Curcumin anti-inflammatory and antitumorigenic effect on arteriovenous fistula maturation in patient with end-stage renal disease with diabetes mellitus

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ABSTRACT

The primary clinical problem of arteriovenous fistula (AVF) is the high maturation failure rate. Curcumin shows anti-inflammatory, antioxidant, antitumorigenic, and antiproliferative effects that may contribute to AVF maturation. This study investigated the effectiveness of curcumin and acetylsalicylic acid on anti-inflammatory and antitumorigenic effects on AVF maturation in patients with End-Stage Renal Disease (ESRD) with diabetes mellitus (DM). The present study is a single-blinded, randomized controlled trial consisting of curcumin, acetylsalicylic acid, or placebo intervention for eight weeks. A total of 67 participants completed the intervention. There was a higher level of nitric oxide (NO) between the curcumin and placebo four weeks (92.58±24.70 vs 75.67±16.50 μmol/L, adjusted p = 0.038) and eight weeks (95.27±28.77 vs 76.66±23.24 μmol/L, adjusted p <0.05) postoperatively. Higher-level of NO between acetylsalicylic acid and placebo (93.65±24.65 vs 75.67±16.50 μmol/L, adjusted p = 0.031) four weeks postoperatively. Curcumin increases NO after four and eight weeks and lowers MMP-9 four weeks after AVF operation. Acetylsalicylic acid increases NO four weeks after AVF surgery on ESRD patients with DM.

INTRODUCTION

End-Stage Renal Disease (ESRD) is a disease with a high global health burden. This disease also has a massive impact on morbidity and mortality by increasing cardiovascular disease, diabetes, and hypertension (Luyckx et al., 2018). A study from the Global Burden of Disease in 2015 estimated that 1.2 million people died from kidney failure in 2015, increasing 32% since 2005 (GBD 2015 Mortality and Causes of Death Collaborators, 2015). Renal replacement therapy, especially hemodialysis, is life-saving where kidney transplants are lim-
Arteriovenous fistula (AVF) is the vascular access of choice for hemodialysis. AVF has a major clinical problem as 20% to 60% of AVFs experience maturation failure (Lok et al., 2020). AVF takes an average time of 3.5 months to reach post-construction maturation (Bylsma et al., 2017). Thus this failure is associated with increased morbidity, the incidence of hospitalization, and repeated interventions to establish vascular access for hemodialysis. The cause of AVF maturation failure is multifactorial, contributing to an abnormal response from the feeding artery and draining vein due to the increased shear stress from the construction of arteriovenous anastomosis (Smith et al., 2012). Patient with diabetes is more likely to encounter AVF failure. Diabetes mellitus (DM) causes endothelial dysfunction in the blood vessels and a higher risk of thrombosis (Yan et al., 2018).

Curcumin is a natural phenol from turmeric (Curcuma sp.). Based on in vitro studies, curcumin has antiproliferative and antitumorigenic effects to prevent proliferation and migration of vascular smooth muscle, neointima hyperplasia, and stenosis that causes the failure of AVF maturation (Yang et al., 2006). Curcumin also shows anti-inflammatory and antioxidant properties that may contribute to remodelling and the development of neointima hyperplasia (Pratama et al., 2020; Hewlings and Kalman, 2017). Studies on curcumin in patients with ESRD patient with DM, however, are limited. Therefore, we investigated the effectiveness of curcumin anti-inflammatory and antitumorigenic effect on arteriovenous fistula maturation in patients with ESRD with DM.

METHODS

Study Population

Patients with ESRD with DM undergoing AVF operation were recruited. Recruitment for the subjects was conducted from July 2019 to March 2020. The inclusion criteria were as follows: 18-70 years old, eGFR of 5 – 25 mL/min/1.73 m², Brachial artery diameter of > 2 mm, triphasic spectrum, and minimal plaque on duplex ultrasonography, cephalic vein diameter of > 2 mm, compressible, and no obstructed flow due to stenosis or thrombosis. Subjects who had previous AVF operation, immunodeficiency, were critically ill, suffering from hepatic disease, and consuming anti-platelet or other antioxidants were excluded from the study. Written informed consent was obtained from all participants before being included in the study.

The study was performed at dr. Cipto Mangunkusumo General Hospital (Jakarta, Indonesia), Hermina Bekasi Hospital (Bekasi, Indonesia), and Hermina Depok Hospital (Depok, Indonesia). Ethical clearance was approved by the Ethics Committee Faculty of Medicine, Universitas Indonesia (KET-353/UN2.F1/ETIK/PPM.00.02/2019).

Study Design

The present study was a single-blinded, randomized controlled trial consisting of curcumin, acetylsalicylic acid, or placebo intervention after AVF operation. Curcumin was given 2000 mg/day (Curcumin plus; Sabinsa corporation, Utah, USA), acetylsalicylic acid 8 mg/day (Aspilet; Medifarma, Depok, Indonesia), and placebo once per day for eight weeks. Randomization was performed using random numbers generator with an equal treatment allocation ratio. Blinding was performed to the doctor by concealing patient intervention. Only the research assistant knew the type of drug received by the patient.

Blood Sample Analysis

The blood sample was extracted post-operation, four weeks post-operation, and eight weeks post-operation. Nitric oxide (NO) is calculated indirectly using Griess reaction at Laboratorium Terpadu Faculty of Medicine, Universitas Indonesia. Matrix metalloproteinase 9 (MMP-9) was measured using the ELISA method and analyzed at Laboratorium of Pharmacology, Faculty of Medicine, Universitas Indonesia. The blood sample collected is transferred to a cooling box and analyzed within 2 hours of blood collection. If the sample arrived outside of work hours, the sample is saved in a cooling box with a temperature of 2 – (-8) °C for less than 24 hours.

Statistical Analysis

Baseline characteristic between subject was analyzed using ANOVA post-hoc Bonferroni or Kruskal Wallis post-hoc Mann Whitney. Serum NO level was analyzed using ANOVA post-hoc Bonferroni and MMP-9 was analyzed using Kruskal Wallis post-hoc Mann Whitney. Results are shown as mean ± SD or median (min; max). ANCOVA analysis is used to adjust for the difference in the baseline characteristic. Statistical significance was defined as p < 0.05. All statistical analyses were performed using IBM SPSS ver. 25.

RESULTS

Baseline Characteristic

A total of 131 participants were assessed for eligibility, 72 participants were eligible and willing to participate in the research, and 67 participants com-
Figure 1: Flow chart of patient selection

Table 1: Baseline characteristic

<table>
<thead>
<tr>
<th></th>
<th>Curcumin (n = 24)</th>
<th>Acetylsalicylic Acid (n = 21)</th>
<th>Placebo (n = 22)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (male)</td>
<td>17</td>
<td>10</td>
<td>7</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>BMI (kg/m2)</td>
<td>23.22 ± 3.42</td>
<td>23.45 ± 4.55</td>
<td>24.45 ± 4.15</td>
<td>0.572</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)*</td>
<td>134 (115; 189)</td>
<td>140 (120; 190)</td>
<td>150 (120; 180)</td>
<td>0.316</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)*</td>
<td>82 (65; 100)</td>
<td>80 (70; 100)</td>
<td>85 (65; 108)</td>
<td>0.797</td>
</tr>
<tr>
<td>Age (year)</td>
<td>55.7 ± 7.27</td>
<td>57.48 ± 8.80</td>
<td>58.05 ± 9.77</td>
<td>0.640</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>25.2 (18; 36)</td>
<td>28 (17; 40.7)</td>
<td>27 (16; 36)</td>
<td>0.114</td>
</tr>
<tr>
<td>Leukocyte (103/μL)</td>
<td>8.045 ± 2.474</td>
<td>8.607 ± 2.305</td>
<td>8.671 ± 1.965</td>
<td>0.600</td>
</tr>
<tr>
<td>Platelet count (103/μL)</td>
<td>255.83 ± 63.12</td>
<td>283.76 ± 65.19</td>
<td>265.23 ± 58.57</td>
<td>0.330</td>
</tr>
<tr>
<td>Random Blood Glucose (mg/dL)*</td>
<td>151 (74; 414)</td>
<td>163 (101; 321)</td>
<td>142 (54; 512)</td>
<td>0.318</td>
</tr>
<tr>
<td>Total Cholesterol (mg/dL)</td>
<td>184 (116; 246)</td>
<td>181 (144; 289)</td>
<td>168 (117; 316)</td>
<td>0.194</td>
</tr>
<tr>
<td>Triglyceride (mg/dL)*</td>
<td>139 (44; 320)</td>
<td>165 (65; 264)</td>
<td>171 (74; 432)</td>
<td>0.515</td>
</tr>
<tr>
<td>HDL (mg/dL)*</td>
<td>43 (24; 98)</td>
<td>40 (27; 75)</td>
<td>43 (23; 160)</td>
<td>0.913</td>
</tr>
<tr>
<td>LDL (mg/dL)</td>
<td>110.71 ± 34.88</td>
<td>118.73 ± 39.21</td>
<td>113.15 ± 35.62</td>
<td>0.391</td>
</tr>
<tr>
<td>Smoking (yes)</td>
<td>9</td>
<td>8</td>
<td>6</td>
<td>0.821</td>
</tr>
<tr>
<td>Brachial artery diameter (mm)</td>
<td>4.30 ± 0.61</td>
<td>4.23 ± 0.59</td>
<td>3.93 ± 0.85</td>
<td>0.144</td>
</tr>
<tr>
<td>Cephalic vein diameter*</td>
<td>3.1 (2.0; 4.8)</td>
<td>3.1 (2.0; 5.2)</td>
<td>2.5 (2.0; 5.4)</td>
<td>0.336</td>
</tr>
</tbody>
</table>

Data shown as mean ± SD unless indicated otherwise *Data shown as median (min; max); BMI, body mass index; HDL, high-density lipoprotein; LDL, low-density lipoprotein
Table 2: NO level after curcumin, acetylsalicylic acid, and placebo intervention

<table>
<thead>
<tr>
<th></th>
<th>Curcumin (n = 24)</th>
<th>Acetylsalicylic acid (n = 21)</th>
<th>Placebo (n = 22)</th>
<th>Adjusted p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Post-operation (μmol/L)</td>
<td>73.78 ± 24.45</td>
<td>77.98 ± 24.55</td>
<td>74.40 ± 21.89</td>
<td>0.756</td>
</tr>
<tr>
<td>4 weeks post-operation (μmol/L)</td>
<td>92.58 ± 24.70a</td>
<td>93.65 ± 24.65b</td>
<td>75.67 ± 16.50a,b</td>
<td>0.019</td>
</tr>
<tr>
<td>8 weeks post-operation (μmol/L)</td>
<td>95.27 ± 28.77c</td>
<td>92.67 ± 23.18</td>
<td>76.6 ± 23.24c</td>
<td>0.040</td>
</tr>
</tbody>
</table>

Data shown as mean ± SD; a,b,c Post-hoc Bonferroni significant difference p <0.05

Table 3: MMP-9 level after curcumin, acetylsalicylic acid, and placebo intervention

<table>
<thead>
<tr>
<th></th>
<th>Curcumin (n = 24)</th>
<th>Acetylsalicylic acid (n = 21)</th>
<th>Placebo (n = 22)</th>
<th>Adjusted p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Post-operation (ng/mL)</td>
<td>50.76(8.24;230.5)</td>
<td>64.39(4.67;266.17)</td>
<td>59.79(4.07;166.12)</td>
<td>0.829</td>
</tr>
<tr>
<td>4 weeks post-operation (ng/mL)</td>
<td>63.54(16.7;93.06)a</td>
<td>69.86(10.15;351.94)</td>
<td>91.12(33.99;244.50)a</td>
<td>0.041</td>
</tr>
<tr>
<td>8 weeks post-operation (ng/mL)</td>
<td>66.94(14.96;383.42)</td>
<td>81.59(15.96;250.25)</td>
<td>72.20(18.50;381.50)</td>
<td>0.824</td>
</tr>
</tbody>
</table>

Data shown as median (min; max); a Post-hoc Mann Whitney significant difference p < 0.05

completed the intervention (Figure 1). Baseline characteristics show no significant difference except for sex (Table 1).

**Nitric Oxide**

The effect of the interventions with NO levels was measured (Table 2). There was no significant increase in NO levels between the three intervention groups after the AVF operation (adjusted p = 0.756). After four weeks postoperatively, there was a significant difference in the increase in NO levels between the curcumin and placebo groups (92.58 ± 24.70 vs 75.67 ± 16.50 μmol/L, adjusted p = 0.038), and between the acetylsalicylic acid group with placebo (93.65 ± 24.65 vs 75.67 ± 16.50 μmol/L, adjusted p = 0.031). At eight weeks postoperatively, the curcumin group still consistently showed a significant increase in higher NO levels than the placebo group (95.27 ± 28.77 vs 76.66 ± 23.24 μmol/L, adjusted p < 0.05). Furthermore, although not statistically significant, the acetylsalicylic acid group showed a higher mean increase in NO levels than placebo (92.67 ± 23.18 vs 76.66 ± 23.24 μmol/L, adjusted p = 0.128).

**Matrix Metalloproteinase 9**

We measured the effect of the intervention with the MMP-9 levels (Table 3). There was no significant difference in MMP-9 levels between the three intervention groups (adjusted p = 0.829) post AVF operation. The curcumin group showed a significantly lower reduction in MMP-9 levels compared to the placebo group at 4 weeks post AVF operation (63.54 (16.7; 93.06) vs 91.12 (33.99; 244.50) ng/mL, adjusted p = 0.005). Furthermore, although not statistically significant, the group given acetylsalicylic acid also showed a lower reduction in MMP-9 levels than placebo at 4 weeks after AVF surgery (69.86 (10.15; 351.94) vs 91.12 (33.99; 244.50) ng/mL, adjusted p = 0.264). There was no significant difference in the reduction in MMP-9 levels between the three intervention groups at eight weeks post AVF surgery.

**DISCUSSION**

AVF construction increases shear stress due to increased blood flow from arteriovenous anastomosis, which will activate NO from the endothelial cells (Smith et al., 2012). The release of NO activate cyclic GMP (cGMP) and protein kinase G (PKG) signals which cause an intracellular influx of calcium to induce vasodilation (Geenen et al., 2016). Moreover, in DM patients, there is an increase in reactive oxygen species from advanced glycation end products due to hyperglycemia which contributes to endothelial dysfunction and decreases endothe-
Lial nitric oxide synthases (eNOS) expression. Thus, efforts to increase NO are essential in the maturation process of AVF, especially in ESRD patients with DM (Tessari et al., 2010; Botker and Møller, 2013).

This study found that the mean serum NO levels on ESRD patients with DM post AVF operation that received curcumin were significantly higher than the placebo group. The results of this study are in line with findings in a study by Santos-parker et al. on a healthy adult and elderly population, which found increases in NO bioavailability after 12 weeks of curcumin supplementation (p = 0.03) (Santos-Parker et al., 2017). This increase occurred either directly or indirectly. An in vitro study by Guo et al. showed curcumin supplementation directly stimulates the production of the eNOS (p <0.01) (Guo et al., 2015). Curcumin also increases NO levels indirectly by increasing antioxidants levels. Antioxidants produced will improve endothelial function, which in subsequent can re-produce NO (Pae et al., 2007). These factors may contribute to the high NO levels in subjects treated with curcumin at 4 and 8 weeks postoperatively.

This study also showed higher NO levels in the acetylsalicylic acid group compared to placebo. The mechanism underlying this phenomenon is that acetylsalicylic acid affects the inhibition of superoxide-mediated NO degradation (Taubert et al., 2004). Acetylsalicylic acid also contains acetylta, which has a structure similar to eNOS. This similarity in structure causes the production of NO from endothelial cells (Schröder, 2009). This is the reason NO levels in patients given acetylsalicylic acid were higher than placebo, although a significant difference was only found at week 4.

MMP is a family of Zn-dependent enzymes that functions in normal and pathological conditions. MMP-9 degrades collagen type IV located on the basement membrane, especially in the vascular smooth muscle wall, stimulating VEGF-A production. VEGF activation trigger migration and proliferation of vascular smooth muscle (Webb et al., 2017; Wang and Khalil, 2018). This explains the underlying mechanism of higher levels of MMP-9 in fail to mature AVF, especially in ESRD patients with DM. Thus, regulation of MMP-9 expression is essential to control neointima hyperplasia (Yabluchanskiy et al., 2013).

In this study, MMP-9 levels decreased significantly after curcumin administration. However, the reduction was only significant at the 4 weeks postoperative and not significant at 8 weeks postoperative. This is because the increase in MMP-9 only occurs transiently in the early stages of inflammation (Berceli et al., 2006). Curcumin inhibits MMP-9 through the protein kinase C (PKC) pathway and depends on AMPK-MAPK by macrophage cells. This inhibition was also facilitated by suppressing the expression of the Extracellular Matrix Metalloproteinase Inducer (EMMPRIN) (Kim et al., 2009). Reduction in MMP-9 levels was also found in other studies conducted on evaluating postoperative skin wound healing processes. In the healing wounds on the skin, there was a decrease in MMP-9 levels and returning to normal values two weeks after the formation of postoperative wounds (Soo et al., 2000). A study by Misra et al. shows a significant increase in expression of MMP-9 activity in venous stenosis 4 weeks after AVF formation in rats (p <0.05) (Misra et al., 2008). Whereas a study by Lee et al. shows no significant different level of MMP-9 after six weeks after AVF formation (p = 0.9) (Lee et al., 2010). No studies have assessed MMP-9 serum in the human population with ESRD and DM. However, preclinical studies with rats as subjects show differences in levels of MMP-9 after AVF formation (Misra et al., 2008).

This study showed no difference in MMP-9 levels between the patient group given acetylsalicylic acid and placebo. This result is similar to Kuliczowski et al., where MMP-9 levels in DM patients with coronary heart disease were not different after administering acetylsalicylic acid (p = 0.29) (Kuliczowski et al., 2017). However, a study by Hua et al. found that giving acetylsalicylic acid significantly inhibits the production of MMP-9 in vitro on macrophage cells by increasing the expression of PPARα / γ in macrophages (p <0.01) (Hua et al., 2009). The difference in MMP-9 levels between these studies can be attributed to the research samples as the study by Hua et al. is conducted in vitro. This indicates that the inhibitory effect of COX enzymes by acetylsalicylic acid does not significantly affect the inhibition of MMP-9 production in endothelial cells AVF.

This present study has several limitations. This study was conducted on small sample size. This study also has a follow-up period of only eight weeks. Patients with DM require a more extended time for AVF to mature compared to patients without DM.

CONCLUSIONS

Curcumin increases NO level after four and eight weeks and lowers MMP-9 four weeks after AVF operation on ESRD patients with DM compared to the placebo group. Acetylsalicylic acid also increases NO level four weeks after AVF surgery on ESRD patients with DM. Limited research has been done to identify curcumin and acetylsalicylic acid anti-inflammatory
and antitumorigenic effect on patients with ESRD and DM. A larger and longer RCT is required to conclude the effect of curcumin and acetylsalicylic acid on patients with ESRD and DM.

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**Conflict of Interest**

The authors declare that they have no conflict of interest.

**REFERENCES**


