PLATELET SEROTONIN-FLUID MEDIATED BRACHIAL ARTERY: INDICATOR FOR ENDOTHELIAL DYSFUNCTION IN MAJOR DEPRESSIVE DISORDER

Arlisa Wulandari¹, Tri Hanggono Achmad², Tuti Wahmurti³, Augustine Purnomowati⁴

¹Department of Psychiatry, Faculty of Medicine, Universitas Jenderal Achmad Yani, West Java, Indonesia.
²Department of Basic Medical Science, Faculty of Medicine, Universitas Padjadjaran, West Java, Indonesia.
³Department of Psychiatry, Faculty of Medicine, Universitas Padjadjaran, West Java, Indonesia.
⁴Department of Cardiology and Vascular Medicine, Faculty of Medicine, Universitas Padjadjaran, West Java, Indonesia.

ABSTRACT

Studies have provided evidence that major depressive disorder (MDD) is associated with an increased risk of vascular diseases. Major depressive disorder has been shown to induce endothelial dysfunction which is believed to be the early process of cardiovascular disease. A possible common denominator between vascular and depressive disease is serotonergic transmission. Platelet serotonin level and assessment of endothelial dysfunction using a non-invasive method could be used as a simple early detection for cardiovascular diseases. This study involved 65 MDD patients (diagnosed using Structured Clinical Interview for DSM-IV Axis I Disorder and had no diagnosis of other psychiatric disorder) as participants, after controlling for age, mean arterial pressure, body mass index, lipid profile, blood glucose and recent or history of cardiovascular diseases. Patients were measured using ELISA for platelet serotonin, while endothelial dysfunction was determined by flow-mediated brachial artery (FMBA). The level of platelet serotonin differed significantly between those in the MDD patients with normal FMBA score and abnormal FMBA score. Platelet serotonin level is associated with and has important role in endothelial dysfunction, and can be used as indicator of endothelial dysfunction in MDD. Endothelial dysfunction in MDD could be detected non-invasively with FMBA.

Key words: endothelial dysfunction, major depressive disorder, platelet serotonin

I. INTRODUCTION

Depressive disorders were pathological conditions characterized by depressed mood or loss of interest and pleasure in activities, got feelings of personal inadequacy, had a low opinion about himself / herself, might feel hopeless and suicidal. Depression led to considerable dysfunction in all areas of life, became significant public health problem and persons with depression were more prone to develop secondary medical disorders (1-3). Depression had been associated with impaired endothelial function in healthy patients, in those at risk for cardiovascular diseases, and in those with established cardiovascular diseases (4-7).

Neurotransmitters had been implicated in both pathophysiology and treatment of mood disorders. The monoamine hypothesis of depression suggested that dysfunction of serotonin may be linked to symptoms in major depressive disorder (8-10). Serotonin (5-HT) was best known as a neurotransmitter involved in mood regulation, but it also directly affected endothelial cells and vascular smooth muscle (11). The serotonin parameters in platelets were frequently used as an indirect way to understand changes in the brain serotonin. Platelets 5-HT levels were supposed to be peripheral models that provide knowledge about central serotonin activity and considered useful biomarkers of the serotonergic synaptic neurotransmission, particularly in psychiatric disturbances such as depression (12,13). One mechanism by which depression might lead to increased cardiovascular disease risk was through effects on endothelial homeostasis (14).
The endothelium played an important role in the development of atherosclerosis and the progression of cardiovascular disease (15). Decreased nitric oxide (NO) production had been suggested to contribute to the reduced endothelium-dependent vasodilatation of depressed patients and, therefore, to the increased risk of cardiovascular disease in patients with major depressive disorder. Endothelium-dependent flow-mediated vasodilatation assessed by brachial artery ultrasound was reduced in depressed adults with or without coronary artery disease (6). In the peripheral circulation, endothelial function could be evaluated in a noninvasive manner from the vasomotor response of the brachial artery using ultrasound. When endothelial dysfunction was present, flow-mediated dilatation might be reduced (16).

The aim of the study was to measure the levels of platelet serotonin in a group of patients with major depressive disorder, and evaluate a possible correlation with the endothelial dysfunction using FMBA.

II. MATERIALS AND METHODS

Samples

The patients were recruited from Dr. Hasan Sadikin Hospital, West Java Mental Hospital, Dustira Hospital and Salamun Hospital in West Java Indonesia through consecutive sampling technique. Subjects diagnosed with major depressive disorder according to the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV). After the initial psychiatric interview to confirm the presence of major depression diagnosis, patients were evaluated with SCID-I (Structured Clinical Interview for DSM-IV Axis I Disorders). This is a semi-structured interview that provides information for the diagnosis of major DSM-IV Axis I psychiatric disorders. SCID-I was translated into Indonesia language and its reliability and validity were established (17). Eighteen to sixty years old patients, never had antidepressant therapy or had not taking antidepressant for the last two weeks were included. Those with a history of depressive disorder or another psychiatric diagnosis, recent or history of cardiovascular diseases, diabetes mellitus, hypertension, obesity, dyslipidemia and smoking were excluded.

As a result, 65 patients were included in the study. The study was approved by the Ethics Committee of the Universitas Padjadjaran. All participants were informed about the study and gave consent to participate in it.

Thirty seven female and twenty eight male patients were included as subjects. The mean (±SD) age of all the patient was 30.6±9.4, patient with normal FMBA (n=25) was 31.5±10.8, patient with abnormal FMBA was 30±8.3, and the difference of the age and sex between the groups was not statistically significant (Table I).
Variable | FMBA score | p
---|---|---
| Normal (n = 25) | Abnormal (n = 40) | 
Age (mean±SD) | 31.5±10.8 | 30±8.3 | 0.79*

Sex
- Male | 9 (36%) | 19 (47.5%) | 0.36**
- Female | 16 (64%) | 21 (52.5%)

*Unpaired t-test

**Chi-square test

**Platelet serotonin measurement**

Blood samples were drawn by venipuncture after an overnight fast into tubes containing EDTA as anticoagulant. Then 2.5 ml of blood was centrifuged for 10 min at 200 x g and room temperature to obtain PRP. Platelet counts were determined on aliquots of pooled PRP diluted in Isotone II and counted twice on a thrombocounter. 200 µL PRP and 800 µL NaCl 0.9% was centrifuged for 2 minutes at 10 000 x g and 4°C. The supernatant was discarded and the pellet was suspended in 1 ml of a mixture containing 4 % perchloric acid and 0.15 % EDTA.

The eluate was used for the determination of platelet serotonin level. The samples were kept frozen at -80 °C until used. The portions kept for serotonin determination in PRP were thawed and centrifuged for 3 min at 2000 x g. Platelet serotonin contents were measured as described previously. An aliquot of the supernatants was applied to the HPLC system. Platelet serotonin contents were expressed as ng/10^9 platelets.

**Fluid Mediated Brachial Artery measurement**

Numerous factors affect flow mediated vascular reactivity, including temperature, food, drugs and sympathetic stimuli, among others. Therefore, subjects should fast for at least 8 to 12 h before the study, and they should be examined in a quiet, temperature-controlled room. Subjects may not exercise neither ingest substances that might affect FMBA such as caffeine, high-fat foods and vitamin C at least 4 to 6 h before the study. All of these confounding factors must be considered in preparing subject in studies that seek to determine the impact of a single intervention. This study was using General Electric-Vivid 7 echocardiography, with 7 - 12 MHz probe/transducer.

FMBA was determined according to Corretti et al as briefly following described (18). The subject was in supine position with the arm in a comfortable position for imaging the brachial artery. The brachial artery is imaged above the antecubital fossa in the longitudinal plane. A segment with clear anterior and posterior intimal interfaces between the lumen and vessel wall is selected for continuous 2D grayscale imaging. During image acquisition, anatomic landmarks such as veins and fascial planes are noted to help maintain the same image of the artery throughout the study. To create a flow stimulus in the brachial artery, a sphygmomanometer (blood pressure) cuff was first placed above the antecubital fossa. A baseline rest image was acquired, and blood flow was estimated by time-averaging the pulsed Doppler velocity signal obtained from a mid-artery sample volume. Thereafter, arterial occlusion was created by cuff inflation to supra systolic pressure. The cuff was inflated to at least 50 mm Hg above systolic pressure to occlude arterial inflow for 5 minutes. The longitudinal image of the artery was recorded continuously from 30 s before to 1 min after cuff deflation. A mid-artery pulsed Doppler signal was obtained upon immediate cuff release and no later than 15 s after cuff deflation to assess hyperemic velocity.

The FMBA results were scored according to Ryliškytė et al scoring in Table II, and divided into normal and abnormal FMBA group (19).
Table II. Flow-mediated dilation ranges in low cardiovascular risk subjects according to age

<table>
<thead>
<tr>
<th>Brachial artery diameter (mm)</th>
<th>FMBA according to patient’s age</th>
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<tbody>
<tr>
<td></td>
<td>25 years</td>
</tr>
<tr>
<td></td>
<td>35 years</td>
</tr>
<tr>
<td>FMBA, (%)</td>
<td>95% C.I.</td>
</tr>
<tr>
<td>2</td>
<td>15.93</td>
</tr>
<tr>
<td>3</td>
<td>13.33</td>
</tr>
<tr>
<td>4</td>
<td>10.72</td>
</tr>
<tr>
<td>5</td>
<td>8.12</td>
</tr>
<tr>
<td>45 years</td>
<td>12.44</td>
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<tr>
<td>55 years</td>
<td>9.84</td>
</tr>
<tr>
<td>65 years</td>
<td>7.24</td>
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<tr>
<td>75 years</td>
<td>4.63</td>
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<tr>
<td>6</td>
<td>12.44</td>
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<td>7</td>
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<td>8</td>
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<td>9</td>
<td>4.63</td>
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<tr>
<td>10</td>
<td>1.15</td>
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</table>

FMBA: Flow-Mediated Brachial Artery

Normative ranges of flow-mediated brachial artery predicted for each age group according to the model established by Ryliškytė et al in a study of low cardiovascular risk patients (Ryliškytė et al, 2003).

Statistical analysis

Results were defined as median values and range (median, range: min-max). Mann Whitney test was used for testing the statistical significance of correlation between platelet serotonin level and FMBA result. Level of significance was set as p < 0.05 for all of the statistical analyses.

III. RESULT

All the patients completed the study. The difference of platelet serotonin level between MDD patients with normal and abnormal FMBA score was shown in Table III and Figure 1.
Table III. The difference of platelet serotonin level between MDD patients with normal and abnormal FMBA score

<table>
<thead>
<tr>
<th>FMBA score</th>
<th>p</th>
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<tbody>
<tr>
<td>normal (n = 25)</td>
<td></td>
</tr>
<tr>
<td>abnormal (n = 40)</td>
<td></td>
</tr>
<tr>
<td>Platelet serotonin</td>
<td>0.0001</td>
</tr>
<tr>
<td>median</td>
<td></td>
</tr>
<tr>
<td>IQR</td>
<td></td>
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<tr>
<td>range (min-max)</td>
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Platelet serotonin level in ng/10⁹ platelet

FMBA: Flow-Mediated Brachial Artery

IV. DISCUSSION

Despite several evidences linking depression to cardiovascular disease, little is known about the patho-etiologies of depressive illness, as interventions could be used to minimize negative outcomes during the critical period of understanding the etiology of the disease. Our study findings are consistent with the plausibility that depression contributes to the progression of the early stages of cardiovascular diseases, the first sign of which is endothelial dysfunction. Previous research has shown that deficient NO signaling is responsible for the endothelial dysfunction observed in depressed individuals (20–23). Mild symptoms of depression may be sufficient to impair endothelial function even in young and healthy people (24).

The question that arises next is how assess the risk of endothelial dysfunction in MDD and what mechanisms underlie the process? Answering these questions will provide deeper insights into how depression increases endothelial dysfunction risk, and theorize the development of efficacious interventions to minimize the impact of depression.

In our study despite the classical risk factor of endothelial dysfunction were excluded (recent or history of cardiovascular diseases, diabetes mellitus, hypertension, obesity, dyslipidemia and smoking), the levels of platelet serotonin in MDD patients had significant correlation with the endothelial dysfunction assessment using FMBA. Our result imply that platelet serotonin could be used in assessing early sign of endothelial dysfunction as with FMBA in MDD.

Platelet serotonin and FMBA might also be use for assess the impact of treatment, especially selective serotonin reuptake inhibitors (SSRIs) treatment. Study assessed effect of SSRI on endothelial dysfunction in young group of women without cardiovascular disease found SSRI treatment had little effect on flow-mediated vascular dilatation (FMD) and platelet aggregation, and unclear whether it decreased nitric oxide production or not (25). There was unique pattern of platelet activation by serotonin in patients with stable coronary artery disease (CAD) (26). Initial concentration of serotonin caused increased platelet activation, but when platelets were stimulated with higher concentration of serotonin, there was a significant decrease in platelet activation. There might be a negative
feedback mechanism which lead to decreased platelet activation and aggregation. SSRI treatment in MDD patients with and without cardiovascular diseases should always consider the importance of assessing whether it affect the endothelial function or not.

Serotonin is an endothelium-dependent vasodilator agents influencing endothelial function (16). One hypothesis that arise is there are genetical influence, the possibility of serotonin transporter (SERT) polymorphism role in the etiology of MDD and cardiovascular diseases because there is increasing evidence that the S allele of SERT polymorphism could increase the risk for cardiac events via its impact on emotion and some studies reveal that there is interaction between SERT activity and NO production (27-30). The possibility that SERT polymorphism influence functions of the vascular endothelium in this regards is subject to be further studied.

In recent years, many studies have investigated polymorphisms in candidate genes in relation to functional characteristics of central or peripheral mechanisms which are involved in the development of cardiovascular diseases. The fact that many of these genes are also discussed as liability genes for depression raises the intriguing question whether an interactive or synergistic effect is responsible for the bidirectional relationship. Future studies of biological markers and genotyping for relevant polymorphisms in genes which are assumed to be involved in both disorders will have to be carried out to shed more light on the interaction between depression and cardiovascular diseases.

V. CONCLUSION
Platelet serotonin levels were associated with and had important role in endothelial dysfunction, and is considered as an indicator of endothelial dysfunction in MDD, beside the non-invasively detection using FMBA.

REFERENCES


