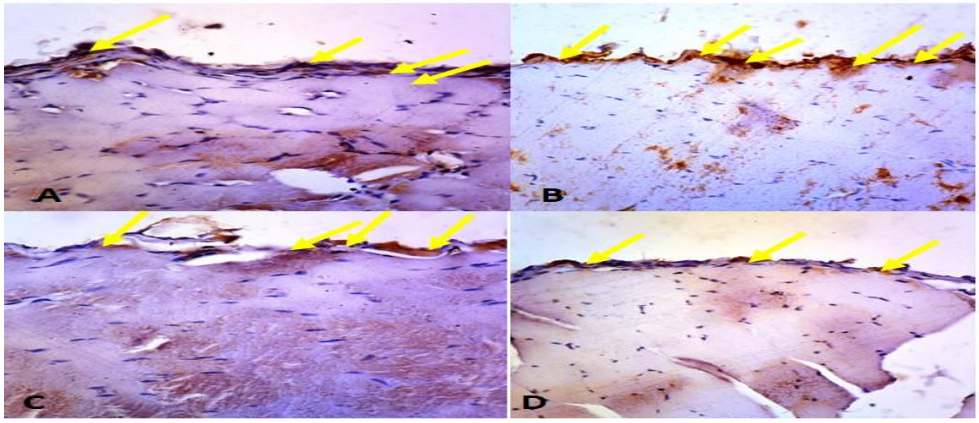

Figure 5. VEGF expression in the peritoneum showed a score of 2 in group I (A); score 3 in group II (B); score 2 in group III (C); and score 2 in group IV (D). Magnification of 100x, arrows indicate VEGF expression in the cytoplasm of mesothel and intercellular cells.0: negative; 1: light; 2: medium; 3: strong.

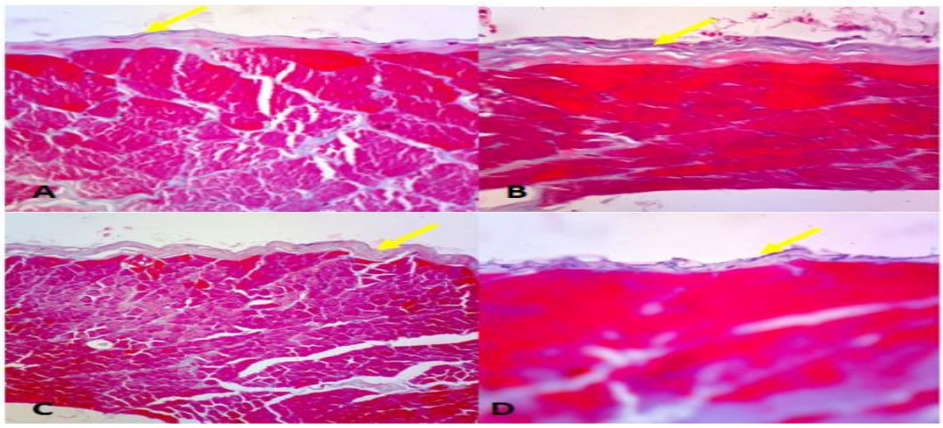

Figure 6. Fibrosis peritoneum with trichome masson staining showed score 0 in group I (A); score 2 in group II (B); score 1 in group III (C) and score 1 in group IV (D). Magnification of 100x, arrows indicate areas of fibrosis.0: scattered thin collagen fibers; 1: thin collagen tape; 2: the collagen band is bigger; 3: Collagen band is thick and dense

DISCUSSION

The objective of this study was to evaluate effect of folic acid to decrease the expression of TGF-ß, VEGF and reduce peritoneal fibrosis.

This study shownthat TGF-β expression were decrease in Spraque Dawley rats in the group of folic acid better than lisinopril group (p = 0.002).

Folic acid could decrease VEGF expression in Spraque Dawley rat (p = 0.01). Decrease of VEGF expression in lisinopril has a lower tendency than folic acid group.

Folic acid may also reduce peritoneal fibrosis of Spraque Dawley rat (p= 0.001). It can reduce peritoneal fibrosis of Dawley Spraque rat better than lisinopril. The mechanism by which the peritoneal fluid of hypertonic dialysis induces structural changes of the peritoneum is not fully understood. In this study, we shownthat high glucose concentrations increased the expression of TGF-β. This is consistent with previous study by Duman, 2001 that shown TGF-β has role in mediating the changes on the peritoneal membranes as high-glucose dialysate. Injury in peritoneum is mediated by angiotensin II and correlated with stimulation of AT receptor I.[9,13]

Activation of TGF-β signalingare complex and involves several mediators. These process including regulatory activation of TGF-β, intracellular signaling, and interaction of DNA transcription factors initiate a change in response to TGF-β. Several Cytokines have the ability to alter TGF-ß response to fibrosis such as IL-1 and IL-6, [14,15,16]

A study by Kariya, *et al* reported that TGF-β was closely related to VEGF in the process of peritoneal fibrosis. TGF-β can directly increase VEGF expression while VEGF are produced by mesothelial cells, endothelial cells, fibroblasts, and macrophages. It can trigger lymphangiogenesis in the peritoneal wall. This shows us that angiogenesis plays an important role in the process of peritoneal fibrosis.[17]

Bio incompatibility of dialysate fluids due to the composition of the high glucose, high osmolarity, and low pH triggers the accumulation of AGE. Furthermore, AGE will bind to its receptor, namely RAGE, which is located in mesothelial cells, fibroblasts, and macrophages.[14]

Uptake of AGE by peritoneal cells including fibroblasts is carried out via RAGE. Peritoneal mesothelial cells produce various typesof proinflammatory cytokines such as TNF-α, IL1, IL6, p50 and growth factors. All types of AGE accumulate in the peritoneum with growth factors, including transforming growth factor-β1 (TGF-β1). Intensity of growth factor expression is proportional to the accumulation of AGE.[18]

Uremia will increase homocysteine levels and NADPH

levels and also trigger inflammatory response by proinflammatory cytokines through ROS activation. Increased proinflammatory cytokines stimulate fibroblasts to produce ECM which leading toultimately results in peritoneal fibrosis.[19]

Folic acid has an important role as a cofactor in carbon metabolism. Deficiency of folate can cause various kinds of disorders in the form of degenerative disorders, cancer, metabolic disorders and psychiatry. Recently, it has beenknown that there is a lot of knowledge that these disorders are related to the inflammatory process, both acute and chronic. Many studies suggest such connections to that folic acid, homocysteine, and inflammation. One suggest that Inflammation will result in cellular damage and proliferation and adversely affect cells such as the carcinogenesis process.[20]

Maintaining the function of the peritoneum is themain step taken to ensure effectiveness of PD process over the long-term. Several steps can be taken to make the peritoneal membrane work properly, and solute regulation and water transport improve peritoneal microcirculation by increasing vasodilator secretion, such as prostaglandin E2 and nitric oxide, peritoneal fibrinolysis regulation, extracellular production, and remodeling matrix, and mechanism local antibacterial defense in the peritoneum.[9,15]

CONCLUSION

Folic acid decreased the expression of TGF-β, VEGF and peritoneum fibrosis compared to control or lisinopril and statistically significant. Folic acid is potential as therapy to reduce peritoneal fibrosis in PD's patients.

This research was still limited in animal models. However, peritoneal fibrosis in ESRD's patient with PD consisted of etiology, pathogenesis, and more complex management. Therefore, further studyin human was still needed to investigate the benefit of folic acid in peritoneal fibrosis.

CONFLICT INTEREST

None

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